PBMCs). Multivariate Cox analyses indicated that a higher proviral load (more than 4 copies/100 PBMCs) is an independent risk factor for progression of ATL, even after adjusting for sex, age, family history of ATL, and other possible risk factors. The result indicated that HTLV-1 carriers with higher HTLV-1 proviral load levels belong to the high-risk group of carriers who develop ATL and in whom any measures to prevent the development of ATL should be instituted.

Nevertheless, the association between HTLV-1 proviral load and disease development remains unclear because a higher proviral load is also an important predictor in patients with HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Further viral markers are needed to determine the function of a higher HTLV-1 proviral load to direct the way to developing ATL or developing HAM/TSP from HTLV-1 carriers.

CONCLUDING REMARKS

Although many prior studies found important epidemiological evidence on ATL and risk factors for the development of ATL in HTLV-1 carriers, limited data are available on the valid annual incidence of ATL from longitudinal prospective studies. Existing predisposing factors are still insufficient to explain the characteristics of ATL oncogenesis. Unknown risk factors may be involved in the acquisition of malignant characteristics of HTLV-1 infected cells. Further well-designed epidemiological studies are needed to fully understand the oncogenesis of ATL.

Even though the incidence of ATL is relatively low among HTLV-1 carriers and a novel promising agent, mogamulizumab (humanized anti-CCR4 monoclonal antibody), is released (Ishida et al., 2003, 2012), preventing new HTLV-1 infections and the development of ATL are major public health concerns in HTLV-1 endemic countries in the world. In Japan, there are approximately one million of HTLV-1 carriers, 1,000 new ATL cases, and 1,000 new deaths from ATL every year. However, only recently has the Japanese government for the first time begun to implement a nationwide comprehensive package of measures covering the prevention of mother-to-child HTLV-1 transmission and the development of medical researches on HTLV-1 and ATL (http://www.kantei.go.jp/foreign/kan/actions/201009/13htlv_e.html). The challenge in the next few years will be to reduce the number of HTLV-1 carriers, to develop an easy method that allows identification of high-risk carriers, and to implement earlier therapeutic interventions for carriers with high-risk markers.

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REFERENCES

Abbaszadegan, M. R., Gholamin, M., Tabatabaee, A., Farid, R., Houshmand, M., and Abbaszadegan, M. (2003). Prevalence of human T-lymphotropic virus type 1 among blood donors from Mashhad, Iran. *J. Clin. Microbiol.* 41, 2593–2595.

Arisaw, K., Katamine, S., Kamihira, S., Kurokawa, K., Sawada, T., Soda, M., Doi, H., Saito, H., and Shirahama, S. (2002). A nested case-control study of risk factors for adult T-cell leukemia/lymphoma among human T-cell lymphotropic virus type-I carriers in Japan. *Cancer Causes Control* 13, 657–663.

Arisawa, K., Soda, M., Endo, S., Kurokawa, K., Katamine, S., Shimokawa, I., Koba, T., Takahashi, T., Saito, H., Doi, H., and Shirahama, S. (2000). Evaluation of adult T-cell leukemia/lymphoma incidence and its impact on non-Hodgkin lymphoma incidence in southwestern Japan. *Int. J. Cancer* 85, 319–324.

Arisawa, K., Soda, M., Ono, M., Uemura, H., Hiyoshi, M., and Suyama, A. (2009). Trends of incidence rate of adult T-cell leukemia/lymphoma in an HTLV-1 endemic area in Japan. *Int. J. Cancer* 125, 737–738.

Au, W. Y., and Lo, J. Y. (2005). HTLV-1-related lymphoma in Hong Kong Chinese. *Am. J. Hematol.* 78, 80–81.

Azarpazhooh, M. R., Hasanpour, K., Ghanbari, M., Rezaee, S. A., Mashkani, B., Hedayati-Moghaddam, M. R., Valizadeh, N., Farid Hosseini, R., Foroghipoor, M., Soltanifar, A., Sahebari, M., Azadmanesh, K., Hassanshahi, G., and Rafatpanah, H. (2012). Human T-lymphotropic virus type 1 prevalence in Northeastern Iran, Sabzevar: an epidemiologic-based study and phylogenetic analysis. *AIDS Res. Hum. Retroviruses* 28, 895–901.

Birmann, B. M., Okayama, A., Kim, N., Arisawa, K., Breen, E. C., Carneiro-Proietti, A. B. F., Falk, K. I., Hanchard, B., Inoue, M., Martínez-Maza, O., Murphy, E. L., Pfeiffer, R. M., Sawada, T., Stuver, S. O., Tsugane, S., Li, H., Suppan, C. A., Mueller, N. E., and Hisada, M. (2011). Altered host immunity, human T lymphotropic virus type I replication, and risk of adult T-cell leukemia/lymphoma: a prospective analysis from the ATL Cohort Consortium. *Retrovirology* 8, A81.

Bitar, N., Hajj, H. E., Houmani, Z., Sabbah, A., Otrock, Z. K., Mahfouz, R., Zaatari, G., and Bazarbachi, A. (2009). Adult T-cell leukemia/lymphoma in the Middle East: first report of two cases from Lebanon. *Transfusion* 49, 1859–1864.

Blattner, W. A., Kalyanaraman, V. S., Robert-Guroff, M., Lister, T. A., Galton, D. A., Sarin, P. S., Crawford, M. H., Catovsky, D., Greaves, M., and Gallo, R. C. (1982). The human type-C retrovirus, HTLV, in Blacks from the Caribbean region, and relationship to adult T-cell leukemia/lymphoma. *Int. J. Cancer* 30, 257–264.

Cabrera, M. E., Labra, S., Catovsky, D., Ford, A. M., Colman, S. M., Greaves, M. F., and Matutes, E. (1994). HTLV-I positive adult T-cell leukaemia/lymphoma (ATLL) in Chile. *Leukemia* 8, 1763–1767.

Cabrera, M. E., Labra, S., Meneses, P., Matutes, E., Cartier, L., Ford, A. M., and Greaves, M. F. (1999). Adult T cell leukemia lymphoma in Chile. A clinical pathologic and molecular study of 26 patients. *Rev. Med. Chil.* 127, 935–944.

Cabrera, M. E., Marinov, N., Guerra, C., Morilla, R., and Matutes, E. (2003). Chronic lymphoproliferative syndromes in Chile. A prospective study in 132 patients. *Rev. Med. Chil.* 131, 291–298.

Cabrera, M. E., Martinez, V., Nathwani, B. N., Muller-Hermelink, H. K., Diebold, J., MacLennan, K. A., Armitage, J., and Weisenburger, D. D. (2012). Non-Hodgkin lymphoma in Chile: a review of 207 consecutive adult cases by a panel of five expert hematopathologists. *Leuk. Lymphoma* 53, 1311–1317.

Carvalho, E. M., and Da Fonseca Porto, A. (2004). Epidemiological and clinical interaction between HTLV-1 and Strongyloides stercoralis. *Parasite Immunol.* 26, 487–497.

Catovsky, D., Greaves, M. F., Rose, M., Galton, D. A., Goolden, A. W., McCluskey, D. R., White, J. M., Lampert, I., Bourikas, G., Ireland, R., Brownell, A. I., Bridges, J. M., Blattner, W. A., and Gallo, R. C. (1982). Adult T-cell lymphoma-leukaemia in Blacks from the West Indies. *Lancet* 1, 639–643.

Chen, P. M., Chiu, C. F., Chiou, T. J., Tzeng, C. H., and Chiang, B. N. (1985). Adult T-cell leukemia. First case reported in Taiwan. *Nippon Ketsueki Gakkai Zasshi* 8, 1035–1041.

Delaporte, E., Dupont, A., Peeters, M., Josse, R., Merlin, M., Schrijvers, D., Hamono, B., Bedjabaga, L., Cheringou, H., Boyer, F., Brun-Vézinet, F., and Larouzé, B. (1988). Epidemiology of HTLV-I in Gabon (Western Equatorial Africa). *Int. J. Cancer* 42, 687–689.

Delaporte, E., Klotz, F., Peeters, M., Martin-Prevel, Y., Bedjabaga, L., Larouzé, B., Nguembi-Mbina, C., Walter, P., and Piot, P. (1993). Non-Hodgkin lymphoma in Gabon and its relation to HTLV-I. *Int. J. Cancer* 53, 48–50.

Etenna, S. L., Caron, M., Besson, G., Makuwa, M., Gessain, A., Mahé, A., and Kazanji, M. (2008). New insights into prevalence, genetic diversity, and proviral load of human T-cell leukemia virus types 1 and 2 in pregnant women in Gabon in equatorial central Africa. *J. Clin. Microbiol.* 46, 3607–3614.

Farias de Carvalho, S. M., Pombo de Oliveira, M. S., Thuler, L. C., Rios, M., Coelho, R. C., Rubim, L. C., Silva, E. M., Reis, A. M., and Catovsky, D. (1997). HTLV-I and HTLV-II infections in hematologic disorder patients, cancer patients, and healthy individuals from Rio de Janeiro, Brazil. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* 15, 238–242.

Gabet, A. S., Mortreux, F., Talarmin, A., Plumelle, Y., Leclercq, I., Leroy, A., Gessain, A., Clity, E., Joubert, M., and Wattel, E. (2000). High circulating proviral load with oligoclonal expansion of HTLV-1 bearing T cells in HTLV-1 carriers with strongyloidiasis. *Oncogene* 19, 4954–4960.

Gérard, Y., Lepere, J. F., Pradinaud, R., Joly, F., Lepelletier, L., Joubert, M., Sainte Marie, D., Mahieu, R., Vidal, A. U., Larregain-Fournier, D., Valensi, F., Moynet, D., de Thé, G., Guillemain, B., Moreau, J. P., and Gessain, A. (1995). Clustering and clinical diversity of adult T-cell leukemia/lymphoma associated with HTLV-I in a remote black population of French Guiana. *Int. J. Cancer* 60, 773–776.

Gessain, A. (1996). "Epidemiology of HTLV-I and associated diseases," in *Human T-Cell Lymphotropic Virus Type 1*, eds P. Höllsberg and D. A. Hafler (Chichester: John Wiley & Sons Ltd.), 33–64.

Gessain, A., Gout, O., Saal, F., Daniel, M. T., Rio, B., Flandrin, G., Sigaux, F., Lyon-Caen, O., Periès, J., and de-Thé, G. (1990). Epidemiology and immunovirology of human T-cell leukemia/lymphoma virus type I-associated adult T-cell leukemia and chronic myelopathies as seen in France. *Cancer Res.* 50, 5692S–5696S.

Gonçalves, D. U., Proietti, F. A., Ribas, J. G., Araújo, M. G., Pinheiro, S. R., Guedes, A. C., and Carneiro-Proietti, A. B. (2010). Epidemiology, treatment, and prevention of human T-cell leukemia virus type 1-associated diseases. *Clin. Microbiol. Rev.* 23, 577–589.

Hanchard, B. (1996). Adult T-cell leukemia/lymphoma in Jamaica: 1986–1995. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* 13, S20–S25.

Hattori, T., Uchiyama, T., Toibana, T., Takatsuki, K., and Uchino, H. (1981). Surface phenotype of Japanese adult T-cell leukemia cells characterized by monoclonal antibodies. *Blood* 58, 645–647.

Hedayati-Moghaddam, M. R., Fathi-moghadam, F., Eftekharzadeh Mashhadi, I., Soghandi, L., and Bidkhori, H. R. (2011). Epidemiology of HTLV-1 in Neyshabour, Northeast of Iran. *Iran. Red Crescent Med. J.* 13, 424–427.

Hinuma, Y., Komoda, H., Chosa, T., Kondo, T., Kohakura, M., Takenaka, T., Kikuchi, M., Ichimaru, M., Yunoki, K., Sato, I., Matsuo, R., Takiuchi, Y., Uchino, H., and Hanaoka, M. (1982). Antibodies to adult T-cell leukemia-virus-associated antigen (ATLA) in sera from patients with ATL and controls in Japan: a nation-wide sero-epidemiologic study. *Int. J. Cancer* 29, 631–635.

Hinuma, Y., Nagata, K., Hanaoka, M., Nakai, M., Matsumoto, T., Kinoshita, D., Shirakawa, S., and Miyoshi, I. (1981). Adult T cell leukemia: antigen in an ATL cell line and detection of antibodies to the antigen in human sera. *Proc. Natl. Acad. Sci. U.S.A.* 78, 6476–6480.

Hisada, M., Okayama, A., Tachibana, N., Stuver, S. O., Spiegelman, D. L., Tsubouchi, H., and Mueller, N. E. (1998a). Predictors of level of circulating abnormal lymphocytes among human T-lymphotropic virus type I carriers in Japan. *Int. J. Cancer* 77, 188–192.

Hisada, M., Okayama, A., Shioiri, S, Spiegelman, D. L., Stuver, S. O., and Mueller, N. E. (1998b). Risk factors for adult T-cell leukemia among carriers of human T-lymphotropic virus type I. *Blood* 92, 3557–3561.

Hoshida, Y., Li, T., Dong, Z., Tomita, Y., Yamauchi, A., Hanai, J., and Aozasa, K. (2001). Lymphoproliferative disorders in renal transplant patients in Japan. *Int. J. Cancer* 91, 869–875.

Hunsmann, G., Bayer, H., Schneider, J., Schmitz, H., Kern, P., Dietrich, M., Büttner, D. W., Goudeau, A. M., Kulkarni, G., and Fleming, A. F. (1984). Antibodies to ATLV/HTLV-1 in Africa. *Med. Microbiol. Immunol.* 173, 167–170.

IARC. (1996). Human immunodeficiency viruses and human T-cell lymphotropic viruses. *IARC Monogr. Eval. Carcinog. Risks Hum.* 67, 1–424.

Ichimaru, M., Kinoshita, K., Kamihata, S., Ikeda, S., Yamada, Y., and Amagasaki, T. (1979). T-cell malignant lymphoma in Nagasaki district and its problems. *Jpn. J. Clin. Oncol.* 9, S337–S346.

Ikeda, N., Inoue, M., Iso, H., Ikeda, S., Satoh, T., Noda, M., Mizoue, T., Imano, H., Saito, E., Katanoda, K., Sobue, T., Tsugane, S., Naghavi, M., Ezzati, M., and Shibuya, K. (2012). Adult mortality attributable to preventable risk factors for non-communicable diseases and injuries in Japan: a comparative risk assessment. *PLoS Med.* 9, e1001160. doi:10.1371/journal.pmed.1001160

Ikeda, S., Momita, S., Kinoshita, K., Kamihira, S., Moriuchi, Y., Tsukasaki, K., Ito, M., Kanda, T., Moriuchi, R., and Nakamura, T. (1993). Clinical course of human T-lymphotropic virus type I carriers with molecularly detectable monoclonal proliferation of T lymphocytes: defining a low- and high-risk population. *Blood* 82, 2017.

Imaizumi, Y., Iwanaga, M., Tsukasaki, K., Hata, T., Tomonaga, M., and Ikeda, S. (2005). Natural course of HTLV-1 carriers with monoclonal proliferation of T lymphocytes ("pre-ATL") in a 20-year follow-up study. *Blood* 105, 903–904.

Ishida, T., Joh, T., Uike, N., Yamamoto, K., Utsunomiya, A., Yoshida, S., Saburi, Y., Miyamoto, T., Takemoto, S., Suzushima, H., Tsukasaki, K., Nosaka, K., Fujiwara, H., Ishitsuka, K., Inagaki, H., Ogura, M., Akinaga, S., Tomonaga, M., Tobinai, K., and Ueda, R. (2012). Defucosylated anti-CCR4 monoclonal antibody (KW-0761) for relapsed adult T-cell leukemia-lymphoma: a multicenter phase II study. *J. Clin. Oncol.* 30, 837–842.

Ishida, T., Utsunomiya, A., Iida, S., Inagaki, H., Takatsuka, Y., Kusumoto, S., Takeuchi, G., Shimizu, S., Ito, M., Komatsu, H., Wakita, A., Eimoto, T., Matsushima, K., and Ueda, R. (2003). Clinical significance of CCR4 expression in adult T-cell leukemia/lymphoma: its close association with skin involvement and unfavorable outcome. *Clin. Cancer Res.* 9, 3625–3634.

Iwanaga, M., Watanabe, T., Utsunomiya, A., Okayama, A., Uchimaru, K., Koh, K. R., Ogata, M., Kikuchi, H., Sagara, Y., Uozumi, K., Mochizuki, M., Tsukasaki, K., Saburi, Y., Yamamura, M., Tanaka, J., Moriuchi, Y., Hino, S., Kamihira, S., Yamaguchi, K., and Joint Study on Predisposing Factors of ATL Development investigators. (2010). Human T-cell leukemia virus type I (HTLV-1) proviral load and disease progression in asymptomatic HTLV-1 carriers: a nationwide prospective study in Japan. *Blood* 116, 1211–1219.

Kamihira, S., Atogami, S., Sohda, H., Momita, S., Yamada, Y., and Tomonaga, M. (1994). Significance of soluble interleukin-2 receptor levels for evaluation of the progression of adult T-cell leukemia. *Cancer* 73, 2753–2758.

Kamihira, S., Iwanaga, M., Doi, Y., Sasaki, D., Mori, S., Tsurda, K., Nagai, K., Uno, N., Hasegawa, H., Yanagihara, K., Morinaga, Y., Tsukasaki, K., and Taniguchi, H. (2012). Heterogeneity in clonal nature in the smoldering subtype of adult T-cell leukemia: continuity from carrier status to smoldering ATL. *Int. J. Hematol.* 95, 399–408.

Kawano, N., Shimoda, K., Ishikawa, F., Taketomi, A., Yoshizumi, T., Shimoda, S., Yoshida, S., Uozumi, K., Suzuki, S., Maehara, Y., and Harada, M. (2006). Adult T-cell leukemia development from a human T-cell leukemia virus type I carrier after a living-donor liver transplantation. *Transplantation* 82, 840–843.

Kchour, G., Makhoul, N. J., Mahmoudi, M., Kooshyar, M. M., Shirdel, A., Rastin, M., Rafatpanah, H., Tarhini, M., Zalloua, P. A., Hermine, O., Farid, R., and Bazarbachi, A. (2007). Zidovudine and interferon-alpha treatment induces a high response rate and reduces HTLV-1 proviral load and VEGF plasma levels in patients with adult T-cell leukemia from North East Iran. *Leuk. Lymphoma* 48, 330–336.

Kchour, G., Tarhini, M., Kooshyar, M. M., El Hajj, H., Wattel, E., Mahmoudi, M., Hatoum, H., Rahimi, H., Maleki, M., Rafatpanah, H., Rezaee, S. A., Yazdi, M. T., Shirdel, A., de Thé, H., Hermine, O., Farid, R., and Bazarbachi, A. (2009). Phase 2 study of the efficacy and safety of the combination of arsenic trioxide, interferon alpha, and zidovudine in newly diagnosed chronic adult T-cell leukemia/lymphoma (ATL). *Blood* 113, 6528–6532

Khameneh, Z. R., Baradaran, M., and Sepehrvand, N. (2008). Survey of the seroprovalence of HTLVI/II in hemodialysis patients and blood donors in Urmia. *Saudi J. Kidney Dis. Transpl.* 19, 838–841.

Koga, Y., Iwanaga, M., Soda, M., Inokuchi, N., Sasaki, D., Hasegawa, H., Yanagihara, K., Yamaguchi, K., Kamihira, S., and Yamada, Y. (2010). Trends in HTLV-1 prevalence and the incidence of adult Tcell leukemia/lymphoma (ATLL) in Nagasaki, Japan. *J. Med. Virol.* 82, 668–674.

Kondo, T., Kono, H., Miyamoto, N., Yoshida, R., Toki, H., Matsumoto, I., Hara, M., Inoue, H., Inatsuki, A., Funatsu, T., Yamano, N., Bando, F., Iwao, E., Miyoshi, I., Hinuma, Y., and Hanaoka, M. (1989). Age- and sex-specific cumulative rate and risk of ATLL for HTLV-I carriers. *Int. J. Cancer* 43, 1061–1104.

Kondo, T., Kono, H., Nonaka, H., Miyamoto, N., Yoshida, R., Bando, F., Inoue, H., Miyoshi, I., Hinuma, Y., and Hanaoka, M. (1987). Risk of adult T-cell leukaemia/lymphoma in HTLV-I carriers. *Lancet* 2, 159.

Kondo, T., Nonaka, H., Miyamoto, N., Yoshida, R., Matsue, Y., Ohguchi, Y., Inouye, H., Komoda, H., Hinuma, Y., and Hanaoka, M. (1985). Incidence of adult T-cell leukemia-lymphoma and its familial clustering. *Int. J. Cancer* 35, 749–751.

Lee, C. W., Chang, M. C., Chang, Y. F., Hsieh, R. K., Lin, J., and Chen, K. S. (2010). Adult T-cell leukemia/lymphoma in Taiwan: an analysis of 17 patients and review of the literature. *Asia Pac. J. Clin. Oncol.* 6, 161–164.

Lee, M., Kim, B. K., Lee, H. B., Park, K. S., Suh, C., Lee, I. H., Bang, Y. I., Kim, S. T., Kim, N. K., Cha, C. Y., Moon, S. B., Cho, H. I., Lee, S. Y., and Takatsuki, K. (1987). Adult T-cell leukemia – the first case in Republic of Korea. *J. Kor. Med. Assoc.* 30, 1146–1152.

Levine, P. H., Dosik, H., Joseph, E. M., Felton, S., Bertoni, M. A., Cervantes, J., Moulana, V., Miotti, A. B., Goberdhan, L. J., Lee, S. L., Daouad, A., DaCosta, M., Jaffe, E. S., Axiotis, C. A., Cleghorn, F. R., Kahn, A., and Welles, S. L. (1999). A study of adult T-cell leukemia/lymphoma incidence in central Brooklyn. *Int. J. Cancer* 80, 662–666.

Liang, R. (1994). HTLV-I associated adult T-cell leukaemia/lymphoma in Hong Kong. *Am. J. Hematol.* 45, 100–101.

Lymphoma Study Group of Japanese Pathologists. (2000). The World Health Organization classification of malignant lymphomas in Japan: incidence of recently recognized entities. *Pathol. Int.* 50, 696–702.

Manns, A., Miley, W. J., Wilks, R. J., Morgan, O. S., Hanchard, B., Wharfe, G., Cranston, B., Maloney, E., Welles, S. L., Blattner, W. A., and Waters, D. (1999). Quantitative proviral DNA and antibody levels in the natural history of HTLV-1 infection. *J. Infect. Dis.* 180, 1487–1493.

Manzari, V., Gradilone, A., Barillari, G., Zani, M., Collalti, E., Pandolfi, F., De Rossi, G., Liso, V., Babbo, P., and Robert-Guroff, M. (1985). HTLV-I is endemic in southern Italy: detection of the first infectious cluster in a white population. *Int. J. Cancer* 36, 557–559.

Marin, O., Hasui, K., Remondegui, C., Sato, E., Aye, M. M., Takenouchi, N., Izumo, S., and Tajima, K. (2002). Adult T-cell leukemia/lymphoma in Jujuy, north-west Argentina. *Pathol. Int.* 52, 348–357.

Meytes, D., Schochat, B., Lee, H., Nadel, G., Sidi, Y., Cerney, M., Swanson, P., Shaklai, M., Kilim, Y., Elgat, M., Chin, E., Danon, Y., and Rosenblatt, J. D. (1990). Serological and molecular survey for HTLV-I infection in a high-risk Middle Eastern group. *Lancet* 336, 1533–1535.

Miller, M., Achiron, A., Shaklai, M., Stark, P., Maayan, S., Hannig, H., Hunsmann, G., Bodemer, W., and Shohat, B. (1998). Ethnic cluster of HTLVI infection in Israel among the Mashhadi Jewish population. *J. Med. Virol.* 56, 269–274.

Mueller, N., Okayama, A., Stuver, S., and Tachibana, N. (1996). Findings from the Miyazaki Cohort Study. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* 13, S2–S7.

Murphy, E. L., Hanchard, B., Figueroa, J. P., Gibbs, W. N., Lofters, W. S., Campbell, M., Goedert, J. J., and Blattner, W. A. (1989). Modelling the risk of adult T-cell leukemia/lymphoma in persons infected with human T-lymphotropic virus type I. *Int. J. Cancer* 43, 250–253.

Nakada, K., Yamaguchi, K., Furugen, S., Nakasone, T., Nakasone, K., Oshiro, Y., Kohakura, M., Hinuma, Y., Seiki, M., Yoshida, M., Matutes, E., Catovsky, D., Ishii, T., and Takatsuki, K. (1987). Monoclonal integration of HTLV-I proviral DNA in patients with strongyloidiasis. *Int. J. Cancer* 40, 145–148.

Ohshima, K., Suzumiya, J., and Kikuchi, M. (2002). The World Health Organization classification of malignant lymphoma: incidence and clinical prognosis in HTLV-1-endemic area of Fukuoka. *Pathol. Int.* 52, 1–12.

Okayama, A., Stuver, S., Matsuoka, M., Ishizaki, J., Tanaka, G., Kubuki, Y., Mueller, N., Hsieh, C. C., Tachibana, N., and Tsubouchi, H. (2004). Role of HTLV-1 proviral DNA load and clonality in the development of adult T-cell leukemia/lymphoma in asymptomatic carriers. *Int. J. Cancer* 110, 621–625.

Paun, L., Ispas, O., Del Mistro, A., and Chieco-Bianchi, L. (1994). HTLV-I in Romania. *Eur. J. Haematol.* 52, 117–118.

Plancoulaine, S., Buigues, R. P., Murphy, E. L., van Beveren, M., Pouliquen, J. F., Joubert, M., Rémy, F., Tuppin, P., Tortevoye, P., de Thé, G., Moreau, J. P., and Gessain, A. (1998). Demographic and familial characteristics of HTLV-1 infection among an isolated, highly endemic population of African origin in French Guiana. *Int. J. Cancer* 76, 331–336.

Plumelle, Y., Gonin, C., Edouard, A., Bucher, B. J., Thomas, L., Brebion, A., and Panelatti, G. (1997). Effect of strongyloides stercoralis infection and eosinophilia on age at onset and prognosis of adult T-cell leukemia. *Am. J. Clin. Pathol.* 107, 81–87.

Poiesz, B. J., Ruscetti, F. W., Gazdar, A. F., Bunn, P. A., Minna, J. D., and Gallo, R. C. (1980). Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc. Natl. Acad. Sci. U.S.A.* 77, 7415–7419.

Pombo de Oliveira, M. S., Loureiro, P., Bittencourt, A., Chiattone, C., Borducchi, D., De Carvalho, S. M., Barbosa, H. S., Rios, M., Sill, A., Cleghorn, F., and Blattner, W. (1999). Geographic diversity of adult t-cell leukemia/lymphoma in Brazil. The Brazilian ATLL Study Group. *Int. J. Cancer* 83, 291–298.

Pombo de Oliveira, M. S., Matutes, E., Schulz, T., Carvalho, S. M., Noronha, H., Reaves, J. D., Loureiro, P., MacHado, C., and Catovsky, D. (1995). T-cell malignancies in Brazil: clinico-pathological and molecular studies of HTLV-I positive and negative cases. *Int. J. Cancer* 60, 823–827.

Portal Site of Official Statistics of Japan. (2012). *Portal Site of Official Statistics of Japan (e-Stat), Developed by Statistics Bureau, Ministry of Internal Affairs and Communications with the collaboration of Ministries and Agencies, and Managed by Incorporated Administrative Agency National Statistics Center.* Available at: http://www.e-stat.go.jp/SG1/estat/GL08020101.do?_toGL08020101_&tstatCode=000001028897&requestSender=dsearch [In Japanese, accessed April 8, 2012]

Pouliquen, J. F., Hardy, L., Lavergne, A., Kafiludine, E., and Kazanji, M. (2004). High seroprevalence of human T-cell lymphotropic virus type 1 in blood donors in Guyana and molecular and phylogenetic analysis of new strains in the Guyana shelf (Guyana, Suriname, and French Guiana). *J. Clin. Microbiol.* 42, 2020–2026.

Proietti, F. A., Carneiro-Proietti, A. B., Catalan-Soares, B. C., and Murphy, E. L. (2005). Global epidemiology of HTLV-I infection and associated diseases. *Oncogene* 24, 6058–6068.

Rafatpanah, H., Hedayati-Moghaddam, M. R., Fathimoghadam, F., Bidkhori, H. R., Shamsian, S. K., Ahmadi, S., Sohgandi, L., Azarpazhooh, M. R., Rezaee, S. A., Farid, R., and Bazarbachi, A. (2011). High prevalence of HTLV-I infection in Mashhad, Northeast Iran: a population-based seroepidemiology survey. *J. Clin. Virol.* 52, 172–176.

Safai, B., Huang, J. L., Boeri, E., Farid, R., Raafat, J., Schutzer, P., Ahkami, R., and Franchini, G. (1996). Prevalence of HTLV type I infection in Iran: a serological and genetic study. *AIDS Res. Hum. Retroviruses* 12, 1185–1190.

Sakuma, T., Satoh, T., Satodate, R., Madarame, T., Onodera, I., Suzuki, Z., and Itoh, C. (1988). Adult T-cell leukemia by probable horizontal transmission from husband to wife. *Jpn. J. Clin. Oncol.* 18, 75–79.

Satake, M., Yamaguchi, K., and Tadokoro, K. (2012). Current prevalence of HTLV-1 in Japan as determined by screening of blood donors. *J. Med. Virol.* 84, 327–335.

Satoh, M., Toma, H., Sugahara, K., Etoh, K., Shiroma, Y., Kiyuna, S., Takara, M., Matsuoka, M., Yamaguchi, K., Nakada, K., Fujita, K., Kojima, S., Hori, E., Tanaka, Y., Kamihira, S., Sato, Y., and Watanabe, T. (2002). Involvement of IL-2/IL-2R system activation by parasite antigen in polyclonal expansion of CD4(+)25(+) HTLV-1-infected T-cells in human carriers of both HTLV-1 and S. stercoralis. *Oncogene* 21, 2466–2475.

Shimoyama, M. (1991). Diagnostic criteria and classification of clinical subtypes of adult T-cell leukaemia-lymphoma: a report from the Lymphoma Study Group (1984–87). *Br. J. Haematol.* 79, 428–437.

Shtalrid, M., Shvidel, L., Korenfeld, R., Duek, A., Landau, Z., and Berrebi, A. (2005). HTLV-1 associated adult T-cell leukemia/lymphoma in Israel: report of two patients of Romanian origin. *Haematologica* 90, ECR13.

Sidi, Y., Meytes, D., Shohat, B., Fenig, E., Weisbort, Y., Lee, H., Pinkhas, J., and Rosenblatt, J. D. (1990). Adult T-cell lymphoma in Israeli patients of Iranian origin. *Cancer* 65, 590–593.

Sonoda, S., Li, H. C., and Tajima, K. (2011). Ethnoepidemiology of HTLV-1 related diseases: ethnic determinants of HTLV-1

susceptibility and its worldwide dispersal. *Cancer Sci.* 102, 295–301.

Tachibana, N., Okayama, A., Ishihara, S., Shioiri, S., Murai, K., Tsuda, K., Goya, N., Matsuo, Y., Essex, M., Stuver, S., and Mueller, N. (1992). High HTLV-I proviral DNA level associated with abnormal lymphocytes in peripheral blood from asymptomatic carriers. *Int. J. Cancer* 51, 593–595.

Tajima, K. (1990). The 4th nationwide study of adult T-cell leukemia/lymphoma (ATL) in Japan: estimates of risk of ATL and its geographical and clinical features. The T- and B-cell Malignancy Study Group. *Int. J. Cancer* 45, 237–243.

Takatsuki, K., Uchiyama, J., Sagawa, K., and Yodoi, J. (1977). "Adult T-cell leukemia in Japan," in *Topics in Hematology*, eds S. Seno, F. Takaku, and S. Irino (Amsterdam: Excerpta Medica), 73–77.

Takezaki, T., Hirose, K., Hamajima, N., Kuroishi, T., and Tajima, K. (1997). Estimation of adult T-cell leukemia incidence in Kyushu District from vital statistics Japan between 1983 and 1982: comparison with a nationwide survey. *Jpn. J. Clin. Oncol.* 27, 140–145.

Talarmin, A., Vion, B., Ureta-Vidal, A, Du Fou, G., Marty, C., and Kazanji, M. (1999). First seroepidemiological study and phylogenetic characterization of human T-cell lymphotropic virus type I and II infection among Amerindians in French Guiana. *J. Gen. Virol.* 80, 3083–3088.

The T- and B-Cell Malignancy Study Group. (1981). Statistical analysis of immunologic, clinical and histopathologic data on lymphoid malignancies in Japan. *Jpn. J. Clin. Oncol.* 11, 15–38.

The T- and B-Cell Malignancy Study Group. (1985). Statistical analyses of clinico-pathological, virological and epidemiological data on lymphoid malignancies with special reference to adult T-cell leukemia/lymphoma: a report of the second nationwide study of Japan. *Jpn. J. Clin. Oncol.* 15, 517–535.

Tian, Y., Kobayashi, S., Ohno, N., Isobe, M., Tsuda, M., Zaike, Y., Watanabe, N., Tani, K., Tojo, A., and Uchimaru, K. (2011). Leukemic T cells are specifically enriched in a unique CD3(dim) CD7(low) subpopulation of CD4(+) T cells in acute-type adult T-cell leukemia. *Cancer Sci.* 102, 569–577.

Tokudome, S., Tokunaga, O., Shimamoto, Y., Miyamoto, Y., Sumida, I., Kikuchi, M., Takeshita, M., Ikeda, T., Fujiwara, K., Yoshihara, M., Yanagawa, T., and Nishizumi, M. (1989). Incidence of adult T-cell leukemia/lymphoma among human T-lymphotropic virus type I carriers in Saga, Japan. *Cancer Res.* 49, 226–228.

Tsuji, T., Sugahara, K., Tsuruda, K., Uemura, A., Harasawa, H., Hasegawa, H., Hamaguchi, Y., Tomonaga, M., Yamada, Y., and Kamihira, S. (2004). Clinical and oncologic implications in epigenetic down-regulation of CD26/dipeptidyl peptidase IV in adult T-cell leukemia cells. *Int. J. Hematol.* 80, 254–260.

Tuppin, P., Lepère, J. F., Carles, G., Ureta-Vidal, A., Gérard, Y., Peneau, C., Tortevoye, P., de Thé, G., Moreau, J. P., and Gessain, A. (1995). Risk factors for maternal HTLV-I infection in French Guiana: high HTLV-I prevalence in the Noir Marron population. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* 8, 420–425.

Uchiyama, T., Yodoi, J., Sagawa, K., Takatsuki, K., and Uchino, H. (1977). Adult T-cell leukemia: clinical and hematologic features of 16 cases. *Blood* 50, 481–492.

Uno, H., Kawano, K., Matsuoka, H., and Tsuda, K. (1988). HLA and adult T-cell leukaemia: HLA-linked genes controlling susceptibility to human T-cell leukaemia vims type I. *Clin. Exp. Immunol.* 71, 211–215.

Veelken, H., Kohler, G., Schneider, J., Dierbach, H., Mertelsmann, R., Schaefer, H. E., and Lubbert, M. (1996). HTLV-I-associated adult T cell leukemia/lymphoma in two patients from Bucharest, Romania. *Leukemia* 10, 1366–1369.

Yamada, Y., Atogami, S., Hasegawa, H., Kamihira, S., Soda, M., Satake, M., and Yamaguchi, K. (2011). Nationwide survey of adult T-cell leukemia/lymphoma (ATL) in Japan. *Rinsho Ketsueki* 52, 1765–1771.

Yamaguchi, K., Kiyokawa, T., Nakada, K., Yul, L. S., Asou, N., Ishii, T., Sanada, I., Seiki, M., Yoshida, M., and Matutes, E. (1988). Polyclonal integration of HTLV-I proviral DNA in lymphocytes from HTLV-I seropositive individuals: an intermediate state between the healthy carrier state and smouldering ATL. *Br. J. Haematol.* 68, 169–174.

Yamaguchi, K., Matutes, E., Catovsky, D., Galton, D. A., Nakada, K., and Takatsuki, K. (1987). Strongyloides stercoralis as candidate co-factor for HTLV-I-induced leukaemogenesis. *Lancet* 2, 94–95.

Yamaguchi, K., Seiki, M., Yoshida, M., Nishimura, H., Kawano, F., and Takatsuki, K. (1984). The detection of human T cell leukemia virus proviral DNA and its application for classification and diagnosis of T cell malignancy. *Blood* 63, 1235–1240.

Yamaguchi, K., Uozumi, K., Taguchi, H., Kikuch, H, Okayama, A., Kamihira, S., Hino, S., Nosaka, K., and Watanabe, T. (2007). Nationwide Cohort Study of HTLV-1 Carriers in Japan: Joint Study on Predisposing Factors of ATL Development (JSPFAD) [Abstract O-23]. *AIDS Res. Hum. Retroviruses* 23, 581–600.

Yamamoto, J. F., and Goodman, M. T. (2008). Patterns of leukemia incidence in the United States by subtype and demographic characteristics, 1997–2002. *Cancer Causes Control* 19, 379–390.

Yashiki, S., Fujiyoshi, T., Arima, N., Osame, M., Yoshinaga, M., Nagata, Y., Tara, M., Nomura, K., Utsunomiya, A., Hanada, S., Tajima, K., and Sonoda, S. (2001). HLA-A*26, HLA-B*4002, HLAB*4006, and HLA-B* 4801 alleles predispose to adult T cell leukemia: the limited recognition of HTLV type 1 tax peptide anchor motifs and epitopes to generate anti-HTLV type 1 tax CD8(+) cytotoxic T lymphocytes. *AIDS Res. Hum. Retroviruses* 17, 1047–1061.

Yoshida, M., Miyoshi, I., and Hinuma, Y. (1982). Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implication in the disease. *Proc. Natl. Acad. Sci. U.S.A.* 79, 2031–2035.

Yoshizumi, T., Shirabe, K., Ikegami, T., Kayashima, H., Yamashita, N., Morita, K., Masuda, T., Hashimoto, N., Taketomi, A., Soejima, Y., and Maehara, Y. (2012). Impact of human T cell leukemia virus type 1 in living donor liver transplantation. *Am. J. Transplant.* 12, 1479–1485.

Zhuo, J., Yang, T., Zeng, Y., and Lu, L. (1995). Epidemiology of anti-human T-cell leukemia virus type I antibody and characteristics of adult T-cell leukemia in China. *Chin. Med. J.* 108, 902–906.

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The roles of acquired and innate immunity in human T-cell leukemia virus type 1-mediated diseases

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Human T-cell leukemia virus type 1 (HTLV-1) causes adult T-cell leukemia (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis in small subsets of HTLV-1 carriers. HTLV-1-specific T-cell responses play critical roles in anti-viral and anti-tumor host defense during HTLV-1 infections. Some HTLV-1 carriers exhibit selective loss or anergy of HTLV-1-specific T-cells at an asymptomatic stage. This is also observed in ATL patients and may therefore be an underlying risk factor of ATL in combination with elevated proviral loads. HTLV-1-specific T-cells often recognize the viral oncoprotein Tax, indicating expression of Tax protein *in vivo*, although levels of HTLV-1 gene expression are known to be very low. A type-I interferon (IFN) response can be induced by HTLV-1-infected cells and suppresses HTLV-1 expression *in vitro*, suggesting a role of type-I IFN response in viral suppression and pathogenesis *in vivo*. Both acquired and innate immune responses control the status of HTLV-1-infected cells and could be the important determinants in the development of HTLV-1-mediated malignant and inflammatory diseases.

Keywords: adult T-cell leukemia, cytotoxic T lymphocytes, human T-cell leukemia virus type 1, HTLV-1-associated myelopathy/tropical spastic paraparesis, type-I interferon

INTRODUCTION

Human T-lymphotropic virus type I (HTLV-1) causes adult T-cell leukemia (ATL) (Hinuma et al., 1981; Uchiyama, 1997) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP; Gessain et al., 1985; Osame et al., 1986) in a subset of infected individuals. While ATL is a malignant lymphoproliferative disease, HAM/TSP presents as a chronic inflammatory neurodegenerative disease. It is not known how one virus can cause two vastly different diseases. Since no disease-specific difference among HTLV-1 strains have been identified (Daenke et al., 1990; Kinoshita et al., 1991), the different pathogenic consequences must be attributed to host factors. Indeed, the two diseases usually occur in different populations of HTLV-1 carriers. Identification of the determinant factors may allow the prediction of disease risks and also the development of prophylactic and therapeutic strategies.

One of the factors that are known to differ between ATL and HAM/TSP patients is the strength of HTLV-1-specific T-cell responses. T-cell responses are activated in HAM/TSP patients, but are weak in ATL patients, and are thus considered to be one of the most important determinants of the disease manifestation. Many investigators, including us, have been investigating HTLV-1-specific cytotoxic T lymphocyte (CTL) responses, and demonstrated the importance of these CTLs on anti-viral and anti-tumor surveillance in HTLV-1-infected hosts (Jacobson et al., 1990; Bangham and Osame, 2005; Kannagi et al., 2005). Based on these studies we concluded that a reduced HTLV-1-specific T-cell response can be an underlying risk factor for the development of ATL.

Another difference between HAM/TSP and ATL patients is the level of HTLV-1 gene expression. Although HTLV-1 mRNA levels are generally low *in vivo*, they are slightly higher in HAM/TSP patients compared with asymptomatic HTLV-1 carriers (ACs; Yamano et al., 2002). The mechanism causing low levels of HTLV-1 gene expression *in vivo* remains unknown. However, we recently demonstrated that HTLV-1 expression is suppressed by non-lymphoid stromal cells through type-I interferon (IFN), indicating that innate immune responses can be another host determinant for HTLV-1-induced diseases (Kinpara et al., 2009). So far there have only been a limited number of studies reporting a type-I IFN response during HTLV-1 infection.

The status of HTLV-1 expression is critical for host immune responses and viral pathogenesis. In particular, HTLV-1 Tax is a multipotent protein that is a main target of T-cell immunity (Jacobson et al., 1990; Kannagi et al., 1991) and can activate NF-κB, a characteristic transcriptional factor in ATL cells and a strong inducer of inflammatory cytokines (Yoshida, 2001; Jeang et al., 2004; Grassmann et al., 2005). The status of Tax expression *in vivo* has been controversial but would be an extremely important factor to measure in order to understand the mechanism of disease development in HTLV-1 infection. Expression of HTLV-1 basic leucine zipper factor (HBZ) encoded by the minus-strand HTLV-1 genome is also an important factor for viral pathogenesis as HBZ elicits indirect effects on tumor development and inflammation (Satou et al., 2011).

In this review, we aim to understand the conflicting findings that have been reported in regard to HTLV-1 expression *in vivo*. We then describe recent findings that add to the knowledge about well-characterized host T-cell responses, followed by a description of the mechanisms that control viral expression. We finally discuss the relationship between HTLV-1 expression, host immune

response, and HTLV-1-mediated malignant and inflammatory diseases.

STATUS OF HTLV-1 EXPRESSION *IN VIVO*

The understanding of HTLV-1 expression *in vivo* has caused much confusion, largely owing to two reasons: (a) HTLV-1 proteins are not detectable in infected cells in the peripheral blood of HTLV-1 carriers; (b) two types of ATL cases exist, and while HTLV-1 expression in ATL cells is conserved in some cases, this expression is lost in other cases (**Figure 1**).

EXPRESSION OF HTLV-1 PROTEINS *IN VIVO*

HTLV-1 mRNA but not proteins are detectable in the peripheral blood mononuclear cells (PBMCs) of HTLV-1-infected individuals including ATL patients (Kinoshita et al., 1989). The presence of HTLV-1 mRNA was also reported in other tissues such as muscle in myositis patients (Tangy et al., 1995; Ozden et al., 2004) or the spinal cord in HAM/TSP patients (Lehky et al., 1995). Therefore, HTLV-1 gene expression does occur *in vivo* at least at a transcriptional level. Furthermore, based on the findings that HTLV-1-infected individuals maintain serum antibodies directed to the HTLV-1 structural Env and Core proteins as well as Tax-specific T-cells, HTLV-1 expression must be occurring also at a protein level *in vivo*. This notion is further supported by the emergence of Tax-specific CTL responses in ATL patients who received hematopoietic stem cells from HTLV-1-negative donors (Harashima et al., 2004, 2005). In these cases, the CTL responses are presumably triggered by the *de novo* exposure of donor-derived T-cells to Tax antigen in the recipients, and resemble an acute infection. These findings suggest that the sensitivity of T-cells recognizing HTLV-1 antigen may be much higher than the detection by serological means such as flow cytometry or immunoblotting, which are dependent on antibody binding. The conflicting arguments concerning HTLV-1 expression might thus continue until more sensitive protein detection methods are developed.

TWO TYPES OF ATL WITH OR WITHOUT HTLV-1 EXPRESSION

HTLV-1 expression in ATL cells immediately after isolation from peripheral blood is very low, and becomes significantly induced only after *in vitro* cultivation (Hinuma et al., 1982).This phenomenon is observed in about half of the ATL cases, regardless of the severity of the disease (Kurihara et al., 2005). A similar induction of viral expression after *in vitro* culture has also been observed in PBMCs from HAM/TSP patients and ACs (Hanon et al., 2000). Recent analysis using quantitative RT-PCR methods confirmed this phenomenon in PBMCs from both ATL and HAM/TSP patients. The data further showed that levels of Tax/Rex mRNA were increased as early as 4 h after initiation of culture, and peaked at 8 h, followed by an increase in Env, Gag/Pol, and other mRNAs (Rende et al., 2011). This finding is consistent with the critical roles of Tax and Rex proteins for viral expression through

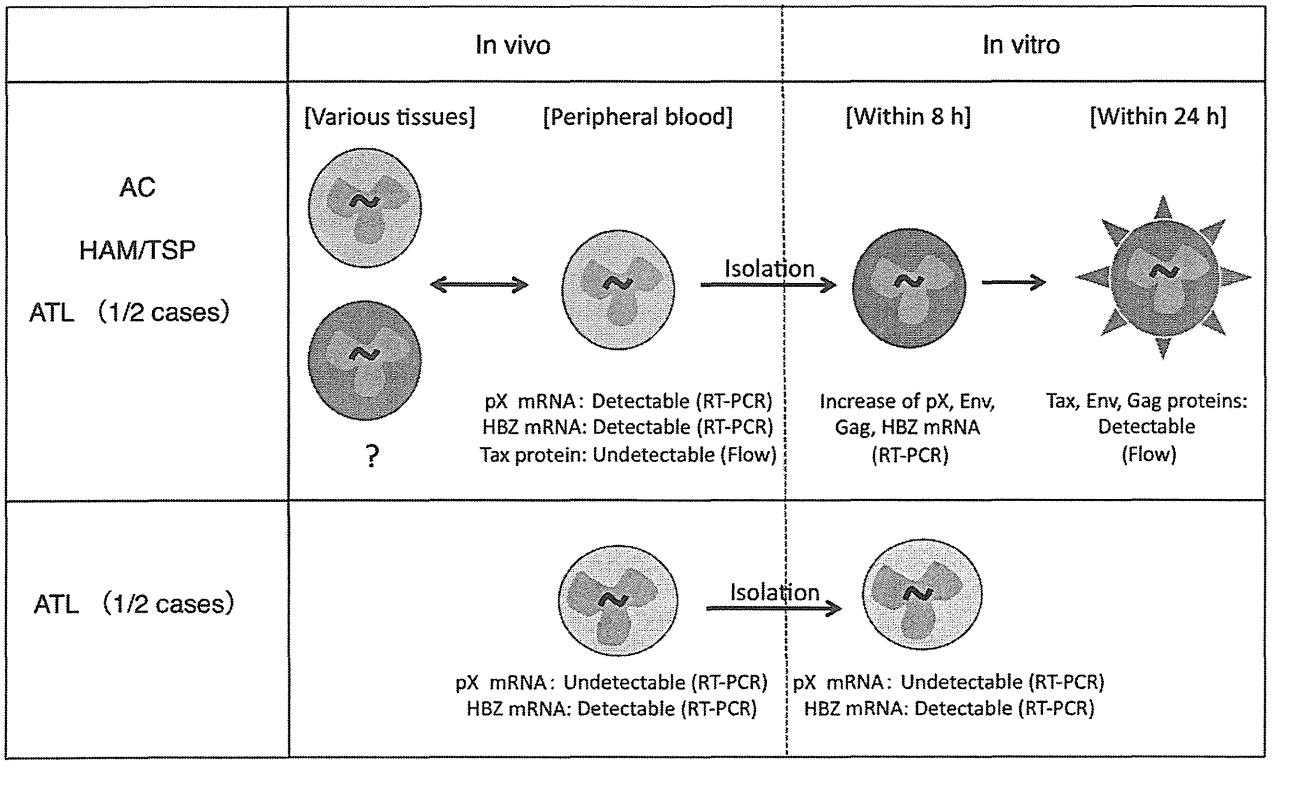

FIGURE 1 | Different status of HTLV-1 expression in infected cells *in vivo* and *in vitro*. HTLV-1-infected cells express viral mRNA in the peripheral blood and can express viral proteins in a short-term culture *in vitro* (top). This phenomenon is observed in most HTLV-1-infected individuals (top), but not in 1/2 cases of ATL (bottom).

transcriptional transactivation, regulation of RNA splicing, and nuclear export of the mRNAs, which were described in previous studies (Yoshida, 2001; Younis and Green, 2005). The rapid induction of viral expression in culture further suggests the presence of a common mechanism transiently suppressing HTLV-1 expression *in vivo*, irrespective of the disease.

In the remaining half of the ATL cases, however, such viral induction does not occur, even after *in vitro* culture. This may be due to genetic and epigenetic changes in the viral genome (Tamiya et al., 1996; Takeda et al., 2004). The malignant phenotype of ATL cells in these cases is presumably attributed to other mechanisms acquired at additional steps of leukemogenesis, independently of HTLV-1 expression.

EXPRESSION OF HBZ IN INFECTED CELLS

In uncultured PBMCs from HTLV-1-infected individuals, expression of the HTLV-1 genome is suppressed as noted above, whereas mRNA of HBZ, the minus-strand HTLV-1-encoded gene, is continuously expressed, irrespective of the disease (Satou et al., 2006). Transcription of HBZ in the absence of Tax implies an indispensable role of HBZ in HTLV-1-infected cells. Interestingly, mice carrying an HBZ transgene under the control of the CD4 promoter often develop lymphoproliferative disease with frequent Foxp3 expression and inflammatory skin lesions (Satou et al., 2011). These features partly resemble the characteristics of ATL.

However, expression of HBZ at a protein level is still controversial. In a study analyzing mRNA kinetics during the initial culture of PBMC from infected individuals, Tax/Rex and other positive-strand transcripts were promptly exported to the cytoplasm after transcriptional induction, while HBZ mRNA was mostly retained in the nucleus (Rende et al., 2011). In addition, HBZ-specific CTLs induced in human leukocyte antigen (HLA)-A2-transgenic mice lysed HBZ peptide-pulsed HLA-A2-positive target cells but not HTLV-1-infected HLA-A2-positive cells (Suemori et al., 2009). These observations suggest that expression of HBZ at a protein level in HTLV-1-infected cells might be limited, even though substantial amounts of HBZ mRNA are expressed. Nevertheless, the presence of T-cells responding to HBZ peptides have been reported in HAM/TSP patients at a low frequency, implying a small amount of HBZ protein synthesis *in vivo* (Hilburn et al., 2011).

HTLV-1 EXPRESSION IN HAM/TSP PATIENTS

HTLV-1 expression is detectable at the transcriptional, but not the protein level in uncultured PBMCs, and such basal levels of mRNA differ among diseases. An early study showed that the pX mRNA/DNA ratio was lower in ATL patients compared with ACs or HAM/TSP patients (Furukawa et al., 1995). This might partly reflect that in 50% of ATL cases the cells lost viral gene expression, as mentioned above. A recent study using a real-time quantitative PCR analysis indicated that HTLV-1 mRNA levels are significantly higher in HAM/TSP patients compared with ACs (Yamano et al., 2002). This is in agreement with high levels of serum antibodies to HTLV-1 in HAM/TSP patients (Dekaban et al., 1994). The detection of HTLV-1-specific antibodies in the cerebrospinal fluid and the pX mRNA in the spinal cord were also reported in HAM/TSP patients (Lehky et al., 1995; Gessain, 1996). These observations indicate that HTLV-1 expression is elevated in HAM/TSP patients generally, and also in the spinal cord.

Little is known about the difference in the levels of HTLV-1 expression between tissues in humans. In transgenic mice carrying the pX gene driven by the HTLV-1 long terminal repeat (LTR), RNA expression of the transgene is detectable only in selected organs, including the central nervous system, eyes, salivary glands, and joints (Iwakura et al., 1991). Transgenic mice and rats with the pX gene often develop arthritis and other collagen-vascular inflammatory conditions (Iwakura et al., 1991; Yamazaki et al., 1997). This is partly explained by Tax-mediated activation of NF-κB, a key transcription factor for multiple inflammatory cytokine production. In addition, a certain WKAH strain exhibits HAM/TSP-like symptoms after HTLV-1 infection. This rat model of HAM/TSP shows increased Tax mRNA expression in the spinal cord before the manifestation of the symptoms, suggesting that viral expression in the spinal cord may be the primary event (Tomaru et al., 1996). Reduced IFN-γ production in the spinal cord has been suggested in this particular rat strain (Miyatake et al., 2006).

DIFFERENT HTLV-1-SPECIFIC T-CELL RESPONSES BETWEEN DISEASES

ANTI-TUMOR AND ANTI-VIRAL SURVEILLANCE BY HTLV-1-SPECIFIC T-CELLS

The strength of the host T-cell response against HTLV-1 differs among diseases. $CD8^+$ HTLV-1-specific CTL responses are activated in HAM/TSP patients but not in ATL patients (Jacobson et al., 1990; Parker et al., 1992; Arnulf et al., 2004; Takamori et al., 2011). These CTLs mainly recognize HTLV-1 Tax and kill HTLV-1-infected cells *in vitro* (Jacobson et al., 1990) (Kannagi et al., 1991). The HTLV-1 envelope protein is also a major target, especially for $CD4^+$ CTLs (Goon et al., 2004). Other viral antigens, including polymerase (Elovaara et al., 1993), TOF, ROF (Pique et al., 2000), and HBZ, (Macnamara et al., 2010) have also been shown to be targets of CTLs. Elimination of $CD8^+$ cells from the PBMCs from HAM/TSP patients induces HTLV-1 expression during subsequent cell culture (Asquith et al., 2005), clearly indicating that $CD8^+$ HTLV-1-specific CTLs contribute to the control of HTLV-1-infected cells.

A series of experiments using a rat model of HTLV-1-infected T-cell lymphoma indicated that inhibition of the T-cell response accelerated tumor development (Hanabuchi et al., 2000), and further showed that vaccination with a Tax-encoding DNA or peptides corresponding to a major epitope for Tax-specific CTLs lead to the eradication of such tumors (Ohashi et al., 2000; Hanabuchi et al., 2001). In a different rat model of HTLV-1 infection, oral HTLV-1 infection induced HTLV-1-specific T-cell tolerance and caused an elevation in the proviral load, while re-immunization of these rats resulted in the recovery of HTLV-1-specific T-cell responses and caused a reduction in the proviral loads (Hasegawa et al., 2003; Komori et al., 2006). Similarly, patients carrying HTLV-1 developed ATL after liver transplantation, when immunosuppressants were administered (Kawano et al., 2006; Suzuki et al., 2006). These findings suggest that HTLV-1-specific T-cells, especially Tax-specific CTLs, play important roles in anti-tumor and anti-viral surveillance in HTLV-1 infection.

The pathological significance of HTLV-1-specific T-cells activated in HAM/TSP patients remains controversial (Jacobson, 1995; Osame, 2002).Activated CTLs produce IFN-γ or TNF-α, which might potentially participate in inflammation in HAM/TSP. However, activation of Tax-specific CTLs could also merely be a result of elevated viral expression in these individuals. HLA-A02-positive individuals in HAM/TSP patients are less frequent compared with the control population, indicating a protective role of HLA-A02 against HAM/TSP. Since HLA-A02 can present a major epitope of HTLV-1 Tax, the strong CTL response induced is thought to mediate the protective effect of HLA-A02 (Jeffery et al., 1999). The association of the protective HLAs with epitopes favoring HBZ-specific CTLs has also been suggested (Macnamara et al., 2010).

SELECTIVE IMPAIRMENT OF HTLV-1-SPECIFIC T-CELLS IN EARLY STAGES OF ATL, A POTENTIAL RISK FOR ATL

We previously identified some major epitopes recognized by HTLV-1-specific CTLs presented by HLA-A2, -A11, or -A24 through analysis of CTLs collected from ATL patients after HSCT or collected from HAM/TSP patients (Kannagi et al., 1992; Harashima et al., 2004, 2005).The identification of these epitopes allowed us to monitor HTLV-1-specific CTLs and analyze their functions *ex vivo* by using antigen/HLA tetrameric complexes. In our study using Tax-specific tetramers on HLA-A2, -A11, or -A24-positive individuals, we detected Tax-specific CTLs in 100% of HAM/TSP patients, 87% of ACs, and 38% of chronic ATL (cATL) patients tested (**Figure 2;** Takamori et al., 2011).

It is interesting that Tax-specific CTLs were detectable also in cATL patients, although their frequency among peripheral $CD8^+$ cells is low. However, these CTLs were anergic as they neither proliferated nor produced IFN-γ upon peptide stimulation. In contrast, Tax-specific CTLs in HAM/TSP patients were highly active even without stimulation, and their response was further enhanced by stimulation.

Amongst ACs, Tax-specific CTLs are detectable in the majority but not a small subset of individuals. Although Tax-specific CTLs detected in ACs are mostly functional, sporadic AC cases with impaired CTL responses to peptide-stimulation analogous to ATL patients have been identified. Interestingly, such functional impairment of CTLs seems selective to HTLV-1-specific responses, as cytomegalovirus (CMV)-specific CTLs remain functional (**Figure 2**; Takamori et al., 2011). Similar dysfunctions of Tax-specific but not CMV-specific CTLs are found in smoldering ATL, an early stage of ATL without clinically apparent lymphoproliferation. These findings suggest that the scarcity and/or dysfunction of Tax-specific CTLs are not merely the result of ATL, but represent host conditions in a subset of HTLV-1 carriers at

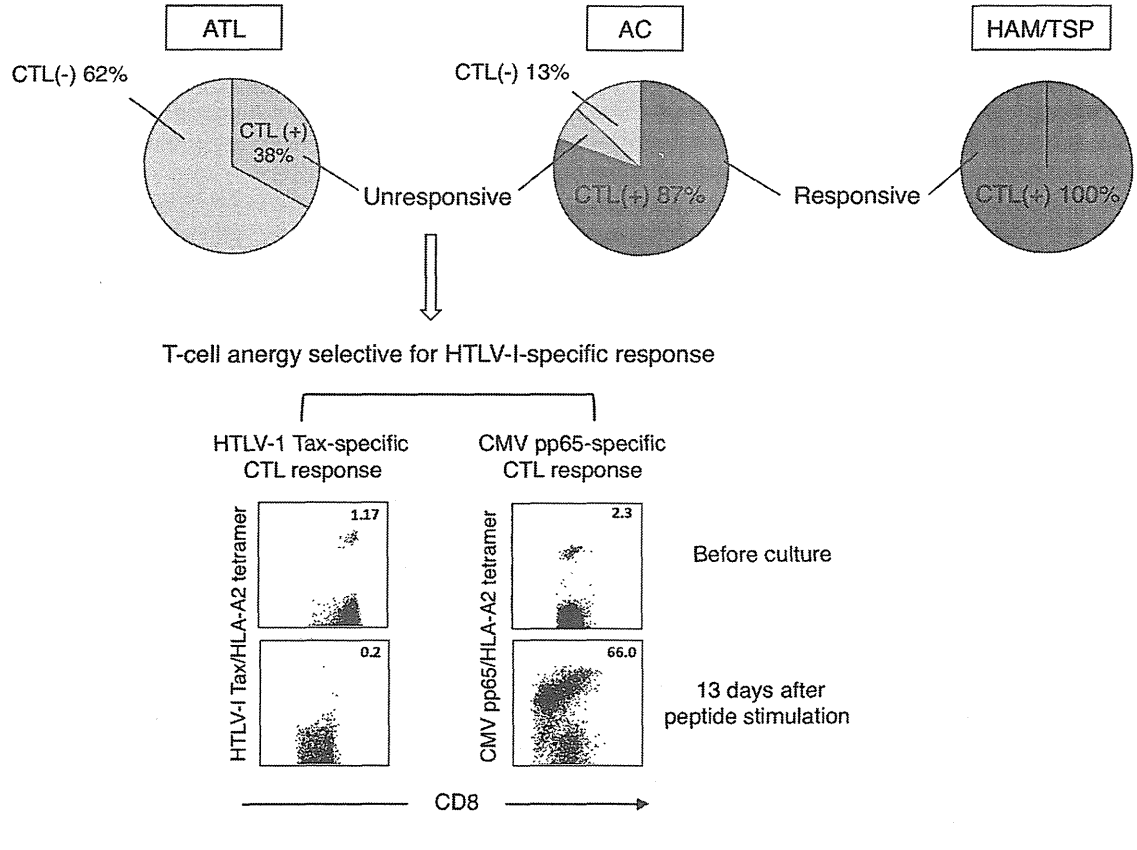

FIGURE 2 | Selective loss or anergy of Tax-specific CTLs in a subset of ACs. Incidences of Tax-specific CTL detection in $CD8^+$ PBMC vary among diseases (top). Tax-specific CTLs detected in HAM/TSP patients and most of ACs (dark blue) were functional, while those in cATL patients and a subset of ACs (light blue) were anergic to antigen stimulation. The CTL anergy was selective for HTLV-1-specific response (bottom; Takamori et al., 2011).

asymptomatic stages. A reduced number and/or dysfunction of Tax-specific CTLs could thus represent an underlying risk factor for the development of ATL.

Epidemiological studies indicated that increased numbers of abnormal lymphocytes or HTLV-1 proviral loads are risk factors for the development of ATL (Tajima, 1990;Hisada et al., 1998). However, elevated HTLV-1 proviral loads are also detected in HAM/TSP patients, and do not discriminate risks for ATL and HAM/TSP (Nagai et al., 1998).The immunological studies described above suggested that insufficiency in host T-cell responses against HTLV-1 might be another risk factor for ATL. We therefore suggest that the combination of elevated proviral loads and low HTLV-1-specific T-cell responses may represent a more selective indicator for the risk of developing ATL.

MECHANISMS OF T-CELL SUPPRESSION IN HTLV-1 INFECTION

It is known that ATL is often associated with severe immune suppression (Tashiro et al., 1992), and a small number of studies reported general immune suppression also in ACs (Imai and Hinuma, 1983). The mechanism of general immune suppression in these individuals is not known. ATL cells are positive for CD4, CD25, CCR4, and frequently express Foxp3, all of which match the phenotype of regulatory T-cells. If ATL cells function as Treg cells, this would be a strong reason for the observed general immune suppression (Karube et al., 2004). There are reports of increased numbers of Foxp3-expressing Treg cells in the HTLV-1-negative cell population in HAM/TSP patients (Toulza et al., 2008). Recent studies reported that HBZ is potentially involved in immune suppression by enhancing TGF-β signaling and suppressing Th1 cytokine production (Zhao et al., 2011; Sugata et al., 2012).

As mentioned above, the insufficient Tax-specific CTL response observed in the early stages of ATL and in a subpopulation of ACs was selective for the response to HTLV-1, and did not affect CMV-targeting CTLs. From this differential effect we deduce that other HTLV-1-specific suppressive mechanisms are active, in addition to the general suppression. In general, antigen-specific T-cell suppression can be induced by immune tolerance and T-cell exhaustion. In HTLV-1 infection, vertical infection could be a reason for T-cell tolerance. In animal models, oral HTLV-1 infection induces T-cell tolerance to HTLV-1, resulting in increased levels of proviral loads (Hasegawa et al., 2003). Since vertical infection of HTLV-1 is established mainly through breast feeding (Kinoshita et al., 1987), it may induce both new-born tolerance and oral tolerance. This might partly explain the epidemiological finding that vertical HTLV-1 infection is one of the risk factors for ATL (Tajima, 1990).

Besides immune tolerance, T-cell suppression can also be caused by T-cell exhaustion, which may be a consequence of continuous expression of HTLV-1 antigens *in vivo*. Expression of PD-1 in Tax-specific CTLs has been reported (Kozako et al., 2009), although the involvement of this molecule in the suppression of CTLs in HTLV-1-infected individuals is still controversial (Takamori et al., 2011). The relevance of other antigens remains unknown, as for example recent studies revealed that Tax-specific CTLs in HAM/TSP patients express reduced levels of Tim3, one of the T-cell exhaustion markers, despite high viral gene expression in these patients (Abdelbary et al., 2011; Ndhlovu et al., 2011).

IMPACT OF TYPE-I IFNs IN CONTROLLING HTLV-1 EXPRESSION

INDUCTION OF TYPE-I IFN RESPONSE BY HTLV-1 INFECTION

Various viruses induce type-I IFN responses. In HTLV-1 infection, however, the number of studies investigating a putative HTLV-1-induced type-I IFN response is limited. One of the reasons is that efficient HTLV-1 infection is mediated mainly through cell–cell contact. A recent report indicated that addition of cell-free HTLV-1 particles propagated using an HTLV-1 molecular clone to plasmacytoid dendritic cells (pDCs) induced a type-I IFN response through Toll-like receptor 7 (TLR7; Colisson et al., 2010). pDCs are a major producer of type-I IFNs, and are reported to be susceptible to HTLV-1 infection (Hishizawa et al., 2004; Jones et al., 2008). In ATL patients, the number of pDCs is decreased, and the remaining pDCs lack the ability to produce IFN-α (Hishizawa et al., 2004).

At cell–cell contacts between HTLV-1-infected T-cells and stromal cells, we found that HTLV-1 induced a type-I IFN response in the stromal cells, suggesting an involvement of pattern recognition molecules other than TLR7. However, the precise mechanisms of HTLV-1-induced type-I IFN responses remain to be clarified.

SUPPRESSION OF HTLV-1 EXPRESSION BY TYPE-I IFNs

HTLV-1 mRNA and protein expression in HTLV-1-infected T-cells are markedly decreased when co-cultured with stromal cells such as epithelial cells and fibroblasts. This suppression of HTLV-1 expression is inhibited by blocking the IFN-α/β receptor, and is therefore thought to be mediated through type-I IFN responses (**Figure 3**; Kinpara et al., 2009).

Interestingly, when infected cells were re-isolated from the co-cultures, viral expression was restored to the original level over the following 48 h (**Figure 3**). This phenomenon resembles the induction of HTLV-1 expression in freshly isolated ATL cells after culture *in vitro*. Type-I IFNs might therefore explain the long-puzzling observation that HTLV-1 expression is suppressed *in vivo*. In support of this notion, viral expression in HTLV-1-infected cells was significantly suppressed when injected into wild-type mice but not into IFN regulatory factor-7-knockout mice, which are deficient in most type-I IFN responses (Kinpara et al., 2009).

In general, type-I IFNs suppress viral replication mostly at post-transcriptional level. Since HTLV-1 transcription is regulated by transactivation of its own LTR, mainly through cyclic AMP (cAMP) response element-like repeats by the Tax protein (Fujisawa et al., 1985; Sodroski et al., 1985), limitation of this protein below a certain level will efficiently reduce HTLV-1 expression to a basal level. Involvement of inducible cAMP early repressor (ICER) and transducer of regulated CREB protein 2 (TORC2) in the inhibition of HTLV-1 transactivation has also been suggested (Newbound et al., 2000; Jiang et al., 2009).

Addition of IFNs alone also elicits suppressive effects in HTLV-1 expression. However, the levels of suppressive effects differ among studies. In HTLV-1-infected cell lines, it has been reported that IFN-α2a decreased HTLV-1 assembly and viral release but not viral protein synthesis (Feng et al., 2003).

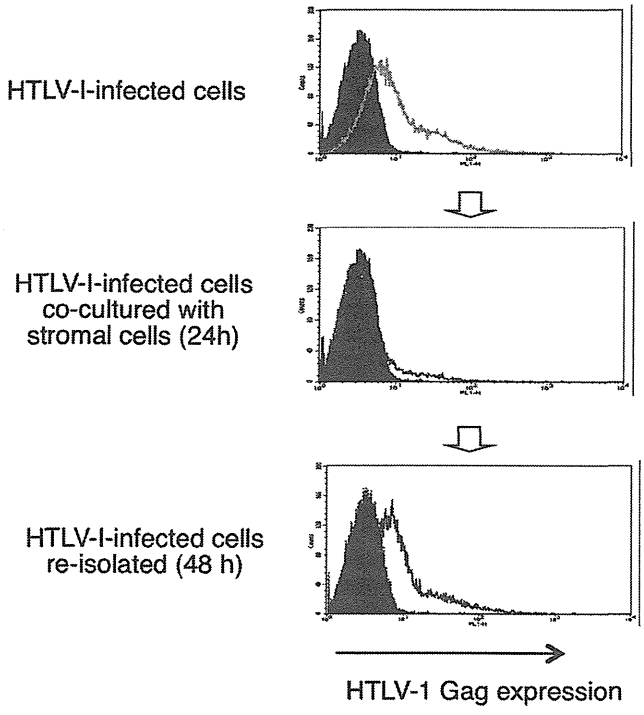

FIGURE 3 | Reversible suppression of HTLV-1 expression by stromal cells through type-I IFNs. HTLV-1 protein expression in HTLV-1-infected T-cells are markedly decreased when co-cultured with stromal cells and recovers after re-isolation from the co-cultures (Kinpara et al., 2009).

RESISTANCE OF HTLV-1 AGAINST TYPE-I IFN SIGNALING

As is the case with many other viruses, HTLV-1 has developed strategies to evade IFN responses. It has been reported that HTLV-1 infection reduces the phosphorylation of tyrosine kinase 2 (TYK2) and signal transducer and transcriptional activator 2 (STAT2; Feng and Ratner, 2008), and that Tax inhibit the induction of IFN-stimulated genes (ISGs) by competing with CREB binding protein/p300 (Zhang et al., 2008). Recent reports also suggest that Tax-mediated up-regulation of suppressor of cytokine signaling 1 (SOCS1) inhibits IFN signaling (Oliere et al., 2010; Charoenthongtrakul et al., 2011). However, expression levels of Tax protein are low *in vivo*, and it is unclear to what extent the evading mechanisms observed *in vitro* are effective *in vivo*.

It has been reported that a combination therapy of AZT and IFN-α is effective for the treatment of ATL (Hermine et al., 1995), indicating that HTLV-1-infected cells retain some susceptibility to IFNs *in vivo*. Intriguingly, this combination of AZT/IFN-α does not affect HTLV-1-infected cells *in vitro* (Bazarbachi et al., 2000), and the mechanistic effect of this therapy is not known. The discrepancy in the therapeutic effects *in vivo* and *in vitro* is presumably due to the different status of HTLV-1-infected cells in the two systems.

AZT/IFN-α is not a radical therapy, and ATL relapses are frequently observed after cessation of the therapy (Hermine et al., 2002), suggesting that AZT/IFN-α may not be cytocidal but rather has static effects on infected cells. Another combination therapy of arsenic trioxide and IFN-α shows more favorable therapeutic effects *in vivo*, and also shows proteolysis of Tax in HTLV-1-infected cells *in vitro* (El Hajj et al., 2010). IFN-α or β alone appears less effective for the treatment of HAM/TSP, but does show some therapeutic effects, especially during the early stages of HAM/TSP (Izumo et al., 1996; Saito et al., 2004).

IFN RESPONSES IN HTLV-1-INFECTED INDIVIDUALS

A recent study revealed up-regulation of SOCS1 in $CD4^+$ cells of HAM/TSP patients, which caused enhanced viral expression through inhibition of type-I IFN signaling (Oliere et al., 2010). At the same time, a different study showed that HTLV-1 Tax up-regulates SOCS1 (Charoenthongtrakul et al., 2011). These findings indicate that up-regulation of SOCS1 might be a result and/or cause of enhanced viral expression in HAM/TSP. Another recent study using gene expression array analysis reported up-regulation of a subset of ISGs, including STAT1, CD64, FAS, and CXCL10, especially in the neutrophil and monocyte fractions from peripheral blood of HAM/TSP patients (Tattermusch et al., 2012). This suggests that type-I IFN responses were induced in these cell populations directly or indirectly by HTLV-1, although type-I IFN production in these cells was not clear. The strong HTLV-1-specific T-cell response in these patients might also cause such effects through IFN-γ production.

The signature of IFN responses in the peripheral blood of HAM/TSP patients left an unanswered question what enhances the basal level of viral expression in these patients. Increased inflammatory cytokines in HAM/TSP patients might be candidates to enhance viral expression, but again these could be a result and/or cause of enhanced HTLV-1 expression.

THE RELATIONSHIP AMONG VIRAL EXPRESSION, HOST IMMUNITY, AND VIRAL PATHOGENESIS

It is speculated that the status of viral expression and host immunity may differ among various tissues *in vivo*. Therefore, it is difficult to estimate HTLV-1 status in the entire body based on the information gained only from peripheral blood. Nevertheless, the recent findings about innate immunity described above provide clues as to how the current knowledge around HTLV-1 expression and host immunity can be integrated, especially when they so closely interact and have both causes and effects on each other.

Type-I IFNs are likely to be the representative factor to control HTLV-1 expression, and HTLV-1-specific T-cells survey infected cells to limit their growth. The suppression of viral expression might interfere with the efficacy of HTLV-1-specific T-cells by reducing the levels of target molecules, even in hosts with a functional HTLV-1-specific T-cell response. The resulting low efficiency of T-cell surveillance would be one of the mechanisms behind persistent HTLV-1 infection, although it seems that T-cells would still contribute to the control of HTLV-1-infected cell growth to some extent.

Despite the negative impact on T-cell surveillance, the suppression of viral expression is important for the host to reduce viral pathogenesis since Tax has a strong ability to activate NF-κB, which is critical for the induction of inflammation or cell growth signaling.

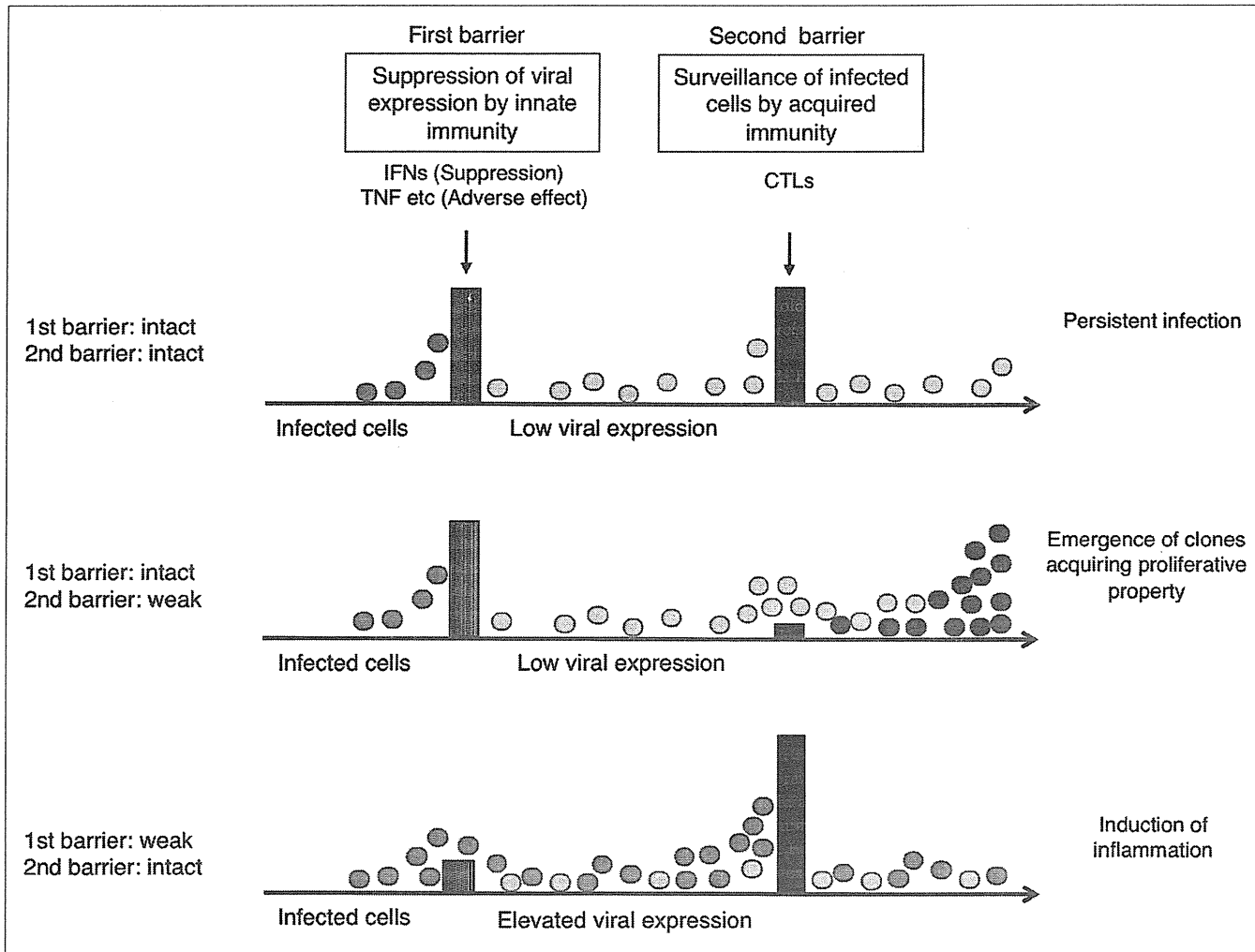

FIGURE 4 | Hypothetical relationship of the host immune balance to the status of HTLV-1-infected cells and viral pathogenesis. If viral expression is well controlled (barrier 1: intact), the viral pathogenesis may not be apparent until malignant cell clones appear through T-cell surveillance (barrier 2). If the T-cell defense is insufficient (barrier 2: weak), the emergence of such clones may occur earlier. If the viral expression is not well controlled (barrier 1: weak), virus-induced inflammation may become apparent, but it also activates HTLV-1-specific T-cells that limit further growth of infected cells.

Supposing that the suppression of viral expression is the first barrier and T-cell surveillance of infected cells is the second barrier in the host defense, the balance of these barriers would influence the status of HTLV-1-infected cells *in vivo*. A conceivable scenario is as follows (**Figure 4**).

If viral expression is well controlled, the viral pathogenesis may not be apparent until malignant cell clones appear through the process of clonal evolution in the infected cell reservoir. This might explain the long incubation time for ATL development. In the absence of effective T-cell responses, the emergence of such clones may occur earlier, as clonal survival may be more likely.

In contrast, if the suppression of viral expression is insufficient, either by insufficient IFN response or increased inflammatory cytokines, viral pathogenesis will become apparent and symptoms will be exhibited, especially in the tissues where viral proteins reach functional levels. In this case, however, the elevated levels of viral expression would also activate HTLV-1-specific T-cells, which potentially limit further growth of infected cells.

CONCLUSION

The status of HTLV-1-specific T-cell response has been shown to be a determinant of HTLV-1-mediated diseases because of its anti-tumor and anti-viral effects. The selective impairment of HTLV-1-specific T-cell responses in early stages of ATL patients implies the presence of HTLV-1-specific suppressive mechanisms. The combination of insufficient HTLV-1-specific T-cell response and elevated proviral load may allow the identification of a group with a high risk for the development of ATL. In addition, vaccines that augment HTLV-1-specific T-cell responses may prove beneficial in reducing the risk in such a subpopulation.

The status of HTLV-1 expression can be another determinant of HTLV-1-mediated diseases. Suppression of viral expression contributes to reduced viral pathogenesis, although it may, at the same time, partially interfere with T-cell surveillance. Host innate immunity, especially type-I IFN, is a candidate for the regulation of viral expression.

Thus, both acquired and innate immunity can be host determinants that modulate HTLV-1-associated diseases. The involvement of the two control systems and their partially conflicting effects on one another may explain why the same virus can cause different diseases after a long incubation time. Further studies will elucidate the precise mechanisms for the regulation of host immunity and viral expression, and thereby provide insights for the prediction of disease risks, as well as new targets for the prevention of HTLV-1-mediated diseases.

REFERENCES

Abdelbary, N. H., Abdullah, H. M., Matsuzaki, T., Hayashi, D., Tanaka, Y., Takashima, H., Izumo, S., and Kubota, R. (2011). Reduced Tim-3 expression on human T-lymphotropic virus type I (HTLV-I) Tax-specific cytotoxic T lymphocytes in HTLV-I infection. *J. Infect. Dis.* 203, 948–959.

Arnulf, B., Thorel, M., Poirot, Y., Tamouza, R., Boulanger, E., Jaccard, A., Oksenhendler, E., Hermine, O., and Pique, C. (2004). Loss of the *ex vivo* but not the reinducible CD8+ T-cell response to Tax in human T-cell leukemia virus type 1-infected patients with adult T-cell leukemia/lymphoma. *Leukemia* 18, 126–132.

Asquith, B., Mosley, A. J., Barfield, A., Marshall, S. E., Heaps, A., Goon, P., Hanon, E., Tanaka, Y., Taylor, G. P., and Bangham, C. R. (2005). A functional CD8+ cell assay reveals individual variation in CD8+ cell antiviral efficacy and explains differences in human T-lymphotropic virus type 1 proviral load. *J. Gen. Virol.* 86, 1515–1523.

Bangham, C. R., and Osame, M. (2005). Cellular immune response to HTLV-1. *Oncogene* 24, 6035–6046.

Bazarbachi, A., Nasr, R., El-Sabban, M. E., Mahe, A., Mahieux, R., Gessain, A., Darwiche, N., Dbaibo, G., Kersual, J., Zermati, Y., Dianoux, L., Chelbi-Alix, M. K., De The, H., and Hermine, O. (2000). Evidence against a direct cytotoxic effect of alpha interferon and zidovudine in HTLV-I associated adult T cell leukemia/lymphoma. *Leukemia* 14, 716–721.

Charoenthongtrakul, S., Zhou, Q., Shembade, N., Harhaj, N. S., and Harhaj, E. W. (2011). Human T cell leukemia virus type 1 Tax inhibits innate antiviral signaling via NF-kappaB-dependent induction of SOCS1. *J. Virol.* 85, 6955–6962.

Colisson, R., Barblu, L., Gras, C., Raynaud, F., Hadj-Slimane, R., Pique, C., Hermine, O., Lepelletier, Y., and Herbeuval, J. P. (2010). Free HTLV-1 induces TLR7-dependent innate immune response and TRAIL relocalization in killer plasmacytoid dendritic cells. *Blood* 115, 2177–2185.

Daenke, S., Nightingale, S., Cruickshank, J. K., and Bangham, C. R. (1990). Sequence variants of human T-cell lymphotropic virus type I from patients with tropical spastic paraparesis and adult T-cell leukemia do not distinguish neurological from leukemic isolates. *J. Virol.* 64, 1278–1282.

Dekaban, G. A., King, E. E., Arp, J., Palker, T. J., and Rice, G. P. (1994). Comparative analysis of the antibody response to the HTLV-I gag and env proteins in HTLV-I asymptomatic carriers and HAM/TSP patients: an isotype and subclass analysis. *Scand. J. Immunol.* 40, 171–180.

El Hajj, H., El-Sabban, M., Hasegawa, H., Zaatari, G., Ablain, J., Saab, S. T., Janin, A., Mahfouz, R., Nasr, R., Kfoury, Y., Nicot, C., Hermine, O., Hall, W., De The, H., and Bazarbachi, A. (2010). Therapy-induced selective loss of leukemia-initiating activity in murine adult T cell leukemia. *J. Exp. Med.* 207, 2785–2792.

Elovaara, I., Koenig, S., Brewah, A. Y., Woods, R. M., Lehky, T., and Jacobson, S. (1993). High human T cell lymphotropic virus type 1 (HTLV-1)-specific precursor cytotoxic T lymphocyte frequencies in patients with HTLV-1-associated neurological disease. *J. Exp. Med.* 177, 1567–1573.

Feng, X., Heyden, N. V., and Ratner, L. (2003). Alpha interferon inhibits human T-cell leukemia virus type 1 assembly by preventing Gag interaction with rafts. *J. Virol.* 77, 13389–13395.

Feng, X., and Ratner, L. (2008). Human T-cell leukemia virus type 1 blunts signaling by interferon alpha. *Virology* 374, 210–216.

Fujisawa, J., Seiki, M., Kiyokawa, T., and Yoshida, M. (1985). Functional activation of the long terminal repeat of human T-cell leukemia virus type I by a trans-acting factor. *Proc. Natl. Acad. Sci. U.S.A.* 82, 2277–2281.

Furukawa, Y., Osame, M., Kubota, R., Tara, M., and Yoshida, M. (1995). Human T-cell leukemia virus type-1 (HTLV-1) Tax is expressed at the same level in infected cells of HTLV-1-associated myelopathy or tropical spastic paraparesis patients as in asymptomatic carriers but at a lower level in adult T-cell leukemia cells. *Blood* 85, 1865–1870.

Gessain, A. (1996). Virological aspects of tropical spastic paraparesis/HTLV-I associated myelopathy and HTLV-I infection. *J. Neurovirol.* 2, 299–306.

Gessain, A., Barin, F., Vernant, J. C., Gout, O., Maurs, L., Calender, A., and De The, G. (1985). Antibodies to human T-lymphotropic virus type-I in patients with tropical spastic paraparesis. *Lancet* 2, 407–410.

Goon, P. K., Igakura, T., Hanon, E., Mosley, A. J., Barfield, A., Barnard, A. L., Kaftantzi, L., Tanaka, Y., Taylor, G. P., Weber, J. N., and Bangham, C. R. (2004). Human T cell lymphotropic virus type I (HTLV-I)-specific CD4+ T cells: immunodominance hierarchy and preferential infection with HTLV-I. *J. Immunol.* 172, 1735–1743.

Grassmann, R., Aboud, M., and Jeang, K. T. (2005). Molecular mechanisms of cellular transformation by HTLV-1 Tax. *Oncogene* 24, 5976–5985.

Hanabuchi, S., Ohashi, T., Koya, Y., Kato, H., Hasegawa, A., Takemura, F., Masuda, T., and Kannagi, M. (2001). Regression of human T-cell leukemia virus type I (HTLV-I)-associated lymphomas in a rat model: peptide-induced T-cell immunity. *J. Natl. Cancer Inst.* 93, 1775–1783.

Hanabuchi, S., Ohashi, T., Koya, Y., Kato, H., Takemura, F., Hirokawa, K., Yoshiki, T., Yagita, H., Okumura, K., and Kannagi, M. (2000). Development of human T-cell leukemia virus type 1-transformed tumors in rats following suppression of T-cell immunity by CD80 and CD86 blockade. *J. Virol.* 74, 428–435.

Hanon, E., Hall, S., Taylor, G. P., Saito, M., Davis, R., Tanaka, Y., Usuku, K., Osame, M., Weber, J. N., and Bangham, C. R. (2000). Abundant tax protein expression in CD4+ T cells infected with human T-cell lymphotropic virus type I (HTLV-I) is prevented by cytotoxic T lymphocytes. *Blood* 95, 1386–1392.

Harashima, N., Kurihara, K., Utsunomiya, A., Tanosaki, R., Hanabuchi, S., Masuda, M., Ohashi, T., Fukui, F., Hasegawa, A., Masuda, T., Takaue, Y., Okamura, J., and Kannagi, M. (2004). Graft-versus-Tax response in adult T-cell leukemia patients after hematopoietic stem cell transplantation. *Cancer Res.* 64, 391–399.

Harashima, N., Tanosaki, R., Shimizu, Y., Kurihara, K., Masuda, T., Okamura, J., and Kannagi, M. (2005). Identification of two new HLA-A*1101-restricted tax epitopes recognized by cytotoxic T lymphocytes in an adult T-cell leukemia patient after hematopoietic stem cell transplantation. *J. Virol.* 79, 10088–10092.

Hasegawa, A., Ohashi, T., Hanabuchi, S., Kato, H., Takemura, F., Masuda, T., and Kannagi, M. (2003). Expansion of human T-cell leukemia virus type 1 (HTLV-1) reservoir in orally infected rats: inverse correlation with HTLV-1-specific cellular immune response. *J. Virol.* 77, 2956–2963.

Hermine, O., Allard, I., Levy, V., Arnulf, B., Gessain, A., and Bazarbachi, A. (2002). A prospective phase II clinical trial with the use of zidovudine and interferon-alpha in the acute and lymphoma forms of adult T-cell leukemia/lymphoma. *Hematol. J.* 3, 276–282.

Hermine, O., Bouscary, D., Gessain, A., Turlure, P., Leblond, V., Franck, N., Buzyn-Veil, A., Rio, B., Macintyre, E., Dreyfus, F., and Bazarbachi, A. (1995). Brief report: treatment of adult T-cell leukemia-lymphoma with zidovudine and interferon alfa. *N. Engl. J. Med.* 332, 1749–1751.

Hilburn, S., Rowan, A., Demontis, M. A., Macnamara, A., Asquith, B., Bangham, C. R., and Taylor, G. P. (2011). *In vivo* expression of human T-lymphotropic virus type 1 basic leucine-zipper protein generates specific CD8+ and CD4+ T-lymphocyte responses that correlate with clinical outcome. *J. Infect. Dis.* 203, 529–536.

Hinuma, Y., Gotoh, Y., Sugamura, K., Nagata, K., Goto, T., Nakai, M., Kamada, N., Matsumoto, T., and Kinoshita, K. (1982). A retrovirus associated with human adult T-cell leukemia: *in vitro* activation. *Gann* 73, 341–344.

Hinuma, Y., Nagata, K., Hanaoka, M., Nakai, M., Matsumoto, T., Kinoshita, K. I., Shirakawa, S., and Miyoshi, I. (1981). Adult T-cell leukemia: antigen in an ATL cell line and detection of antibodies to the antigen in human sera. *Proc. Natl. Acad. Sci. U.S.A.* 78, 6476–6480.

Hisada, M., Okayama, A., Tachibana, N., Stuver, S. O., Spiegelman, D. L., Tsubouchi, H., and Mueller, N. E. (1998). Predictors of level of circulating abnormal lymphocytes among human T-lymphotropic virus type I

carriers in Japan. *Int. J. Cancer* 77, 188–192.

Hishizawa, M., Imada, K., Kitawaki, T., Ueda, M., Kadowaki, N., and Uchiyama, T. (2004). Depletion and impaired interferon-alpha-producing capacity of blood plasmacytoid dendritic cells in human T-cell leukaemia virus type I-infected individuals. *Br. J. Haematol.* 125, 568–575.

Imai, J., and Hinuma, Y. (1983). Epstein–Barr virus-specific antibodies in patients with adult T-cell leukemia (ATL) and healthy ATL virus-carriers. *Int. J. Cancer* 31, 197–200.

Iwakura, Y., Tosu, M., Yoshida, E., Takiguchi, M., Sato, K., Kitajima, I., Nishioka, K., Yamamoto, K., Takeda, T., Hatanaka, M., Yamamoto, H., and Sekiguchi, T. (1991). Induction of inflammatory arthropathy resembling rheumatoid arthritis in mice transgenic for HTLV-I. *Science* 253, 1026–1028.

Izumo, S., Goto, I., Itoyama, Y., Okajima, T., Watanabe, S., Kuroda, Y., Araki, S., Mori, M., Nagataki, S., Matsukura, S., Akamine, T., Nakagawa, M., Yamamoto, I., and Osame, M. (1996). Interferon-alpha is effective in HTLV-I-associated myelopathy: a multicenter, randomized, double-blind, controlled trial. *Neurology* 46, 1016–1021.

Jacobson, S. (1995). Human T lymphotropic virus, type-I myelopathy: an immunopathologically mediated chronic progressive disease of the central nervous system. *Curr. Opin. Neurol.* 8, 179–183.

Jacobson, S., Shida, H., Mcfarlin, D. E., Fauci, A. S., and Koenig, S. (1990). Circulating CD8+ cytotoxic T lymphocytes specific for HTLV-I pX in patients with HTLV-I associated neurological disease. *Nature* 348, 245–248.

Jeang, K. T., Giam, C. Z., Majone, F., and Aboud, M. (2004). Life, death, and tax: role of HTLV-I oncoprotein in genetic instability and cellular transformation. *J. Biol. Chem.* 279, 31991–31994.

Jeffery, K. J., Usuku, K., Hall, S. E., Matsumoto, W., Taylor, G. P., Procter, J., Bunce, M., Ogg, G. S., Welsh, K. I., Weber, J. N., Lloyd, A. L., Nowak, M. A., Nagai, M., Kodama, D., Izumo, S., Osame, M., and Bangham, C. R. (1999). HLA alleles determine human T-lymphotropic virus-I (HTLV-I) proviral load and the risk of HTLV-I-associated myelopathy. *Proc. Natl. Acad. Sci. U.S.A.* 96, 3848–3853.

Jiang, S., Inada, T., Tanaka, M., Furuta, R. A., Shingu, K., and Fujisawa, J. (2009). Involvement of TORC2, a CREB co-activator, in the *in vivo*-specific transcriptional control of HTLV-1. *Retrovirology* 6, 73.

Jones, K. S., Petrow-Sadowski, C., Huang, Y. K., Bertolette, D. C., and Ruscetti, F. W. (2008). Cell-free HTLV-1 infects dendritic cells leading to transmission and transformation of CD4(+) T cells. *Nat. Med.* 14, 429–436.

Kannagi, M., Harada, S., Maruyama, I., Inoko, H., Igarashi, H., Kuwashima, G., Sato, S., Morita, M., Kidokoro, M., Sugimoto, M., Funahashi, M., Osame, M., and Shida, H. (1991). Predominant recognition of human T cell leukemia virus type I (HTLV-I) pX gene products by human CD8+ cytotoxic T cells directed against HTLV-I-infected cells. *Int. Immunol.* 3, 761–767.

Kannagi, M., Harashima, N., Kurihara, K., Ohashi, T., Utsunomiya, A., Tanosaki, R., Masuda, M., Tomonaga, M., and Okamura, J. (2005). Tumor immunity against adult T-cell leukemia. *Cancer Sci.* 96, 249–255.

Kannagi, M., Shida, H., Igarashi, H., Kuruma, K., Murai, H., Aono, Y., Maruyama, I., Osame, M., Hattori, T., Inoko, H., and Harasa, S. (1992). Target epitope in the Tax protein of human T-cell leukemia virus type I recognized by class I major histocompatibility complex-restricted cytotoxic T cells. *J. Virol.* 66, 2928–2933.

Karube, K., Ohshima, K., Tsuchiya, T., Yamaguchi, T., Kawano, R., Suzumiya, J., Utsunomiya, A., Harada, M., and Kikuchi, M. (2004). Expression of FoxP3, a key molecule in CD4CD25 regulatory T cells, in adult T-cell leukaemia/lymphoma cells. *Br. J. Haematol.* 126, 81–84.

Kawano, N., Shimoda, K., Ishikawa, F., Taketomi, A., Yoshizumi, T., Shimoda, S., Yoshida, S., Uozumi, K., Suzuki, S., Maehara, Y., and Harada, M. (2006). Adult T-cell leukemia development from a human T-cell leukemia virus type I carrier after a living-donor liver transplantation. *Transplantation* 82, 840–843.

Kinoshita, K., Amagasaki, T., Hino, S., Doi, H., Yamanouchi, K., Ban, N., Momita, S., Ikeda, S., Kamihira, S., Ichimaru, M., Katamine, S., Miyamoto, T., Tsuji, Y., Ishimaru, T., Yamabe, T., Ito, M., Kamura, S., and Tsuda, T. (1987). Milk-borne transmission of HTLV-I from carrier mothers to their children. *Jpn. J. Cancer Res.* 78, 674–680.

Kinoshita, T., Shimoyama, M., Tobinai, K., Ito, M., Ito, S., Ikeda, S., Tajima, K., Shimotohno, K., and Sugimura, T. (1989). Detection of mRNA for the tax1/rex1 gene of human T-cell leukemia virus type I in fresh peripheral blood mononuclear cells of adult T-cell leukemia patients and viral carriers by using the polymerase chain reaction. *Proc. Natl. Acad. Sci. U.S.A.* 86, 5620–5624.

Kinoshita, T., Tsujimoto, A., and Shimotohno, K. (1991). Sequence variations in LTR and env regions of HTLV-I do not discriminate between the virus from patients with HTLV-I-associated myelopathy and adult T-cell leukemia. *Int. J. Cancer* 47, 491–495.

Kinpara, S., Hasegawa, A., Utsunomiya, A., Nishitsuji, H., Furukawa, H., Masuda, T., and Kannagi, M. (2009). Stromal cell-mediated suppression of human T-cell leukemia virus type 1 expression *in vitro* and *in vivo* by type I interferon. *J. Virol.* 83, 5101–5108.

Komori, K., Hasegawa, A., Kurihara, K., Honda, T., Yokozeki, H., Masuda, T., and Kannagi, M. (2006). Reduction of human T-cell leukemia virus type 1 (HTLV-1) proviral loads in rats orally infected with HTLV-1 by reimmunization with HTLV-1-infected cells. *J. Virol.* 80, 7375–7381.

Kozako, T., Yoshimitsu, M., Fujiwara, H., Masamoto, I., Horai, S., White, Y., Akimoto, M., Suzuki, S., Matsushita, K., Uozumi, K., Tei, C., and Arima, N. (2009). PD-1/PD-L1 expression in human T-cell leukemia virus type 1 carriers and adult T-cell leukemia/lymphoma patients. *Leukemia* 23, 375–382.

Kurihara, K., Harashima, N., Hanabuchi, S., Masuda, M., Utsunomiya, A., Tanosaki, R., Tomonaga, M., Ohashi, T., Hasegawa, A., Masuda, T., Okamura, J., Tanaka, Y., and Kannagi, M. (2005). Potential immunogenicity of adult T cell leukemia cells *in vivo*. *Int. J. Cancer* 114, 257–267.

Lehky, T. J., Fox, C. H., Koenig, S., Levin, M. C., Flerlage, N., Izumo, S., Sato, E., Raine, C. S., Osame, M., and Jacobson, S. (1995). Detection of human T-lymphotropic virus type I (HTLV-I) tax RNA in the central nervous system of HTLV-I-associated myelopathy/tropical spastic paraparesis patients by in situ hybridization. *Ann. Neurol.* 37, 167–175.

Macnamara, A., Rowan, A., Hilburn, S., Kadolsky, U., Fujiwara, H., Suemori, K., Yasukawa, M., Taylor, G., Bangham, C. R., and Asquith, B. (2010). HLA class I binding of HBZ determines outcome in HTLV-1 infection. *PLoS Pathog.* 6, e1001117. doi: 10.1371/journal.ppat.1001117

Miyatake, Y., Ikeda, H., Ishizu, A., Baba, T., Ichihashi, T., Suzuki, A., Tomaru, U., Kasahara, M., and Yoshiki, T. (2006). Role of neuronal interferon-gamma in the development of myelopathy in rats infected with human T-cell leukemia virus type 1. *Am. J. Pathol.* 169, 189–199.

Nagai, M., Usuku, K., Matsumoto, W., Kodama, D., Takenouchi, N., Moritoyo, T., Hashiguchi, S., Ichinose, M., Bangham, C. R., Izumo, S., and Osame, M. (1998). Analysis of HTLV-I proviral load in 202 HAM/TSP patients and 243 asymptomatic HTLV-I carriers: high proviral load strongly predisposes to HAM/TSP. *J. Neurovirol.* 4, 586–593.

Ndhlovu, L. C., Leal, F. E., Hasenkrug, A. M., Jha, A. R., Carvalho, K. I., Eccles-James, I. G., Bruno, F. R., Vieira, R. G., York, V. A., Chew, G. M., Jones, R. B., Tanaka, Y., Neto, W. K., Sanabani, S. S., Ostrowski, M. A., Segurado, A. C., Nixon, D. F., and Kallas, E. G. (2011). HTLV-1 tax specific CD8 T cells express low levels of Tim-3 in HTLV-1 infection: implications for progression to neurological complications. *PLoS Negl. Trop. Dis.* 5, e1030. doi: 10.1371/journal.pntd.0001030

Newbound, G. C., O'Rourke, J. P., Collins, N. D., Andrews, J. M., Dewille, J., and Lairmore, M. D. (2000). Repression of tax-mediated human t-lymphotropic virus type 1 transcription by inducible cAMP early repressor (ICER) protein in peripheral blood mononuclear cells. *J. Med. Virol.* 62, 286–292.

Ohashi, T., Hanabuchi, S., Kato, H., Tateno, H., Takemura, F., Tsukahara, T., Koya, Y., Hasegawa, A., Masuda, T., and Kannagi, M. (2000). Prevention of adult T-cell leukemia-like lymphoproliferative disease in rats by adoptively transferred T cells from a donor immunized with human T-cell leukemia virus type 1 Tax-coding DNA vaccine. *J. Virol.* 74, 9610–9616.

Oliere, S., Hernandez, E., Lezin, A., Arguello, M., Douville, R., Nguyen, T. L., Olindo, S., Panelatti, G., Kazanji, M., Wilkinson, P., Sekaly, R. P., Cesaire, R., and Hiscott, J. (2010). HTLV-1 evades type I interferon antiviral signaling by inducing the suppressor of cytokine signaling 1 (SOCS1). *PLoS Pathog.* 6, e1001177. doi: 10.1371/journal.ppat.1001177

Osame, M. (2002). Pathological mechanisms of human T-cell lymphotropic

virus type I-associated myelopathy (HAM/TSP). *J. Neurovirol.* 8, 359–364.

Osame, M., Usuku, K., Izumo, S., Ijichi, N., Amitani, H., Igata, A., Matsumoto, M., and Tara, M. (1986). HTLV-I associated myelopathy, a new clinical entity. *Lancet* 1, 1031–1032.

Ozden, S., Cochet, M., Mikol, J., Teixeira, A., Gessain, A., and Pique, C. (2004). Direct evidence for a chronic CD8 T-cell-mediated immune reaction to tax within the muscle of a human T-cell leukemia/lymphoma virus type 1-infected patient with sporadic inclusion body myositis. *J. Virol.* 78, 10320–10327.

Parker, C. E., Daenke, S., Nightingale, S., and Bangham, C. R. (1992). Activated, HTLV-1-specific cytotoxic T-lymphocytes are found in healthy seropositives as well as in patients with tropical spastic paraparesis. *Virology* 188, 628–636.

Pique, C., Ureta-Vidal, A., Gessain, A., Chancerel, B., Gout, O., Tamouza, R., Agis, F., and Dokhelar, M. C. (2000). Evidence for the chronic *in vivo* production of human T cell leukemia virus type I Rof and Tof proteins from cytotoxic T lymphocytes directed against viral peptides. *J. Exp. Med.* 191, 567–572.

Rende, F., Cavallari, I., Corradin, A., Silic-Benussi, M., Toulza, F., Toffolo, G. M., Tanaka, Y., Jacobson, S., Taylor, G. P., D'Agostino, D. M., Bangham, C. R., and Ciminale, V. (2011). Kinetics and intracellular compartmentalization of HTLV-1 gene expression: nuclear retention of HBZ mRNAs. *Blood* 117, 4855–4859.

Saito, M., Nakagawa, M., Kaseda, S., Matsuzaki, T., Jonosono, M., Eiraku, N., Kubota, R., Takenouchi, N., Nagai, M., Furukawa, Y., Usuku, K., Izumo, S., and Osame, M. (2004). Decreased human T lymphotropic virus type I (HTLV-I) provirus load and alteration in T cell phenotype after interferon-alpha therapy for HTLV-I-associated myelopathy/tropical spastic paraparesis. *J. Infect. Dis.* 189, 29–40.

Satou, Y., Yasunaga, J., Yoshida, M., and Matsuoka, M. (2006). HTLV-I basic leucine zipper factor gene mRNA supports proliferation of adult T cell leukemia cells. *Proc. Natl. Acad. Sci. U.S.A.* 103, 720–725.

Satou, Y., Yasunaga, J., Zhao, T., Yoshida, M., Miyazato, P., Takai, K., Shimizu, K., Ohshima, K., Green, P. L., Ohkura, N., Yamaguchi, T., Ono, M., Sakaguchi, S., and Matsuoka, M. (2011). HTLV-1 bZIP factor induces T-cell lymphoma and systemic inflammation *in vivo*. *PLoS Pathog.* 7, e1001274. doi: 10.1371/journal.ppat.1001274

Sodroski, J., Rosen, C., Goh, W. C., and Haseltine, W. (1985). A transcriptional activator protein encoded by the x-lor region of the human T-cell leukemia virus. *Science* 228, 1430–1434.

Suemori, K., Fujiwara, H., Ochi, T., Ogawa, T., Matsuoka, M., Matsumoto, T., Mesnard, J. M., and Yasukawa, M. (2009). HBZ is an immunogenic protein, but not a target antigen for human T-cell leukemia virus type 1-specific cytotoxic T lymphocytes. *J. Gen. Virol.* 90, 1806–1811.

Sugata, K., Satou, Y., Yasunaga, J., Hara, H., Ohshima, K., Utsunomiya, A., Mitsuyama, M., and Matsuoka, M. (2012). HTLV-1 bZIP factor impairs cell-mediated immunity by suppressing production of Th1 cytokines. *Blood* 119, 434–444.

Suzuki, S., Uozumi, K., Maeda, M., Yamasuji, Y., Hashimoto, S., Komorizono, Y., Owatari, S., Tokunaga, M., Haraguchi, K., and Arima, N. (2006). Adult T-cell leukemia in a liver transplant recipient that did not progress after onset of graft rejection. *Int. J. Hematol.* 83, 429–432.

Tajima, K. (1990). The 4th nationwide study of adult T-cell leukemia/lymphoma (ATL) in Japan: estimates of risk of ATL and its geographical and clinical features. The T- and B-cell Malignancy Study Group. *Int. J. Cancer* 45, 237–243.

Takamori, A., Hasegawa, A., Utsunomiya, A., Maeda, Y., Yamano, Y., Masuda, M., Shimizu, Y., Tamai, Y., Sasada, A., Zeng, N., Choi, I., Uike, N., Okamura, J., Watanabe, T., Masuda, T., and Kannagi, M. (2011). Functional impairment of Tax-specific but not cytomegalovirus-specific CD8 T lymphocytes in a minor population of asymptomatic human T-cell leukemia virus type 1-carriers. *Retrovirology* 8, 100.

Takeda, S., Maeda, M., Morikawa, S., Taniguchi, Y., Yasunaga, J., Nosaka, K., Tanaka, Y., and Matsuoka, M. (2004). Genetic and epigenetic inactivation of tax gene in adult T-cell leukemia cells. *Int. J. Cancer* 109, 559–567.

Tamiya, S., Matsuoka, M., Etoh, K., Watanabe, T., Kamihira, S., Yamaguchi, K., and Takatsuki, K. (1996). Two types of defective human T-lymphotropic virus type I provirus in adult T-cell leukemia. *Blood* 88, 3065–3073.

Tangy, F., Vernant, J. C., Coscoy, L., Ossondo, M., Bellance, R., Zaninovic, V., Cartier, L., Brahic, M., and Ozden, S. (1995). A search for human T-cell leukemia virus type I in the lesions of patients with tropical spastic paraparesis and polymyositis. *Ann. Neurol.* 38, 454–460.

Tashiro, T., Yamasaki, T., Nagai, H., Kikuchi, H., and Nasu, M. (1992). Immunological studies on opportunistic infection and the development of adult T-cell leukemia. *Intern. Med.* 31, 1132–1136.

Tattermusch, S., Skinner, J. A., Chaussabel, D., Banchereau, J., Berry, M. P., Mcnab, F. W., O'Garra, A., Taylor, G. P., and Bangham, C. R. (2012). Systems biology approaches reveal a specific interferon-inducible signature in HTLV-1 associated myelopathy. *PLoS Pathog.* 8, e1002480. doi: 10.1371/journal.ppat.1002480

Tomaru, U., Ikeda, H., Ohya, O., Abe, M., Kasai, T., Yamasita, I., Morita, K., Wakisaka, A., and Yoshiki, T. (1996). Human T lymphocyte virus type I-induced myeloneuropathy in rats: implication of local activation of the pX and tumor necrosis factor-alpha genes in pathogenesis. *J. Infect. Dis.* 174, 318–323.

Toulza, F., Heaps, A., Tanaka, Y., Taylor, G. P., and Bangham, C. R. (2008). High frequency of CD4+FoxP3+ cells in HTLV-1 infection: inverse correlation with HTLV-1-specific CTL response. *Blood* 111, 5047–5053.

Uchiyama, T. (1997). Human T cell leukemia virus type I (HTLV-I) and human diseases. *Annu. Rev. Immunol.* 15, 15–37.

Yamano, Y., Nagai, M., Brennan, M., Mora, C. A., Soldan, S. S., Tomaru, U., Takenouchi, N., Izumo, S., Osame, M., and Jacobson, S. (2002). Correlation of human T-cell lymphotropic virus type 1 (HTLV-1) mRNA with proviral DNA load, virus-specific CD8(+) T cells, and disease severity in HTLV-1-associated myelopathy (HAM/TSP). *Blood* 99, 88–94.

Yamazaki, H., Ikeda, H., Ishizu, A., Nakamaru, Y., Sugaya, T., Kikuchi, K., Yamada, S., Wakisaka, A., Kasai, N., Koike, T., Hatanaka, M., and Yoshiki, T. (1997). A wide spectrum of collagen vascular and autoimmune diseases in transgenic rats carrying the env-pX gene of human T lymphocyte virus type I. *Int. Immunol.* 9, 339–346.

Yoshida, M. (2001). Multiple viral strategies of HTLV-1 for dysregulation of cell growth control. *Annu. Rev. Immunol.* 19, 475–496.

Younis, I., and Green, P. L. (2005). The human T-cell leukemia virus Rex protein. *Front. Biosci.* 10, 431–445.

Zhang, J., Yamada, O., Kawagishi, K., Araki, H., Yamaoka, S., Hattori, T., and Shimotohno, K. (2008). Human T-cell leukemia virus type 1 Tax modulates interferon-alpha signal transduction through competitive usage of the coactivator CBP/p300. *Virology* 379, 306–313.

Zhao, T., Satou, Y., Sugata, K., Miyazato, P., Green, P. L., Imamura, T., and Matsuoka, M. (2011). HTLV-1 bZIP factor enhances TGF-beta signaling through p300 coactivator. *Blood* 118, 1865–1876.

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Molecular hallmarks of adult T cell leukemia

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The molecular hallmarks of adult T cell leukemia (ATL) comprise outstanding deregulations of signaling pathways that control the cell cycle, resistance to apoptosis, and proliferation of leukemic cells, all of which have been identified by early excellent studies. Nevertheless, we are now confronted the therapeutic difficulties of ATL that is a most aggressive T cell leukemia/lymphoma. Using next-generation strategies, emerging molecular characteristics such as specific surface markers and an additional catalog of signals affecting the fate of leukemic cells have been added to the molecular hallmarks that constitute an organizing principle for rationalizing the complexities of ATL. Although human T cell leukemia virus type 1 is undoubtedly involved in ATL leukemogenesis, most leukemic cells do not express the viral protein Tax. Instead, cellular gene expression changes dominate homeostasis disorders of infected cells and characteristics of ATL. In this review, we summarize the state of the art of ATL molecular pathology, which supports the biological properties of leukemic cells. In addition, we discuss the recent discovery of two molecular hallmarks of potential generality; an abnormal microRNA pattern and epigenetic reprogramming, which strongly involve the imbalance of the molecular network of lymphocytes. Global analyses of ATL have revealed the functional impact of crosstalk between multifunctional pathways. Clinical and biological studies on signaling inhibitory agents have also revealed novel oncogenic drivers that can be targeted in future. ATL cells, by deregulation of such pathways and their interconnections, may become masters of their own destinies. Recognizing and understanding of the widespread molecular applicability of these concepts will increasingly affect the development of novel strategies for treating ATL.

Keywords: HTLV-1, ATL, genome, epigenetics, miRNA, signal transduction

INTRODUCTION: CURRENT STATUS OF ADULT T CELL LEUKEMIA RESEARCH

Adult T cell leukemia (ATL), which is derived from human T cell leukemia virus type 1 (HTLV-1)-infected CD4+ T cells, is an aggressive T cell leukemia/lymphoma with the worst poor prognosis (Yamaguchi and Watanabe, 2002). Endemic expansion of HTLV-1 infection and related ATL onset have been observed in Japan (Iwanaga et al., 2010). The diverse clinical features of ATL have led to its sub-classification into acute, lymphoma, chronic, and smoldering subtypes. ATL has a poor prognosis with a mean survival time of 13 months and is refractory to currently available combination chemotherapy (Yamada et al., 2001). It is therefore essential to develop a novel treatment strategy, in particular, a molecular targeting therapy. ATL is incurable because we do not have complete understanding of its molecular basis, leading to the lack of molecular targeting. Although HTLV-1 is an apparent causative agent of ATL, several studies have demonstrated that viral gene expression is rare, except for the expression of the HTLV-1 antisense gene product HBZ (Gaudray et al., 2002). Mounting evidence has shown that ATL does not contain the somatic mutant genes that can explain its aggressiveness. However, it is evident that ATL cells possess multiple deregulations of genome and gene regulation, namely the molecular hallmarks of ATL, which should be targeted (**Figure 1**). We believe that normal cells acquire a succession of molecular hallmarks as they progressively evolve into a neoplastic state and that the multistep process of human pathogenesis can be rationalized by the need of incipient cancer cells for acquiring traits that enable them to become leukemogenic and ultimately malignant. In this review, we first summarize the essence of each molecular hallmarks of ATL cells. Basic molecular analyses and next-generation global analyses of ATL samples have revealed the molecular traits of ATL. We address new developments that broaden the scope of the conceptualization and describe recent advances of science to acquisition of the molecular mechanistic underpinnings. Finally, we discuss the possibility and future direction of treating ATL.

MOLECULAR HALLMARKS CONTROLLED BY TAX AND HBZ

Since 1985, numerous excellent studies have indentified signaling abnormalities in HTLV-1-associated cells, mainly induced by HTLV-1 Tax. In general, T cell disorders include several deregulations of cellular processes that regulates the cell cycle, cell proliferation, and cell survival. Tax has been shown to disrupt all these cellular processes. The classical oncogenic function of Tax was first demonstrated in a study of cell cycle regulation. Tax was found to inhibit the cyclin-dependent kinase (CDK) inhibitor (CKI) CDKN2A ($p16^{INK4A}$) by physical interaction (Suzuki et al., 1996). Several subsequent studies also revealed the mitogenic activity of Tax exerted through the stimulation of G1 to S phase transition (Akagi et al., 1996; Neuveut et al., 1998; Schmitt et al., 1998;

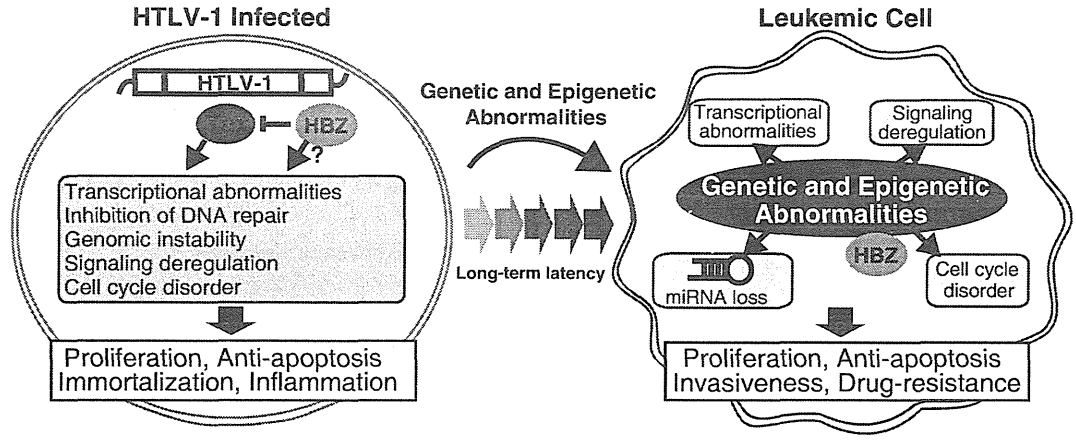

FIGURE 1 | Transition from HTLV-1-infected cell to transformed leukemic cell. In early and latent clinical phases, the HTLV-1 Tax and HBZ mainly act as driving forces for the molecular hallmarks of infected cells. After a long-term latency, leukemic cells have acquired genetic and epigenetic abnormalities, which lead to deregulations of gene expression pattern, cell cycling, signaling activation, and miRNA expression. These molecular changes consequently induce cellular hallmark capabilities of ATL such as chronic proliferation, apoptotic resistance, multiple organ invasion, and drug resistance.

Suzuki et al., 1999; Iwanaga et al., 2001; Haller et al., 2002; Liang et al., 2002). Tax also affects a cohort of cell cycle-related proteins, including CDKs, CDKN1A, CDKN1B, and CDKN2A, by regulating their expression or by physical interaction. Tax also participates in genetic damage (Jeang et al., 1990; Ressler et al., 1997; Kao and Marriott, 1999). Because of particular functions of Tax that can interact with many host factors (Interactome; Boxus et al., 2008; Simonis et al., 2012), Tax can activate several signaling pathways and lead to abnormal gene expression (a Tax-dependent molecular hallmark) and overproduction of several cytokines. Especially, IL-2 and its receptor are important for T cell activation. IL-2 signals through its receptor are primarily delivered by two molecular families, the Janus tyrosine kinases (JAKs) and signal transducers and activators of transcription (STATs), whose activation leads to lymphocyte proliferation. Tax can also activate the NF-κB and NFAT pathways responsible for the predominant expression of IL-2 and the IL-2 receptor (Ballard et al., 1988; Ruben et al., 1988; Hoyos et al., 1989; McGuire et al., 1993; Good et al., 1996). These findings implicate the IL-2–IL-2 receptor autocrine loop in ATL; however, several studies have shown that alterations of the loop alone are not sufficient to ensure the maintenance and proliferation of ATL cells because most Tax- or HTLV-1-immortalized T cells still require exogenous IL-2 and do not express detectable amounts of either IL-2 mRNA or protein (Akagi and Shimotohno, 1993; Chung et al., 2003; Chen et al., 2010). Similarly, IL-15 and its receptor and IL-13 and its receptor have been associated with Tax-expressing cells because similar to IL-2, they activate the STAT pathways (Azimi et al., 1999; Mariner et al., 2001; Chung et al., 2003; Wäldele et al., 2004). IL-6 is also transduced by Tax through the NF-κB pathway (Villiger et al., 1991; Muraoka et al., 1993). Because IL-6 mainly participates in inflammatory signaling, HTLV-1 infection can induce cytokine-dependent inflammation, which is frequently observed in ATL as well as HTLV-1-associated myelopathy/Tropical spastic paraparesis (HAM/TSP; Oh et al., 2011). OX40, a member of the TNF-receptor superfamily, is specifically expressed in HTLV-1-infected cells, whose expression is induced by the Tax-NF-κB pathway. Tax may play a role in leukemic cell infiltration in addition to cell adhesion *in vivo* (Imura et al., 1997). Tax affects not only the abovementioned signaling pathways but also the TGF-β pathway (Kim et al., 1990; Höllsberg et al., 1994; Arnulf et al., 2002; Lee et al., 2002). It has been recently shown that TGF-β signaling is activated by HBZ by binding with Smad 2/3 (Zhao et al., 2011). TP53 is the master regulator of the cell cycle that guards against DNA damage by inducing the transcription of several genes. Tax can inhibit TP53 functioning in multiple ways (Grassmann et al., 2005).

Strong NF-κB activation is the outstanding hallmark provided by Tax. NF-κB represents a family of inducible transcription factors that regulate diverse biological processes, including the growth and survival of both T cells and non-lymphoid cells. Transcriptional activation of genes such as several cytokines and apoptosis-resistance factors plays an important role in immunity. Tax acts as an intracellular stimulator of IKK by physical interaction, leading to persistent activation of NF-κB-mediated transcription. The Tax/IKK complex formation relies on the physical interaction between Tax and the IKK regulatory subunit IKKγ. The Tax/IKKγ interaction is required for recruiting Tax to the IKK catalytic subunits and for Tax-mediated IKK activation (Sun and Yamaoka, 2005). Recent studies have identified cellular proteins that are important for Tax-mediated NF-κB activation, such as NRP/Optineurin and TAX1BP1 (Journo et al., 2009; Shembade et al., 2011), and the ubiquitin-specific peptidase USP20 (Yasunaga et al., 2011). Subcellular localization of Tax also predominantly controls Tax-mediated NF-κB activation (Fryrear et al., 2009). Given that NF-κB governs the expression of a large array of cellular genes that control various cellular functions, the phenotypes of HTLV-1-infected cells are dominated by Tax-mediated abnormal activation.

Tax also activates several signaling pathways through key transcriptional factors such as CREB, SRF, and AP-1. It does not directly

bind to promoter or enhancer DNA, however, disruption of these pathways causes serious gene expression disorders (Grassmann et al., 2005).

It should be also noted that HTLV-1 antisense product HBZ seems to be involved in leukemogenesis; its expression is sustained in leukemic cells. *In vitro* and *in vivo* studies have demonstrated that the growth-promoting activity of HBZ RNA may play an important role in oncogenesis by HTLV-I (Satou et al., 2006). Furthermore, transgenic expression of HBZ in CD4+ T cells induces T cell lymphomas and systemic inflammation in mice. HBZ directly induces *Foxp3* gene transcription, and the increased CD4+Foxp3+ T_{reg} cells in HBZ transgenic mice are functionally impaired, suggesting that the expression of HBZ in CD4+ T cells may be a key mechanism of HTLV-1-induced neoplastic and inflammatory diseases (Satou et al., 2011).

Taking together with these mounting evidences, Tax and HBZ undoubtedly contribute to leukemogenesis in HTLV-1-infected T cells. However, as a low rate of incidence, clinical observation implies that HTLV-1 itself does not have a strong capacity of leukemogenesis in contrast with other animal leukemia viruses.

CHROMOSOMAL CHANGES AND GENE ALTERATIONS IN ATL

Tax is not expressed in most ATL cases because HTLV-1 provirus is substantially silenced by proviral defect and/or epigenetic mechanism (Tamiya et al., 1996; Koiwa et al., 2002; Taniguchi et al., 2005). However, leukemic cells possess very similar traits to Tax-expressing cells (**Figure 1**). The paradoxical truth, i.e., memory of Tax, still remains to be elucidated. Investigation of established ATL cell lines and primary ATL samples has led to the identification of the molecular hallmarks of leukemic cells, which may partially explain their malignant characteristics.

From 1980s, chromosomal analyses of clinical cases were reported. Therefore, we know that ATL is characterized by various abnormal chromosomes. Kamada et al. (1992) reported that 96% of ATL cases had an abnormal chromosome pattern, suggesting that a genomic catastrophe underlies the clinical and molecular characteristics of ATL, which is consistent with all other cancers. In 2000s, global analysis has been available and whole genomic analysis could be challenged. Comparative genomic hybridization (CGH) revealed the genomic distinctions between the clinical subtypes; patients with aggressive ATL (acute and lymphoma) have a higher number of chromosomal imbalances, losses, and gains than those with indolent ATL (chronic and smoldering). Thus, genomic abnormalities are prognostic factors (Tsukasaki et al., 2001a). High-resolution analyses based on microarrays unveiled the genomic properties of ATL (Oshiro et al., 2006) and shed light on the genomic characteristics that affect the gene expression pattern (**Figure 2**).

In cancer cells, genetic mutations and deletions are generally observed in genes important for cell cycle regulation, cell survival, and cell proliferation. In ATL, the accumulation of genetic deletions in the gene encoding the cell cycle regulator CDKN2A have been reported (Hatta et al., 1995; Uchida et al., 1996). Southern blotting analyses of samples from large cohort studies (114 patients) also demonstrated that the *CDKN2* family (*p15* and *p16*) is lost in more frequently observed in the acute type (33.8%) than in the chronic type of ATL (5.4%; Yamada et al., 1997). Patients

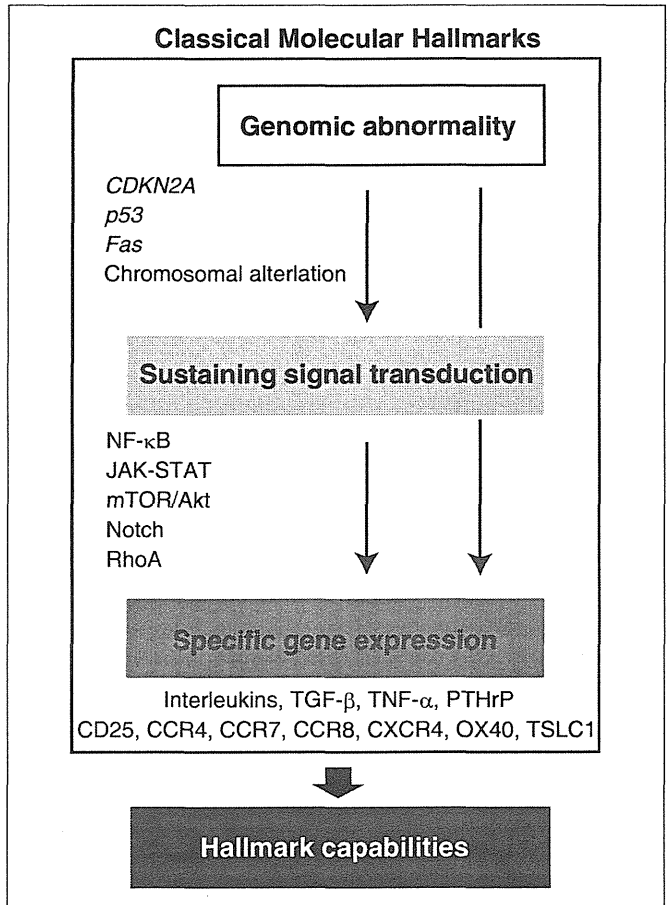

FIGURE 2 | Acquired molecular hallmarks of ATL. From the beginning of ATL discovery, indicated molecular hallmarks have been suggested. Hierarchical regulation of the gene expression has been expected. Abnormal expressions of these cytokines and receptors as well as various proteins that act as anti-apoptotic factors or proliferation agents are responsible for the malignant phenotypes as hallmark capabilities.

with these genetic deletions showed reduced survival compared with those without deletions. *CDKN2A* transcription is also regulated in an epigenetic manner. Abnormal DNA methylation has been during ATL progression (Nosaka et al., 2000).

Alterations of other cell cycle-regulating genes are unusual. Mutations of *CDKN1B* ($p27^{Kip1}$) is rare in non-Hodgkin's lymphoma and ATL (Morosetti et al., 1995). Mutations of *Rb* gene have also been observed in a few ATL patients (Hatta et al., 1997). Extensive analyses have revealed that 26–40% of ATL cases have a *p53* gene mutation, which is much less than that observed in other cancers (Nagai et al., 1991; Cesarman et al., 1992; Sakashita et al., 1992; Nishimura et al., 1995). Gene expression analysis of ATL samples showed that CDKN1A ($p21^{Cip1}$) is commonly down regulated in ATL (Watanabe et al., 2010). In contrast, Tax activates $p21^{Cip1}$ expression in HTLV-1-infected cells, which may be an important mechanism for stopping the host at the G1/S boundary and repairing damaged DNA before entering the S phase entry (Cereseto et al., 1996; de La Fuente et al., 2000). Although $p21^{Cip1}$ transcription may be activated by the Tax–CREB pathway in infected cells,

it seems to be epigenetically silenced in several cancers, including ATL. Thus, these gene alterations and related expression patterns are important molecular hallmarks of ATL as well as other cancers. Of note, no ATL cell has been shown to have both mutations, suggesting that either *p16* or *p53* mutation may be sufficient to promote the more aggressive phenotype of leukemic cells (Tawara et al., 2006). In addition, as many ATL patients carry a wild-type *p53*, TP53 activation by antagonists such as Nutlin-3a may be a promising strategy for ATL therapy (Hasegawa et al., 2009). The ability to resist apoptotic cell death is conferred by *Fas* gene mutation (Tamiya et al., 1998; Maeda et al., 1999). Indeed, Fas-negative ATL cells are resistant to adriamycin-induced apoptosis *in vitro*, which is consistent with the finding that ATL in this case is resistant to chemotherapy.

Single nucleotide polymorphism (SNP) is another possible cause for developing ATL. TNF-α polymorphism may be associated with increased susceptibility to development of ATL in HTLV-1 carriers (Tsukasaki et al., 2001b). An immunological study also suggested that the HLA haplotype is related to ATL progression (Yashiki et al., 2001). Currently, whole SNP can be detected by next-generation sequencing and other array-based techniques. Global understanding of SNPs in ATL patients may provide us with the genetic basis of the familial clustering of ATL.

By utilizing global survey techniques for analyzing the gene copy number, several research groups have detected specific gene copy number patterns in ATL. CGH methods first demonstrated gains at 14q32 and 2p16-22 in ATL cell lines (Ariyama et al., 1999). Fluorescence *in situ* hybridization (FISH) analysis with several yeast artificial chromosome (YAC), BAC, and PAC clones also mapped the breakpoints in ATL cell lines with 6q aberrations (Tagawa et al., 2002). Clinical subtype-specific genomic alterations in aggressive ATL were also identified by array-based CGH (Oshiro et al., 2006). The lymphoma type of ATL had significantly more frequent gains at 1q, 2p, 4q, 7p, and 7q and losses at 10p, 13q, 16q, and 18p, whereas the acute type showed a gain of 3/3p. *CARMA1* is also a possible target gene for 7p22 amplification in the lymphoma type but not in the acute type of ATL. In contrast, BCL11B is expressed in the acute type of ATL, regardless of 14q32 gain/amplification; however, there is no or low expression of the gene in the lymphoma type of ATL. Taken together, these findings suggest that the acute and lymphoma types of ATL are genomically distinct. The physiological molecular hallmarks of ATL have been identified by integrating the gene copy number and gene expression analyses findings, and these are described below.

GLOBAL GENE EXPRESSION ANALYSES OF ATL

Genetic alterations and other modulations enhance specific gene expression signatures. Classically, based on a comparison between normal T cells and ATL cell lines as well as primary leukemic cells, ATL has been shown to have a distinct gene expression pattern that may reflect its clinical pathogenesis. In addition, global expression analyses, mainly based on microarray, have identified emerging molecular hallmarks that can possibly be targeted in ATL therapies.

First, Harhaj et al. (1999) performed gene array analysis of HTLV-1-immortalized cells and reported up- and downregulated genes. Ruckes et al. (2001) performed suppressive subtractive hybridization that enabled the isolation of novel sequences derived from unknown genes. They identified a number of genes linked to Tax transformation and ATL leukemogenesis. Systematic comparison of gene expression between cultured cells from patients with acute ATL and that of stimulated peripheral T lymphocytes revealed 346 cDNA clones, which included the genes encoding IL-2 receptor α chain and $p21^{Cip1}$. They also included a dual-specific protein phosphatase (PAC1), an interferon-inducible factor (ISG15), a basic helix–loop–helix transcription factor (DEC-1), and the secreted anti-apoptotic chemokine I-309. Pise-Masison et al. (2002) also reported the results of analyses performed using an Affymetrix Hu6800 GeneChip. They identified 763 genes with differentially regulated expression in HTLV-1 cell lines.

Tsukasaki et al. (2004) directly analyzed the gene expression patterns of fresh ATL samples (three acute types and one chronic type) using high-density oligonucleotide DNA arrays. A total of 203 genes that included ribosomal proteins, proteasome subunits, translation factors, immunophilins, heat shock proteins, and genes important for DNA replication were found to be upregulated in ATL cases, and 91 genes were downregulated. Sasaki et al. (2005) also determined the expression profiles of more than 12,000 genes in eight cases of the acute type of ATL using microarray analysis, and they found that 192 genes were upregulated more than twofold compared with healthy CD4+ and CD4+/CD45RO+ T cells. In particular, the expression of tumor suppressor in lung cancer 1 (TSLC1), caveolin 1, and prostaglandin D2 synthase was increased by more than 30-fold. Interestingly, comparison of gene expression between established cell lines with or without HTLV-1 infection led to the identification of the specific HTLV-1-related cell surface marker CD70, which is also expressed in freshly isolated leukemic cells (Baba et al., 2008).

Integrated analyses of the genome and its expression revealed more detailed data for the acquisition of molecular and physiological information. Choi et al. (2007) isolated CD4+ T cells from ATL patients and profiled their gene expression using DNA microarrays containing >44,000 probe sets. Changes in the chromosomal copy number were examined for 24 cell specimens using microarrays harboring approximately 50,000 probe sets. Coordinated analysis revealed a novel molecular target, the HGF–MET pathway. Ligation of MET by increased plasma HGF levels may confer a growth advantage on ATL cells through the phosphatidylinositol 3-kinase (PI3K) and Ras pathways.

We performed further global integrated analyses of samples from ATL patients using mRNA expression array ($n = 52$), microRNA (miRNA) microarray ($n = 40$), and DNA copy number analysis using SNP-based array ($n = 168$; Yamagishi et al., 2012). Each analysis revealed many novel molecular characteristics of ATL (data available in the Gene Expression Omnibus database). Analysis of this massive dataset derived from primary ATL samples led to the conclusion that genetic and epigenetic imbalances are involved in ATL leukemogenesis through miRNA and the signaling pathways responsible for leukemic cell survival, proliferation, and invasiveness. Taken together, these findings clearly indicate that disrupted gene expression is a molecular hallmark of ATL. Diverse abnormalities have been found in each of these comprehensive studies; however, several gene alterations and other critical events have been commonly implicated as determinants of the gene

expression pattern. Abnormal expression of different cytokines, their receptors, and various proteins that act as anti-apoptotic factors or proliferation agents is responsible for malignant phenotypes as hallmark capabilities (**Figure 2**). From the spreading intelligence, we have to acquire the genuine molecular targets harboring in the bare bones of ATL.

SIGNALING NETWORKS IN ATL: MAIN DRIVING FORCES

The cellular complexity of signaling networks confers robustness to specific gene expression and biological functions. This is supported by the fact that the inhibition of a signaling pathway can strictly suppress abnormal gene expression. The functional characteristics of ATL cells, such as chronic proliferation, abnormal survival, and penetrating multiple organs, is supported by aberrant gene expression, as described above.

Activation of NF-κB signaling is the most outstanding hallmark of both HTLV-1-infected cells and leukemic cells. Abnormal activation and deficient negative control of the NF-κB pathway strongly contributes to prolonged survival, proliferation, and invasiveness, all of which are observed in ATL cells. Ruben et al. (1988) found that Tax can activate IL-2 receptor gene expression in a NF-κB-dependent manner. Following this, several studies clearly demonstrated that Tax can activate NF-κB activity through a multistep (Sun and Yamaoka, 2005). Strong and persistent activation of NF-κB signaling was then demonstrated in leukemic cell lines that did not express Tax (Mori et al., 1999). In transformed ATL cells, both the canonical and non-canonical NF-κB pathways are persistently activated because NF-κB inducing kinase (NIK) is aberrantly expressed in ATL (Saitoh et al., 2008). NIK plays a central role in non-canonical NF-κB signaling by IKKα phosphorylation (Thu and Richmond, 2010). Very recently, we found a novel linkage between NF-κB activation and miRNA deregulation. Comprehensive gene expression analysis and *in vitro* experiments revealed that miRNA-31 (miR-31) can regulate NIK expression through the 3′ untranslated region (UTR). In ATL cells, miR-31 expression is genetically and epigenetically silenced, which in turn induces constitutive NF-κB activation through NIK expression (**Figure 3**; Yamagishi et al., 2012). Because current evidence clearly indicates that miR-31 dominates NF-κB activity in T cells, manipulating cellular miR-31 levels may be a novel molecular approach to reduce NF-κB activity and induce apoptosis.

Numerous efforts have demonstrated that NF-κB inhibitory agents may be suitable for treating ATL. Inhibitors such as bortezomib (Satou et al., 2004), Bay 11-7082 (Mori et al., 2002), DHMEQ (Watanabe et al., 2005), ACHP (Sanda et al., 2006), and IMD0354 (Uota et al., 2012) have the ability to kill leukemic cells. Fludarabine, a purine analog that has significant activity in B cell malignancies, also inhibits the NF-κB activity in ATL (Nishioka et al., 2007). Tax degradation by arsenic trioxide and IFN-α treatments is also useful for inhibiting NF-κB in HTLV-1-infected cells (El-Sabban et al., 2000). In the near future, gene manipulation for example by siRNA targeting NIK may be a promising strategy for inhibiting NF-κB activity with strict specificity (Yamagishi et al., 2012). Because NF-κB prevention showed good results in a xenograft model of cell lines with or without Tax (Dewan et al., 2003; Watanabe et al., 2005; Ohsugi et al., 2007a), molecular targeting therapy based on the NF-κB pathway is a promising new ATL treatment. Of note, NF-κB inhibition by DHMEQ can also remove

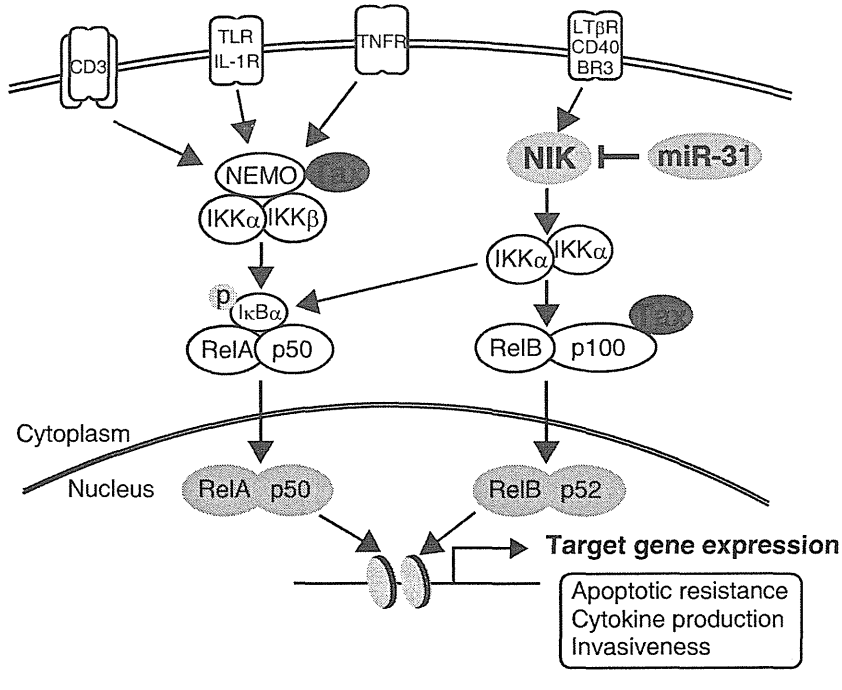

FIGURE 3 | NF-κB signaling pathway in ATL. Compared to normal T cell, HTLV-1-infected cell shows intense activity of NF-κB pathway through the Tax interactome. In contrast, leukemic cell sustains strong NF-κB activity without Tax by acquisition of miR-31-dependent NIK expression. Activation of the non-canonical pathway ultimately joins canonical pathway, resulting in transactivation of numerous target genes important for cell survival, cytokine production, and invasiveness.

virus-carrying cells from carrier peripheral blood mononuclear cell (PBMC) samples (Watanabe et al., 2005).

Why is NF-κB important for ATL cell survival? One of the target genes is BCL-XL, which is expressed when NF-κB is stimulated. In HTLV-1-infected cell lines, BCL-XL is expressed through the Tax-NF-κB pathway. Interestingly, fresh ATL samples show BCL-XL overexpression (Nicot et al., 2000). Given that BCL-XL is a principal anti-apoptotic protein as a BCL-2 family member, persistent BCL-XL expression is one of the molecular capability of apoptotic resistance, which may contribute to clinical chemoresistance. Indeed, inhibition of the NF-κB pathway by NIK depletion leads to impaired BCL-XL levels and apoptotic cell death in ATL (Yamagishi et al., 2012). In addition, a recent study has implicated that both BFL1 (BCL2A1) and BCL-XL are responsible for ATL cell survival. BFL1 expression is regulated by Tax and HBZ through the NF-κB and AP-1 pathways, respectively (Macaire et al., 2012).

The JAK–STAT pathway, which is universal and essential to cytokine receptor signaling, is also one of the best understood signal transduction cascades in several cell types, including ATL cells. The JAK–STAT pathway mediates signaling by cytokines, which control cell survival, proliferation, and differentiation. Constitutive JAK activation leads to persistent activation of STAT transcription factors, and several cancers exhibit constitutive STAT activation in the absence of JAK- or STAT-activating mutations. JAK–STAT activation in HTLV-1-infected cells was first reported in 1995 (Migone et al., 1995). Takemoto et al. (1997) demonstrated that STAT-3 and STAT5 are activated in freshly isolated ATL cells. However, functional redundancy was also reported. Blockade of the JAK3–STAT5 pathway by AG490 failed to inhibit the proliferation of HTLV-1-transformed T cell lines, despite activation of the pathway (Kirken et al., 2000). Recently, a JAK2–STAT5 inhibitor, AZ960, was shown to have an anti-proliferative effect on HTLV-1-infected and ATL cell lines (Yang et al., 2010). However, which gene is transactivated by STAT remains unknown. The biological relevance of the JAK–STAT pathway in ATL remains to be elucidated.

EMERGING SIGNALING PATHWAYS AND INHIBITORY AGENTS

In addition to the abovementioned pathways, several signaling pathways, including the AP-1, NFAT, and CREB pathways, may be involved in the functional characteristics of ATL (Hall and Fujii, 2005). These are activated by Tax. Leukemic cells may have certain activities in each of these pathways.

The RhoA family also participates in Tax-mediated immortalization. A recent study employing a proteomic approach to identify Tax-binding proteins in a HTLV-1-infected T cell line identified direct interactions between Tax and several small GTPases, including RhoA, Rac1, and Cdc42, all of which are involved in a wide variety of cellular processes, including cytoskeleton organization and transcriptional activation (Wu et al., 2004). Recently, our global gene expression analysis as well as miRNA study strongly suggested that RhoA is overexpressed in ATL cells. A decreased miR-31 level may contribute to RhoA expression and migration activity (Yamagishi et al., 2012). The gene expression signature determined from patient samples could identify the additional signaling pathways involved in leukemogenesis (authors' unpublished data).

Recently, several studies have demonstrated that ATL shows emerging signaling activation, which is well established as oncogenic signaling in other types of cancer. Typically, the Notch pathway, which is the signaling pathway implicated in T cell acute lymphoblastic leukemia (T-ALL), is also activated in ATL (Pancewicz et al., 2010). More than 30% of ATL cases show activating mutations in *NOTCH1*, leading to reduced CDC4/Fbw7-mediated degradation and stabilization of the intracellular cleaved form of Notch1 (ICN1). Inhibition of Notch signaling by a γ-secretase inhibitor reduced tumor cell proliferation and tumor formation in ATL-engrafted mice. It is therefore suggested that activated Notch may be important for ATL pathogenesis.

TGF-β signaling is also reportedly important. Increased TGF-β production was observed in freshly isolated ATL cells, and this was at least partially mediated by AP-1 (Niitsu et al., 1988; Kim et al., 1990). Nevertheless TGF-β could inhibit the growth of normal T lymphocytes, HTLV-1-infected cells were resistant to this inhibition (Höllsberg et al., 1994). In ATL cells, transcription factor 8 (TCF8) is frequently disrupted by several mechanisms, primarily by epigenetic dysregulation. TCF8 mutant mice frequently develop invasive CD4+ T cell lymphomas *in vivo*. Downregulation of TCF8 expression in ATL cells *in vitro* is associated with resistance to TGF-β1, suggesting that escape from TGF-β1-mediated growth inhibition is important in the pathogenesis of ATL (Hidaka et al., 2008). The same research group also reported that ZEB1 downregulation and Smad7 overexpression contribute to resistance to TGF-β1-mediated growth suppression in ATL (Nakahata et al., 2010). ZEB1 plays a critical role in regulating the epithelial–mesenchymal transition (EMT) in several solid cancers. The general function of ZEB1 in lymphocytes, particularly in association with leukemogenesis and TGF-β signaling, is a very intriguing issue.

If a chemical drug that can inhibit cellular signaling can induce apoptosis specifically, it is probable that leukemic cell survival is supported by the targeted signaling. Recent studies of inhibitors have indicated that mTOR signaling may be involved in ATL. mTOR is a serine/threonine-specific protein kinase that is located downstream of the PI3K/Akt pathway. Deregulation of mTOR signaling is implicated in a range of cancers due to its roles in cell survival and proliferation, protein synthesis and breakdown, membrane trafficking, and protein kinase C signaling (Zoncu et al., 2011). Ikezoe et al. (2007) used several inhibitors to show that the PI3K/Akt/mTOR pathway is activated in ATL cell lines as well as fresh ATL cells. In addition, a resent study reported that the mTOR complex 1 (mTORC1) inhibitor everolimus has a dramatic inhibitory effect on the growth of HTLV-1-positive and HTLV-1-negative malignant T cells, whereas normal resting or activated T cells are resistant to it (Darwiche et al., 2011). Furthermore, in addition to HTLV-1-infected cells in which Tax activates the Akt pathway (Liu et al., 2001), transformed leukemic cells in which Tax is not expressed may have some Akt activity. The most important point is how these pathways are constitutively activated in ATL. The functional importance of this pathway and its therapeutic potential will be addressed in the near future.

In the context of inhibitory agents, a higher response rate following azidothymidine/interferon α (AZT/IFNα) treatment of ATL patients has been reported in several human trials (Gill et al., 1995; Bazarbachi et al., 2010). AZT treatment of ATL patients