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# Lymphoproliferative disorders developing after transplantation and their relation to simian T-cell leukemia virus infection

**H. P. J. D. Stevens[1, 2], L. Holterman[2], A. G. M. Haaksma[2], M. Jonker[2], and J. L. Heeney[2]**

[1] Department of Plastic and Reconstructive Surgery, University Hospital Dijkzigt, Rotterdam,
[2] Department of Chronic and Infectious Diseases, Rijswijk, The Netherlands

**Abstract.** In this report the role of the HTLV-1-like simian T-cell leukemia virus (STLV) during the development of posttransplantation lymphoproliferative disorders (PTLPD) is described. To prevent rejection of an allogeneic transplant in 12 rhesus monkeys cyclosporin A (CyA), prednisone, and/or lymphocyte-specific monoclonal antibodies were used for immunosuppression. Seven monkeys died during the experiment between 22 and 179 days postoperatively. At autopsy in 4 monkeys PTLPD were found. In each case, STLV provirus was acquired during the experiment, either from the blood transfusions or allograft donors. Seroconversion of anti-STLV titers occurred in 3 monkeys. However, Southern blot analysis showed the presence of STLV provirus at the DNA level in all PTLP tissues. PTLPD morphology and phenotype varied significantly. In conclusion, for the first time the oncogenic potential of STLV is identified in a rhesus monkey transplantation model. Moreover, the importance of screening blood and organ donors for HTLV-1 must be emphasized.

**Key words:** Lymphoma – Immunosuppression – Transplantation – Simian/human T-cell leukemia virus (STLV, HTLV)

It has been reported that following clinical transplantation under cyclosporin A (CyA) and prednisone immunosuppression in man, lymphoproliferative disorders frequently develop [4]. Associations of the proliferative lymphoid lesions in transplant patients have been made with Epstein-Barr virus (EBV) [2] but, to our knowledge, never with HTLV-1. In primate species, HTLV-like viruses, designated simian T-cell leukemia viruses (STLV), occur with great frequency, especially in Old World primates. Studies revealed that more than 90% sequence homology exists between these viruses, which have a marked similarity in genomic structure and morphology [12]. A spectrum of lymphoproliferative diseases has been described in a variety of STLV-infected nonhuman primates [11]. In this study, in *Macaca mulatta,* the role of STLV during the development of posttransplantation lymphoproliferative disorders (PTLPD) was investigated.

*Offprint requests to:* J. L. Heeney, Department of Chronic and Infectious Diseases, ITRI-TNO, PO Box 5815, 2280 HV Rijswijk, The Netherlands

## Materials and methods

*Experimental design.* To prevent rejection of allografts in 12 mature, outbred rhesus monkeys *(Macaca mulatta)* who had received a successfully transplanted allogeneic radial side of the hand, strong immunosuppressive therapy was administered [6]. In this respect, a daily maintenance dose of 25 mg/kg CyA was given, starting 1 day preoperatively. In addition, a high dose of steroids (Di-Adreson-$F_{aquosum}$ = DAF, 12 mg/kg daily) was administered for the first 3 postoperative days and the dosage tapered slowly until a maintenance dose of 1 mg/kg daily was reached after 12 days.

If rejection occurred and could be confirmed histologically, half of the affected allograft recipients were treated by increasing DAF to 12 mg/kg daily, followed by tapering of the DAF dose as described above. In the other monkeys, rejection was treated with a combination of 7 monoclonal antibodies (MAbs), administered as an (iv) bolus injection for a period of 10 days. MAbs were specific for CD3+, CD4+, CD8+, and MHC class II-DR positive antigens and crossreactive with rhesus monkey lymphocytes. This cocktail of MAbs has a strong immunosuppressive potential [8].

Additionally, the effect of preoperative fully mismatched blood transfusions was tested. Six animals received three third party blood transfusions, consisting of 20 ml of fresh whole citrated blood from random donors, administered at biweekly intervals before transplantation. A combination of the aforementioned immunosuppressive treatments resulted in four different treatment groups I-IV with three monkeys per group (see Table 1).

Following successful surgery, 7 animals became terminally ill during the posttransplantion period. They were euthanized, and a complete autopsy was performed.

*Histology and immunohistochemistry.* The presence or absence of PTLP disorders was confirmed histologically and classified morphologically according to the NCI working formulation. For this purpose, tissue sections harvested at autopsy were fixed in buffered formalin and processed routinely for histology on hematoxylin and

**Table 1.** Allograft recipients that died after transplantation of the radial side of the hand (rhesus monkey): anti-STLV titers in serum, immunosuppressive therapy administered, and cause of death

| | Monkey | Anti-STLV titer | Protocol | Duration of basic immunosuppressive therapy (days)[a] | Duration of antirejection therapy (days) | Cause of death |
|---|---|---|---|---|---|---|
| I | 2799 | + | MAbs | 79 | 1 × 1[b] | shock |
| | 4023 | sc | & | 121 | 2 × 10 | sepsis/PTLP disorder |
| | 2988 | sc | transf. | 22 | 1 × 7[b] | multicentric PTLP disorder |
| II | 3439 | lt | MAbs, | 97 | 2 × 20 | follicle center cell PTLP disorder |
| | 3308 | – | no transf. | 179 | – | multicentric PTLP disorder |
| III | 2I | – | DAF & | 29 | 1 × 8[b] | sepsis |
| | 3310 | nt | transf. | 85 | – | sepsis |
| IV | | | DAF, no transf. | | | |

[a] Daily maintenance doses of immunosuppression consisted of 25 mg/kg CyA and 1 mg/kg DAF
[b] The proposed duration of antirejection therapy (10 days for MAb therapy and 12 days for DAF therapy) was not completed
STLV, simian T-cell leukemia virus; PTLP disorder, posttransplantation lymphoproliferative disorder; cs, seroconversion; lt, low titer; nt, not tested; MAbs (in groups I & II), antirejection therapy consisted of a 10-day course of a combination of 7 monoclonal antibodies; transf. (in groups I & III), 3 third party blood transfusions were given to the recipient, preoperatively; DAF (in groups III & IV), antirejection therapy consisting of an increase in steroid treatment

azofloxine (H/A) stained sections. Parallel biopsy material was snap-frozen in liquid nitrogen-chilled isopentane and used for immunohistochemical studies. This technique and the semiquantitative method for scoring distribution and intensity of staining have been described previously [7]. A selection of MAbs was used to demonstrate expression of the following antigens: CD2, CD4, CD8, *k*-light chain, *l*-light chain, MHC class II antigens, and a proliferation-associated nuclear antigen. In control incubations, the primary antibody was omitted.

*Serological assessment of HTLV-1-related virus infections.* Serum samples to determine titers of STLV-specific antibodies were obtained (1) prior to preoperative third party blood transfusions, (2) following transfusions but before transplantation, and (3) several months posttransplantation. For the assessment of STLV-specific antibodies a commercially obtained HTLV-1 enzyme-linked immunoenzyme assay (ELISA; Du Pont de Nemours International S.A., Geneva) was used which had been demonstrated to be cross-reactive with STLV (J.L. Heeney, personal communication). To confirm further these ELISA results and the relatedness of these viruses to HTLV-1, various serum samples found to be ELISA-positive were tested for antigenic similarity on HTLV-1 Western blots and the results compared with serum from HTLV-1-infected patients.

*Assessment of HTLV-1-like provirus infection at the DNA level.* Remaining PTLP tissue was used to prepare DNA for Southern blot analysis. Probing of the DNA was done with $_{32}$P-labeled, 9 kb full length (minus the long termnal repeats) pCS-HTLV-1 clone (D. Derse, NCL, Frederick, Md.) or partial fragments consisting of the envelope or *tax* regions.

*Statistical analysis.* When appropriate, Fisher's exact-test, two sample *t*-test and log-rank test were performed for hypothesis testing. Differences were considered significant if $P < 0.05$.

## Results

### *Findings at necropsy*

Allograft survival times after transplantation of the radial side of the hand ranged from 21 to 179 days (Table 1). Detailed information on technical, immunological, and functional aspects of these experiments is described elsewhere [5, 6]. Unfortunately, 7 monkeys died during the experimental period.

In treatment group I (pretransplant blood transfusions and with MAbs antirejection therapy if indicated) 3 monkeys died, monkey # 2799 due to an irreversible shock directly after the first administration of MAbs (79 days postoperatively); monkey # 4023 due to sepsis, 10 days after onset of the second episode of antirejection treatment. In this monkey at autopsy, widespread lymphoproliferation was observed. Monkey # 2988 died due to multicentric PTLP disorder 22 days after operation, its allograft being fully rejected despite 7 days of MAbs administration.

In group II (no pretransplant blood transfusions but with MAbs antirejection therapy if indicated), monkey # 3439 died due to a malignant follicle center cell PTLP disorder, 22 days after onset of the second episode of rejection treatment with MAbs. Monkey # 3308 died before rejection had occurred from a multicentric PTLP disorder 179 days postoperatively.

In group III (pretransplant blood transfusions and DAF antirejection treatment if indicated) monkey # 2I died due to sepsis 29 days postoperatively, and monkey # 3310 suffered from sepsis 85 days following transplantation.

In group IV (no pretransplant blood transfusions and DAF antirejection therapy if indicated), no monkeys died during experiment. However, it should be noted that allograft survival times in this group were shorter than in the other treatment groups, and thus they received immunosuppression therapy for a shorter period of time.

In the 4 monkeys which were found to have PTLP disorders, all lymphoproliferation was multicentric with variable morphologic characteristics ranging from a lymphoreticular to follicular center cell morphology.

### *Influence of immunosuppressive treatment and case history*

The addition of MAbs therapy to baseline immunosuppression did not have a significant effect on the occurrence of death in general ($P > 0.05$, Fisher's exact test) [5].

Though the population treated was small, an enhanced predisposition to PTLP disorder development ($P = 0.03$, one-sided Fisher's exact test) was noted in monkeys which received MAb antirejection therapy, possibly as a consequency of additional, potent immunosuppression in addition to baseline therapy. Other factors in the case history of each animal like age, sex, CyA therapy whole blood trough levels, kidney donorship, and experimental low-dose radiation of the testis were examined for a possible correlation with PTLP disorder development but were found to be nonsignificant ($P > 0.05$, Fisher's exact test) [5].

*Relevance of anti-STLV titers to PTLP disorder development*

All animals were tested for the presence of anti-STLV antibodies before, during, and after the experiment. Four animals were seropositive before the experiment. Four animals acquired anti-STLV antibodies in the course of the experiment (Table 1). In each case of PTLP disorder development, the STLV provirus was acquired during the experimental period. This was clearly evident serologically in two of these animals, and a third which developed a low titer shortly before death.

Serologically, virus transmission could be traced to have come from STLV-positive monkeys, either by preoperative blood transfusions or by allograft donation through the demonstration of seroconversion for STLV titers in transplant recipients.

Animals which retrospectively had serologic evidence of STLV infection prior to the experiment ($n = 4$) did not develop PTLP disorders, suggesting that active STLV infection during immunosuppressive therapy was an important factor in PTLP disorder development.

*Immunohistochemical staining of lymphoproliferative tissue*

Frozen sections of lesions collected at necropsy were assessed for phenotypic characteristics. In each case the staining pattern varied and was frequently complicated by a mixed population of residual benign leukocytes. All cases stained strongly positively for the MHC class-II DR marker, and a small mixed population of light-chain-positive cells were frequently seen in each case. In the frozen tissues available to us to study, there were not enough sections of homogenous lymphoproliferative tissue to make a conclusive statement of the PTLP disorder phenotype.

*Evidence of STLV infection at the DNA level*

Southern blot analysis revealed that in all 4 monkeys with a PTLP disorder, STLV provirus was present. A common 5.4-kb band in the lymphoid tissue was present which hybridized with the full length HTLV-1 probe. Using partial HTLV-1 fragments as probes, it was confirmed that under conditions of high stringency the rhesus STLV had envelope, polymerase, and *gag* regions which were highly homologous with HTLV-1. We were not able to demonstrate consistently hybridization of the Tax region of HTLV-1 with rhesus tumor DNA samples, suggesting that differences in this region exist at the molecular level.

## Discussion

The incidence of lymphoma in normal healthy individuals infected with HTLV-1 is low. Less than 1 % of HTLV-1-infected people are believed to develop cancer under normal conditons over an entire lifespan, although the virus transforms cells in vitro [3]. In this transplantation study, however, for the first time, the oncogenic potential of a HTLV-1-like virus in rhesus macaques is identified. Furthermore, several important problems are distinguished concerning the selection of transplantation donors and recipients and the immunosuppressive therapy used.

Four of 7 animals which died following allogeneic transplantation of the radial side of the hand developed lymphoproliferative disorders which contained STLV provirus at the DNA level. All 4 monkeys were seronegative before transplantation. In 2 of the 4 cases, it could be demonstrated serologically that STLV was acquired from the blood transfusions or organ donor. In another monkey, which did not receive any preoperative blood transfusion the graft came from a seronegative donor. These data suggest that infection was acquired during the period of intensive posttransplantation immunosuppressive therapy. Moreover, infection might also be possible via other routes than blood or an allograft. In this respect, it is interesting to note that monkeys in this study were housed 4 by 4 in separate cages in the same room during experiment.

If PTLP disorder development in this species infected with STLV is analogous to HTLV-1-associated lymphomas in man, then one would expect to identify a mature CD4 + T-cell phenotype [10]. However, the morphologic and phenotypic characteristics of these PTLP disorders in *Macaca mulatta* varied significantly. Results in this study therefore suggest that under these circumstances in rhesus monkeys STLV causes PTLP disorders of a more diverse cell type.

It has been reported that primary EBV infection carries a higher risk than reactivated infection in the development of PTLP disorders [2], but also anti-CD3 in itself does seem to precipitate lymphoid tumor development [9]. In our study, the finding that MAbs antirejection therapy showed an enhanced predisposition to PTLP disorder development ($P = 0.03$, one-sided Fisher's exact test) coincided with the fact that in each case of PTLP disorder development, STLV provirus was acquired during the experiment. This might indicate that two different mechanisms increased the chance of lymphoid proliferation, resulting in the variable morphologic characteristics of these disorders.

Furthermore, it should be noted that PTLP disorders in this study arose during high dose cyclosporin A treatment, which by itself can cause or permit tumor development in strongly immunosuppressive protocols [4]. Recently, it has been described that cyclosporin A acts

through nuclear protiens involved in T-cell activation [1], some of which interact with the HTLV-1 *tax* gene, suggesting a mechanism by which T-cell cancer can develop.

This relationship between CyA and MAb (anti-CD3) therapy, HTLV-1 (STLV-1) infection, and PTLP disorder development in rhesus monkeys indicates the possible clinical risk of treating HTLV-1-infected patients with an immunosuppressive protocol which includes these drugs. Based on the findings in this transplantation model in *Macaca mulata,* the importance of screening blood or organ donors for HTLV-1 must be emphasized.

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