Cutaneous Manifestations of Human T Cell Leukemia Virus Type I Infection in an Experimental Model

R. Mark Simpson, Michel Leno, Beth Schmidt Hubbard, and Thomas J. Kindt

Skin diseases ranging from infective dermatitis to cutaneous lymphoma have been associated with human T cell leukemia virus (HTLV) type I. A generalized exfoliative papillated dermatopathy occurred in a rabbit 20 months into a course of chronic HTLV-I infection. Biopsies revealed epidermotropic T cell infiltrates, including Sezary-like cells, that resulted in a pattern mimicking cutaneous T cell lymphoma. HTLV-I was isolated from affected skin, and virus expression was detected in cutaneous cultures. Sezary-like cells also occurred in circulation. Interleukin-2-independent lymphocyte cultures, established from blood exhibiting elevated CD8 T cell levels and CD25 expression, had polyclonal integration of provirus. The findings are similar to those in evolving adult T cell leukemia lymphoma and may represent a prelymphomatous change. The cutaneous lymphoproliferative lesion resulted from HTLV-I infection and further establishes the New Zealand White rabbit inoculated with the RH/K34 T cell line as a suitable model for investigation of HTLV-I pathogenesis.

Human T cell leukemia virus (HTLV) type I, initially isolated from a patient with a cutaneous T cell lymphoma [1], has been shown to cause adult T cell leukemia lymphoma (ATLL) [2] and other disorders, including a progressive neuro-myelopathy known as tropical spastic paraparesis—HTLV-associated myelopathy [3]. ATLL is a heterogeneous T cell lymphoproliferative disease syndrome that can be manifested as several clinical types, including smoldering (evolving), chronic, acute, and lymphoma forms [3]. Cutaneous lymphoid infiltrates occur in 40%-70% of HTLV-I-infected persons with lymphoproliferative disease and may appear in any of the four clinical types [4]. Skin involvement is frequently the presenting clinical sign of chronic and smoldering ATLL, types that are considered preleukemic. Some patients pass through more than one clinical stage; during progression to the acute form, changes in HTLV-I proviral integration from polyclonal to monoclonal can occur [3]. Evidence also suggests that childhood HTLV-I infective dermatitis may advance to acute ATLL [5]. Furthermore, HTLV-I is associated with a variety of other lymphoproliferative diseases, including cutaneous T cell lymphomas [3]. Several investigators have looked at the potential role of retrovirus in cutaneous T cell lymphomas, such as mycosis fungoides and the Sezary syndrome [4, 6, 7]. A clonal progression similar to HTLV-I lymphoproliferative disease has been suggested for cutaneous T cell lymphomas, which typically have a long indolent succession with disease confined to the skin until late in the disease course [4].

The precise mechanism of lymphomagenesis by retroviruses such as HTLV-I has not been defined [3]. Viral oncogenesis studies in animals have yielded important clues to disease mechanisms, and investigation of human retroviral pathogenesis could potentially profit from controlled infection studies in animals. The HTLV-I infection model using the laboratory rabbit has proven beneficial for study of HTLV infection and pathogenesis [8–10]. Like HTLV-I in humans, infection in rabbits is generally benign with disease produced only in limited circumstances. Most reports focus on acute ATLL-like disease after inoculation of infected cell lines [8, 9]. The ability of the rabbit to develop lymphoproliferative diseases after an extended period of infection on a scale similar to that observed in humans has not been demonstrated. Here we report a cutaneous lymphoproliferative disease in a rabbit chronically infected with HTLV-I that has clinical, immunopathologic, and virologic features suggesting a pathogenesis similar to human lymphoproliferative disease.

Materials and Methods

A mature male Pasteurella-free New Zealand White rabbit (X138) was given a single intravenous inoculation of $2 \times 10^8$ viable HTLV-I–infected T cells. The source of the HTLV-I was the infected rabbit T cell line RH/K34. RH/K34 was derived using an infected T cell line (RHT-16) isolated from a rabbit inoculated with MT-2, the human T cell line infected with HTLV-I [11]. RH/K34 is capable of transmitting infection to rabbit and human lymphocytes in vitro.

In animal inoculation studies, routine complete blood cell counts and serum biochemistry panels were done (Maryland Medical Lab-
oratories, Baltimore). CD4, CD8, and CD25 (rabbit-specific monoclonal antibodies; Spring Valley Laboratories, Woodbine, MD) blood lymphocyte subpopulations were analyzed by single-parameter flow cytometry [11]. Skin punch biopsies were bisected, and portions were cultured in vitro and preserved by fixation in formalin or glutaraldehyde or were snap frozen in liquid nitrogen. Diced whole skin specimens and isolated blood mononuclear cells were cultured under conditions appropriate for RH/K34 [11]. Preserved skin was processed routinely for light microscopy. Skin was also used in an indirect immunofluorescence assay by application of CD3 monoclonal antibody to frozen sections (antibody CD3PC3/188A; gift of M. Jones, John Radcliffe Hospital, Oxford, UK) to detect T cells as previously described [12]. Transmission electron microscopy was done on freshly fixed skin, pelleted cultured skin cells, and freshly fixed blood leukocyte samples (JFE Electron Microscopy Services, Brookeville, MD).

Evaluation of HTLV-I infection status included assays for serum antibodies and for presence of virus in tissue and blood. Serum samples were analyzed by Western blot (Cambridge Biotech, Worcester, MA) for the presence of antibodies to HTLV-I using biotinylated goat anti-rabbit IgG (GIBCO BRL, Gaithersburg, MD) as secondary antibody. Supernatants from skin cultures were assayed for the presence of HTLV-I p24\textsuperscript{ag} protein using a commercial antigen capture assay (Coulter, Hialeah, FL). DNA from skin and cultured peripheral blood mononuclear cells was isolated [11] and analyzed by polymerase chain reaction (PCR) for HTLV-I env sequences as described [10]. Reactions were verified by hybridization with an HTLV-I-specific env probe, 5'-TCCTTGCAGGACCATTGCATCTCCGTACGCTAGCACC-3', that was labeled and detected using a chemiluminescence assay according to manufacturer's instructions (Amersham, Arlington Heights, IL). Additional DNA samples were digested with EcoRI (New England Biolabs, Beverly, MA) and analyzed by Southern blot using a \textsuperscript{32}P-labeled 8.3-kb HTLV-I probe [10]. Southern blot analysis was used for evaluation of class I and II major histocompatibility (MHC) antigen complex genotype [13].

Results

New Zealand White rabbits inoculated with the HTLV-I-transformed rabbit T cell line RH/K34 typically die of acute ATLL-like disease [9]. Disease occurs after inoculation of a threshold dose of RH/K34 without regard to MHC matching between inoculum and host [13a]. In a series of experiments that examined the detailed pathogenesis of the acute phase of experimental ATLL (unpublished data), rabbit X138 was given a single intravenous inoculation of a generally lethal dose of viable HTLV-I-infected RH/K34 T cells (2 \times 10\textsuperscript{6}) and survived the acute phase. Rabbit X138 maintained chronic HTLV-I infection (see below) and developed generalized exfoliative papillated dermatopathy \textasciitilde20 months after inoculation (figure 1A).

We biopsied cutaneous lesions of rabbit X138 and control skin from HTLV-I-infected rabbits inoculated with a \gamma-irradiated lethal dose of RH/K34 (10\textsuperscript{4} rads) or with cell-free virus isolated from RH/K34 (dose particle count, 2 \times 10\textsuperscript{9}) and healthy uninoculated rabbit skin. The major histologic alteration in biopsies of X138, serially collected over 6 months, was the presence of a monomorphic population of lymphocytes that primarily affected the epidermis and epithelium of the outer root sheath of hair follicles. Lymphocytes were present individually, occasionally in aggregates (Pautrier's microabscess), and often obscured the dermoepidermal junction. Lymphocytes were also diffusely scattered in the superficial dermis, and the infiltrate was associated with papillated epidermal hyperplasia, hyperkeratosis, and keratin plugging of follicular infundibula (figure 1B). No lesions were observed in other HTLV-I-infected or in uninoculated rabbit skin biopsies.

Indirect immunofluorescence assay of rabbit X138 skin indicated the lymphoid infiltrates were primarily composed of T cells. Transmission electron microscopy of biopsied skin revealed individual and clustered infiltrating lymphocytes with Sezary cell-like morphology amid keratinocytes (figure 1C). Lymphocytes with irregular nuclear margins (\textlesssim5%) were visible in stained blood smears, and ultrastructural examination of freshly fixed blood leukocyte (buffy coat) preparations disclosed Sezary-like lymphocytes in circulation (figure 1D). Immunophenotypic analysis of lysed whole blood uncovered elevated levels of circulating CD8 T cells (1021/\mu L whole blood; normal reference range, 72–742/\mu L, unpublished data) and the X138 blood lymphocyte population expressed CD25, the interleukin-2 receptor (figure 1E).

Evidence of HTLV-I infection in rabbit X138 was provided by detection of HTLV-I serum antibodies at multiple times after inoculation (figure 2A) and by amplification of HTLV-I env sequence in DNA samples analyzed in two separate reactions prepared from freshly preserved skin lesions biopsied at different postinoculation intervals (figure 2B). Southern blot analysis of DNA samples from interleukin-2-independent cultured X138 blood lymphocytes revealed a diffuse pattern of HTLV-I-specific hybridization that was interpreted as indicating polyclonal proviral integration. Even though HTLV-I was found in X138 skin by PCR and virus isolation, no clonal pattern of proviral integration was detected in X138 skin samples by Southern blot. Hybridization of DNA from the RH/K34 inoculum yielded the pattern expected for the monoclonally integrated provirus. Supernatant from skin maintained in culture for \textasciitilde1 month and assayed for the presence of HTLV-I p24\textsuperscript{ag} protein provided evidence of virus expression in cutaneous lesions. By ultrastructural examination, we found small numbers of type C retrovirus particles (\textlesssim100 nm in diameter) typical of HTLV-I amid pelleted cultured skin cells. Molecular analysis of MHC genotypes revealed differences between X138 and RH/K34 using probes for class I and II (DQ\textalpha) genes.

Discussion

After an extended period of chronic asymptomatic infection, T cell lymphoproliferative disease with phenotypic manifestations strikingly reminiscent of cutaneous T cell lymphoma (especially mycosis fungoides and the Sezary syndrome [3, 6])
developed in a rabbit that survived a usually lethal dose of RH/K34. In addition to cutaneous T cell lymphoma, features were similar to smoldering ATLL, especially in instances in which monoclonality cannot always be demonstrated (e.g., early disease) [3, 7, 14]. Isolation of tissues from rabbit X138 bearing Sezary-like cells [6] yielded evidence of HTLV-I. These findings provide further credence for the possible role of HTLV-I in some types of cutaneous T cell lymphoma [7].

Evolution of monoclonality in lymphoproliferative disease may not be detected in early lesions by Southern blot analysis and may coexist with oligoclonal and polyclonal disease [14]. Our inability to detect the monoclonal integration pattern of the RH/K34 provirus and the polyclonality of T cells cultured from X138 were inconsistent with transplantation of RH/K34 as the cause of the cutaneous T lymphoproliferative disease in X138. Had RH/K34 persisted, the distinctive monoclonal proviral integration pattern would have been displayed by Southern blot. X138 and RH/K34 differ in their MHC genotypes, and such mismatches typically result in the subacute rejection of other closely related HTLV-I–infected T cell lines by New Zealand White rabbits [9] (unpublished data).

Evidence that the cutaneous T lymphoproliferative disease described was caused by HTLV-I infection was supported by the similarity of lesions to those associated with human HTLV-I infection, by the detection of retrovirus particles, and by the HTLV-I proteins and sequences in lesions of an inoculated animal. While spontaneous lymphosarcomas occur in rabbits (some associated with type C retrovirus particles in one rabbit colony [15]), no lymphomas have been detected in our colony for the past 4 years. Furthermore, no cases of cutaneous T cell lymphoma were reported in a recent review of laboratory rabbit diseases [16].

The amplified HTLV-I proviral sequence in skin of rabbit X138 likely represents the presence of infected T cells in epidermotropic lymphoid infiltrates. Despite serologic and molecular evidence of HTLV-I infection in rabbits without cutaneous
lymphoproliferative disease (rabbits X60 and X62, unpublished data), DNA samples extracted from their skin biopsies yielded no evidence of HTLV-I on repeated PCR analyses. This inability to amplify HTLV-I env in skin samples from infected rabbits lacking cutaneous lymphoproliferative disease supports our contention that detection of HTLV-I in X138 was not due to inadvertent amplification of infected cells circulating in cutaneous vasculature; rather, coupled with virus isolation, the finding indicates that the cutaneous T lymphoproliferative lesion was in all likelihood a direct consequence of HTLV-I infection. The possible involvement of a second agent, however, has not been excluded in experimental or spontaneous ATLL.

Cutaneous lymphoproliferative disease mimicking smoldering ATLL or other cutaneous lymphoma occurred in an HTLV-I–infected New Zealand White rabbit that escaped the lethal manifestations typically produced in an acute ATLL model [9, 13a]. This finding further demonstrates strong correlates between the biology of HTLV-I infection in New Zealand White rabbits and human disease. Further study is needed to determine events important in the initiation and progression of the experimental disease and to learn the role of host response. This information may yield new insight into mechanisms of HTLV-I pathogenesis in lymphoproliferative diseases.

Acknowledgments

We thank Robert W. Dunstan, Vanessa M. Hirsch, Mary Ann Robinson, and Tong Mao Zhao for scholarly contributions; Florence Bowers, Jan Endlich, Michele Fain, and Roy Teller for critical advice and technical assistance; and Andrea Barnes and Charles Y. Davis, Jr., for veterinary care and medical photography in the animal facility.

References