T-Cell Lymphoma Caused by Herpesvirus Saimiri C488 Independently of ie14/vsag, a Viral Gene with Superantigen Homology

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The immediate-early gene ie14/vsag of herpesvirus saimiri has homology with murine superantigens. We compared the pathogenesis of infection with either ie14/vsag deletion mutants or wild-type virus C488 in cottontop tamarin monkeys (Saguinus oedipus). Two weeks after infection, all animals developed acute T-cell lymphomas independently of the presence of the viral ie14/vsag gene.

Herpesvirus saimiri is a DNA tumor virus of New World primates and harbors a series of genes with homology to cellular counterparts. Among them is the immediate-early gene ie14/vsag, whose product resembles cellular and retroviral superantigens in mice (1, 12). As previously shown, the gene product IE14/Vsag is capable of binding to human major histocompatibility complex class II molecules and of stimulating the proliferation of primary human T cells, similar to superantigens (14). However, selectivity for human Vβ chains has not been observed (11, 13, 14).

Human T cells are transformed to stable growth by herpesvirus saimiri C488 (2). The transformed human cells carry the viral genome as nonintegrated episomes without virion production and express only a few viral genes, which are strongly induced after T-cell stimulation: the transformation-associated gene ORF13/IL17, whose product resembles cellular and retroviral superantigens (7, 8, 11). Although classified as a viral immediate-early gene, ie14/vsag was shown not to be required for virus replication (11, 12). Moreover, the deletion of ie14/vsag from virus strain C488 did not affect its capacity to transform simian or human T cells to stable growth in culture (11). To determine if this gene was essential for T-cell lymphoma development in vivo, we compared the pathogenesis in cottontop tamarins infected with wild-type virus carrying an intact ie14/vsag gene with that in cottontop tamarins infected with mutant viruses having this gene deleted.

The ie14/vsag deletion mutants of strain C488, 14-3.11 and 14-4.6, which lack most of the ie14/vsag coding sequence, were generated in independent experiments in order to minimize any bias from spontaneous mutations elsewhere in the herpesvirus genome (3, 5, 11). After approval by the Institutional Animal Care and Use Committee (Biomedical Research Centre, Rijswijk, The Netherlands) wild-type C488 and mutants 14-3.11 and 14-4.6 (10⁷ PFU in 1 ml of cell-free culture supernatant in Dulbecco modified Eagle medium) were individually injected into two naive, purpose-bred Saguinus oedipus monkeys. An intravenous infection at a high dose was done in order to exclude artifacts due to limiting conditions of infection. The animals were mature (400 to 500 g) and in good physical health. Animals R207 and R217 received wild-type virus, B222 and B240 got mutant 14-3.11, and B225 and R222 got mutant 14-4.6. On day 15 or 16 the animals were euthanatized when illness was evident. Autopsy was performed, followed by histopathological examination. Blood samples (1.5 ml each) were taken prior to infection, at weekly intervals, and before euthanasia. Virus isolation experiments were performed on all blood samples obtained after infection. Cells from peripheral blood and from autopsy samples were cultured without interleukin-2 in a mixture of half RPMI 1640 and half CG medium (Vitromex, Selters, Germany) and supplemented with fetal bovine serum (10%), glutamine, and gentamicin (6). Stably growing cells were analyzed by genomic PCR for ie14/vsag and for the neighboring gene ORF13/IL17 as a positive control (11). Standard flow cytometry analysis was performed with the cell lines and with fresh peripheral blood mononuclear cells (PBMC).

For this purpose, the following monoclonal antibodies, which are directed against human epitopes and cross-react with S. oedipus cells, were used: αCD2 (αLeu5b, SS; 2; Becton-Dickinson, Heidelberg, Germany), αCD3 (LT3, kindly provided by A. Filatov, Moscow, Russia), αCD4 (αLeu-3a, SK3; Becton-Dickinson or MT301; Dako, Hamburg, Germany), αCD8 (MT1014, kindly provided by E. Rieber, Dresden, Germany), αCD14 (αMY4, 32A1-2; Coulter, Krefeld, Germany), αCD20 (αLeu16, L27; Becton-Dickinson), αCD25 (2A3; Becton-Dickinson), αCD28 (αLeu28, L293; Becton-Dickinson), αCD29 (K20; Dako), αCD38 (αLeu17, HB-7; Becton-Dickinson), and αHLA-DR (L243; Becton-Dickinson).

All six animals developed evidence of disease rapidly and almost simultaneously at day 15 or 16 after infection, when they became apathetic and inappetent. In addition, animals R222, B222, B225, and R207 developed severe diarrhea. At necropsy, extranodal solid tumors were not apparent. However, severely enlarged mesenteric lymph nodes were observed in animals B222, B240, R207, R217, and R222. In the same animals, the kidneys had an irregular red-and-white-speckled appearance, suggesting lymphomatous infiltration of renal tissue. The adrenals of animals B222, B240, and R217 were...
hyperemic and hemorrhagic. Evidence for enteropathy was
detected at the necropsies of animals B222, B240, R207, R217,
and R222. Fresh PBMC were analyzed by whole-blood flow
cytometry. CD4⁺-cell counts, in particular the relative number
of memory-type CD4⁺CD29⁺ cells, increased moderately af-
after virus infection (Fig. 1). Neither double-staining reactions
with antibody pairs directed to CD14/CD4, CD20/HLA-DR,
CD2/HLA-DR, CD2/CD25, and CD2/CD38 nor the absolute
numbers of lymphocytes, T cells (CD2¹), and B cells (CD20⁺)
revealed further significant changes after infection. The abso-
lute numbers of granulocytes and monocytes decreased during
the course of infection in most animals; however, individual
variation was large. Peripheral cells of each blood sample and
cells from various organs (thymus, spleen, liver, and kidney and
axillar, mesenteric, and inguinal lymph nodes) were cultured in
order to expand the lymphoma cells and to isolate the virus. At
day 7 after infection, most PBMC samples yielded continu-
ously proliferating T-cell lines, whereas virus isolations re-
mained negative. Two weeks after infection, herpesvirus
saimiri was recovered from all animals by cocultivation of
PBMC with owl monkey kidney cells (6). Stably growing T-cell
cultures were regularly obtained from PBMC (day 14 and at
death) and from the thymus, spleen, and lymph nodes at au-
topsy. These cell lines expressed surface markers which are
typically found on activated T cells (Fig. 2). All T cells ex-
pressed CD8. The percentage of CD8⁺ cells coexpressing CD4
varied from 10 to 100%, depending on the cell line but was
independent of the virus genotype used for infection. The cell

FIG. 1. Flow cytometry values from fresh blood. The relative CD4⁺-cell
counts increased moderately in infected animals. This increase was most evident
for the relative numbers of memory-type CD4⁺CD29⁺ cells. For this analysis,
fresh PBMC were costained with monoclonal antibodies directed to CD4 and
CD29. dpi, days postinfection.

FIG. 2. Surface phenotype of tumor cell lines. The surface phenotype is
shown for the thymus derived T-cell lines R217T (wild type-infected), B240T
(deletion mutant 14-3.11), and R222T (deletion mutant 14-4.6). The histograms
show fluorescence intensity in logarithmic scale on the x axis and cell numbers in
linear scale on the y axis. MHC, major histocompatibility complex.

FIG. 3. Presence of viral DNA in tumor cell lines from the thymus and the
axillar lymph nodes. Virus DNA was demonstrated in ex vivo T-cell lines from all
animals. Whereas cells from wild type-infected animals were positive both for orf13/vil17 and ie14/
vsag, those from deletion mutant-infected monkeys contained orf13/vil17 but not ie14/vsag (Fig. 3). Histopathological eval-
uation confirmed the diagnosis of peripheral pleomorphic T-cell
lymphoma with follicular lysis and infiltration of multiple or-
Although tumor cells can be recovered from the peripheral blood, the cell-type distribution in PBMC seems rather normal. Besides the transformation-associated genes stpC and tip (4, 10), ie14/vsag was one of the leading candidates to contribute to transformation and pathogenesis (1, 12). However, as demonstrated in this study, deletion of this gene did not alter the course of disease, which emphasizes the assumed relevance of stpC/tip for T-cell leukemogenesis by herpesvirus saimiri. We conclude that ie14/vsag is dispensable for lytic virus replication, in vitro transformation, and pathogenicity if nonlimiting infection conditions are applied. It remains to be seen whether ie14/vsag plays a role in perinatal infection or apathogenic persistence in squirrel monkeys (Saimiri sciureus). A similar constellation seems relevant for early transmission of mouse mammary tumor virus. In this context, a Vβ-specific function of the superantigen homolog IE14/V Sag in the natural host remains to be elucidated.

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FIG. 4. T-cell lymphoma in S. oedipus. Formalin-fixed tissue sections were stained with hematoxylin and eosin. (a and b) Germinal centers with follicular B-cell areas. The photographs shown in panels a and c were obtained from C488 wild-type-infected animals, and the photographs shown in panels b and d are from animals infected with ie14/vsag deletion mutant viruses. Original magnification, ×40.