Modification of the In Vitro Replication of the Human Immunodeficiency Virus HIV-1 by TPSg, a Polysaccharide Fraction Isolated from the Cupressaceae *Thuja occidentalis* L. (Arborvitae)*, **

S. H. Gohla [1], R. A. Zeman [5], M. Bögel [2], E. Jurkiewicz [3], S. Schrum [1], H. D. Haubeck [4], H. Schmitz [2], G. Hunsmann [3], and R. D. Neth [1]

Introduction

The acquired immune deficiency syndrome (AIDS) is caused by an infection with the human immunodeficiency virus (HIV-1) [2–8]. CD4-positive lymphocytes were shown to be one major target in HIV-1 infections [9–10]. Apart of $CD4^+$ cell depletion, the functional impairment of the T-cell system also plays an important role in the progress of this disease [11, 12, 13].

Two distinct approaches to controlling HIV-1 infections have been explored so far, specifically, inhibition of the reverse transcriptase and inhibition of HIV-1 replication.

For the first approach, inhibition of the virus replication, 3′-azido-3′-deoxythymidine (AZT) [15] and its nucleoside analogues [16, 17], suramin and its derivatives [18], phosphonoformic acid [19], and antimoniotungstate [20] have been used. Inhibition of virus replication was demonstrated on the other hand using interferon-α [21, 22], AL721 [23], D-penicillamine [24], amphotericin analogues [25], dextrane sulfate [36], chondroitine sulfate [36, 42], Avarone [27], Avarol [27], and synthetic oligonucleotides [26].

The need to obtain an effective principle for the treatment of AIDS prompted the search for selective and nontoxic anti-HIV-1 agents even in medicinal plants. Some extracts with anti-HIV-1 properties have been isolated from medicinal plants of Chinese folk remedies [46], for instance, *Altherantera philoxeroides* [44], *Viola yedoensis* [45], and the chemically partially defined prunellin isolated from *Prunella vulgaris* [43]. Most of these extracts and partially purified substances have shown in vitro anti-HIV-1 properties accompanied by some cytotoxic activities [43–46]. Lai et al. have reported a dose-dependent modification of the viral replication of HIV-1-infected CR10, CEM, and U937 cells by two defined extracts (PC6 and PC7) from the Japanese white pine (*Pinus parvifloria* Sieb. et Zucc.) [49]. In previous studies, extracts from *Thuja occidentalis* L. (Arborvitae), another plant in the cedar/pine family, were shown to be in vitro inhibitors of plant pathogenic viruses and human herpes simplex viruses (HSV-1 strain) [34, 35].

In the present paper we are dealing with a new substance, the g fraction of

[1] 2nd Medical Clinic, Department of Clinical Chemistry, UKE, Martinistraße 52, 2000 Hamburg 20, FRG.
[2] Bernhard-Nocht Institute, Department of Virology, Bernhard-Nocht-Straße 74, 2000 Hamburg 4, FRG.
[3] DPZ, German Primate Center, Department of Virology and Immunology, Kellnerweg 4, 3400 Göttingen, FRG.
[4] Technical University of Aachen, Department of Clinical Chemistry and Pathobiochemistry, Pauwelsstraße 30, 5100 Aachen, FRG.
[5] Laboratory of Tumor Cell Biology, National Cancer Institute (NCI), National Institutes of Health and Human Services (NIH), Bethesda, Maryland, USA.
* Parts of this study have been supported by the Karl und Veronika Carstens Stiftung im Stifterverband für die Deutschen Wissenschaften.
** Awarded with the "Henry Kaplan Award 1990 (Cell Biological Session)".

Haematology and Blood Transfusion Vol. 35
Modern Trends in Human Leukemia IX
R. Neth et al. (Eds.)

thujapolysaccharides (TPSg), and its ability to modify HIV-1 replication in both human MT-2 and MT-4 cells as measured by determination of reverse transcriptase (RT) activity, cell growth (both MT-4 cell system), and the expression of HIV-1-specific proteins by indirect immunofluorescence (MT-2 cell system).

Materials and Methods

Virus and Cell Lines

The HIV-1 strain $HTLV_{IIIb}$ used for the MT-2 experiments was obtained from culture supernatants of virus-producing H9 cells, as previously described [4].

MT-2 cells were maintained in RPMI 1640 (Gibco, Eggenstein, FRG) containing 15% fetal calf serum. MT-2 is a HTLV-1-preinfected human T-cell leukemia line and has been shown to be highly susceptible to infections with HIV-1 [28, 47].

MT-2 cells have been used as target cell lines for in vitro HIV-1 infection experiments using indirect immunofluorescence assays [39].

H9 cells used as the HIV-1 source for the MT-2 experiments were also maintained in RPMI 1640 (Gibco, Eggenstein, FRG) containing 15% fetal calf serum. This cell line was a kind gift from M. Popovic (NCI, Bethesda, Maryland, USA).

MT-4 cells were kept in Click-RPMI medium (Biochrom, Berlin, FRG) containing 10% (v/v) complement-inactivated fetal bovine serum (Seromed, Berlin, FRG) and antibiotics.

MT-4 cells are highly susceptible to in vitro HIV-1 infections [39], too. For the in vitro infection experiments with MT-4 cells, the $HTLV_{IIIb}$ strain of HIV-1 was used. HIV-1 has been generated on Jurkat cells as described in detail elsewhere [41]. Jurkat cells were also grown in RPMI 1640 medium (Gibco, Karlsruhe, FRG) with the supplements described above.

Virus Titration

For virus titration on MT-2 cells, cell-free supernatants were harvested from HIV-1-infected H9 cells. The virus titration was performed by indirect immunofluorescence. The quantitative determination of the infectious capability of the HIV-1 stocks was performed according to the method described by Kaerber et al. [31]. The HIV-1 preparations for the MT-2 experiments were shown to have a titer of 1×10^7 $TCID_{50}$/ml. In the MT-4 system, a final infectious activity of 100 $TCID_{50}$ for each well was used.

Indirect Immunofluorescence

For immunofluorescence experiments, both freshly HIV-1-infected and noninfected MT-2 cells were used and incubated for 12 days at 37 °C. For preparing the cell smears, HIV-1-infected and noninfected MT-2 cells were contrifuged for 10 min at 250 g. The supernatants were removed and the sediments resuspended in phosphate-buffered saline (PBS). Cell smears were performed on 10-well multitest slides (Flow Lab., Meckenheim, FRG). The slides are air dried and fixed for 10 min in acetone at −20 °C. A standardized HIV-1-positive human serum was used as reagent.

Cell smears of HIV-1-infected and noninfected MT-2 cells were incubated for 60 min in a moist chamber at 37 °C with titrated serum of an AIDS patient (25 µl/well; dilution 1:20) [29, 30]. HIV-1-positive cells were visualized after incubation with FITC-conjugated goat anti-human immunoglobulin G (AHS Deutschland, Bereich Merz and Dade, Munich, FRG) for 30 min (25 µl/well; dilution 1:200).

As negative controls, sera of noninfected human individuals were used. The specific reaction was determined by fluorescence microscopical evaluation.

Determination of RT activity

Uninfected MT-4 cells or MT-4 cells infected with HIV-1 were treated with

various concentrations of TPSg and incubated for 5 days under standard conditions. For the RT inhibition assay, HIV-1 was harvested from infected Jurkat cells by centrifugation. The virus was then suspended in PBS at pH 7.2 and mixed with the same amount of ultrapure glycerol (Serva, Heidelberg, FRG). Different final concentrations of TPSg were examined in 50 m*M* Tris-HCl pH 7.8, 5 m*M* dithiothreitol (DTT), 25 m*M* Mg^{2+}, 30 m*M* KCl, 6% Triton X-100, 1 µg polyrC:oligodG, 9 µ*M* dGTP, 1 µCi [^{32}P]dGTP and lysed HIV-1. The RT assay was performed according to the procedure described previously [40, 42].

The influence of TPSg on virus production in infected MT-4 cells was monitored by RT activity in culture supernatants. The virus was prepared from the supernatants by centrifugation as described above and the RT assay was performed as shown.

[^{3}H]Thymidine Incorporation

[^{3}H]Thymidine incorporation experiments were performed according to standard procedures to measure HIV-1-specific cytopathic effects on MT-4 cells [39]. The MT-4 assay was performed in 96-well microtiter plates as described previously [42]. 3×10^4 MT-4 cells/well were incubated with TPSg at 625 µg/ml, 62.5 µg/ml, 6.25 µg/ml and 625 ng/ml final concentrations, with or without HIV-1.

The concentration of the infectious particles used was 100 $TCID_{50}$ for each well. Fresh Click-RPMI medium was added to each well 3 days after set-up. 5 days after infection, 0.1 µCi [^{3}H]thymidine (Amersham-Buchler, Brunswick, FRG; specific activity 185 GBq/mmol) was added to the cultures. The cells were harvested 20 h later on glass fiber filters (Whatman GFC, UK) using a Scatron cell harvester and dried. After addition of scintillation cocktail (PPO, POPOP, and toluene; Roth, Karlsruhe FRG) filters were counted in a β-liquid scintillation counter.

The results were expressed as the arithmetic mean in counts per minutes of triplicate determinations.

As an alternative to the determination of the cellular DNA synthesis in the MT-4 assay, the cell growth was measured on day 5 after infection. Cell viability was assayed microscopically in a hematocytometer by trypan blue exclusion experiments and the RT activity was measured in the supernatant of the cultures.

Preparation of TPSg

Thujapolysaccharides, g-fraction (TPSg), from the Cupressaceae *Thuja occidentalis* L. (Arborvitae) was prepared as described in detail elsewhere (EPO 315182). TPSg was stored up to use lyophilized at −20 °C. TPSg was reconstituted in the appropriate cell culture media and was sterilized directly before use using 0.2 µm filter systems (Sartorius, FRG).

Results

The anti-HIV-1 activity and cytotoxicity of the polysaccharide fraction TPSg was examined in MT-2 and MT-4 cell culture systems. The ability of TPSg to inhibit the HIV-1-specific RT was also examined. Finally, the 50% inhibitory concentration (IC_{50}) of TPSg on MT-4 cells was determined.

Protection of HIV-1-Dependent Cytopathic Effects by TPSg

TPSg inhibited HIV-1-dependent cell death at final concentrations of 625 µg/ml (Fig. 1). At this concentration TPSg was shown to be completely nontoxic for MT-4 cells, which had not been infected with HIV-1 (Figs. 1, 2).

This result was confirmed by comparing the cell growth of TPSg-treated infected and noninfected MT-4 cells (Fig. 2). These experiments were performed in triplicate and repeated three times.

Inhibition of HIV-1 Expression by TPSg

HIV-1-specific viral antigen expression was measured by indirect immuno-

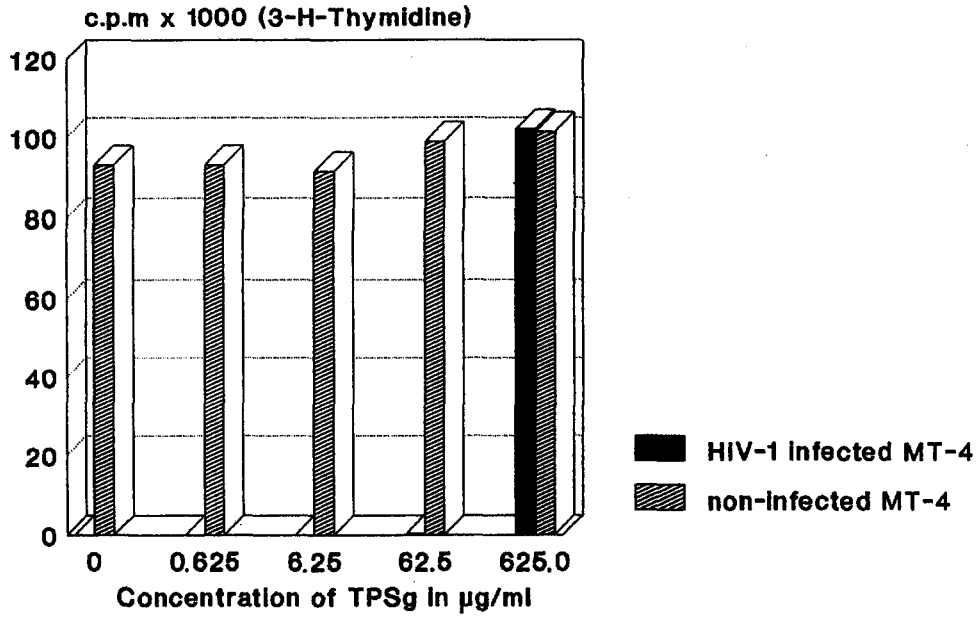

Fig. 1. Anti-HIV-1 activity of TPSg in the MT-4 cell assay. The anti-HIV-1 activity of various concentrations of TPSg is expressed as the [^{3}H]thymidine incorporation into HIV-1-infected and noninfected MT-4 cells (median of three experiments). The cells were treated with final concentrations of 625 ng/ml to 625 µg/ml

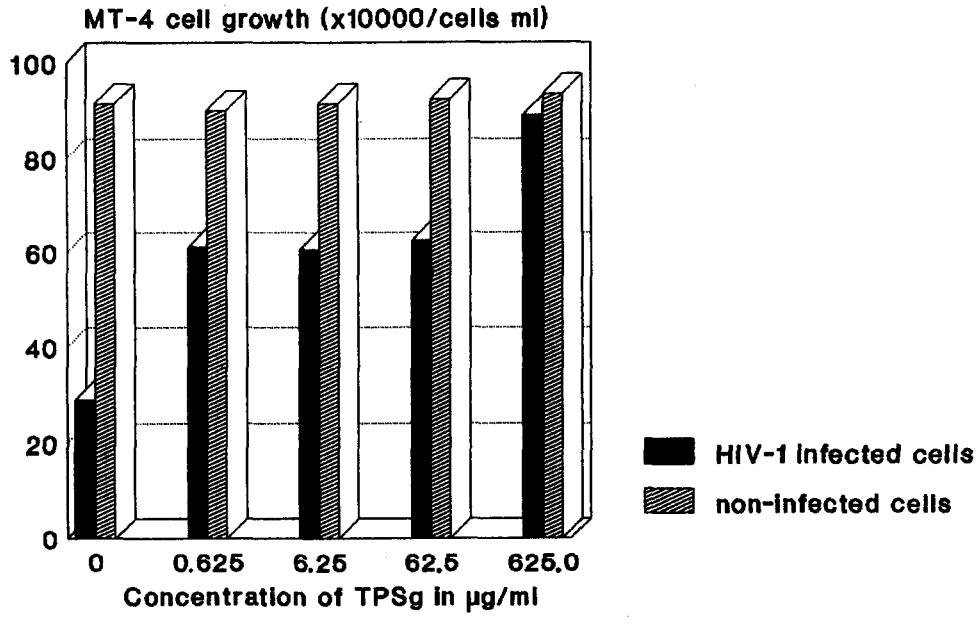

Fig. 2. Effect of TPSg on growth of MT-4 cells. The numbers of noninfected and HIV-1-infected cells were examined (media of three experiments). The cells were treated with TPSg at final concentrations of 625 ng/ml to 625 µg/ml

fluorescence. The inhibitory effect of TPSg was tested on freshly HIV-1-infected MT-2 cells.

TPSg was shown to inhibit HIV-1-specific antigen expression on freshly infected MT-2 cells in a dose-dependent manner (Figs. 3, 4). TPSg did not alter viral antigen expression at a concentration of 0.625 µg/ml (99.6% ± 0.5%). A significant reduction in HIV-1 antigens measured by immunofluorescence was observed at a concentration of 6.25 µg/ml 69.8% ± 10.8% of HIV-1 infected MT-2 cells expressed HIV-1-specific antigens).

Only 0.4% of all HIV-1-infected MT-2 cells counted (200 cells/slide) were shown

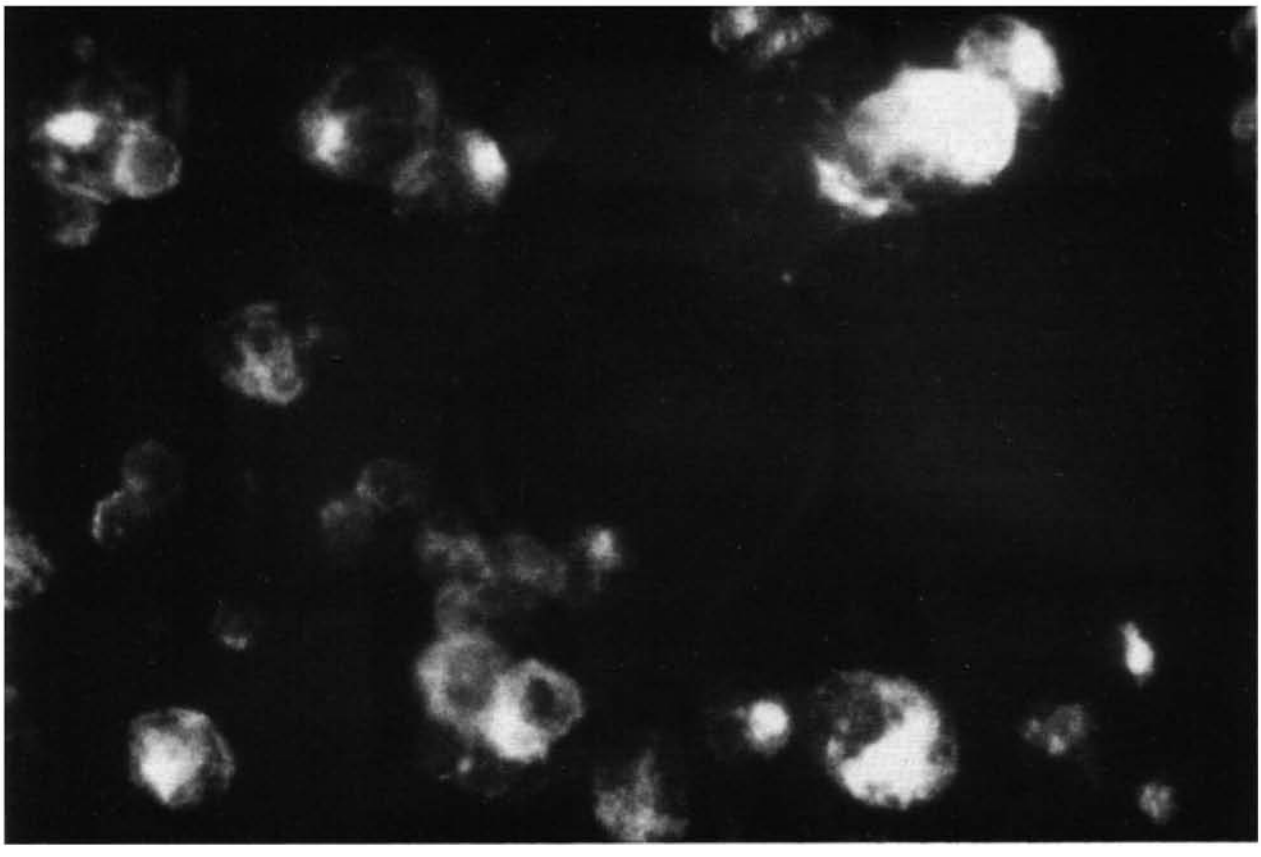

Fig. 3. Indirect immunofluorescence of freshly HIV-1-infected MT-2 cells. The cells were prepared as described in "Material and Methods." They were labelled with an anti-serum against HIV-1 and FITC-conjugated goat anti-human IgG. This micrograph shows the non-TPSg-treated freshly HIV-1-infected MT-2 cells after 5 days of incubation. × 500

to express HIV-1-specific antigens at final concentrations of 62.5 μg/ml, and an inhibition of 99.94% ± 0.08% of HIV-1 expression was measured at the final TPSg concentrations of 625 μg/ml.

Inhibition of RT Activity by TPSg

As an additional approach, HIV-1 replication was determined by measuring RT activity in the supernatants of HIV-1-infected MT-4 cells 5 days after infection. In uninfected MT-4 cells, no RT activity was detected in the culture medium after an incubation period of 5 days. In contrast to HIV-1-infected MT-4 cells not treated with TPSg, no RT-dependent dGMP incorporation was found in supernatants of infected MT-4 cells treated with final concentrations of TPSg of up to 62.5 μg/ml (Fig. 5).

In addition, the inhibition of RT activity was measured with disrupted HIV-1. TPSg was found to be active against the enzyme (Fig. 6) with a IC_{50} of 300 μg/ml.

Discussion

Several authors have reported antiretroviral activities of plant extracts, for example, extracts of *Prunella vulgaris* [43], *Alternanthera philoxeroides* [44], *Viola yedoensis* [45], *Gerardia savaglia* [49] and some Chinese medicinal herbs [46, 50, 51]. Lai et al. have reported a dose-dependent modification of the viral replication of HIV-1-infected CR10, CEM, and U937 cells by two defined extracts (PC6 and PC7) of the Japanese white pine (*Pinus parvifloria* Sieb. et Zucc.), a plant of the pine family [49]. Previously, extracts of *Thuja occidentalis* L., another plant belonging to the cedar/pine family, were shown to inhibit the cytolytic activity of herpes simplex virus

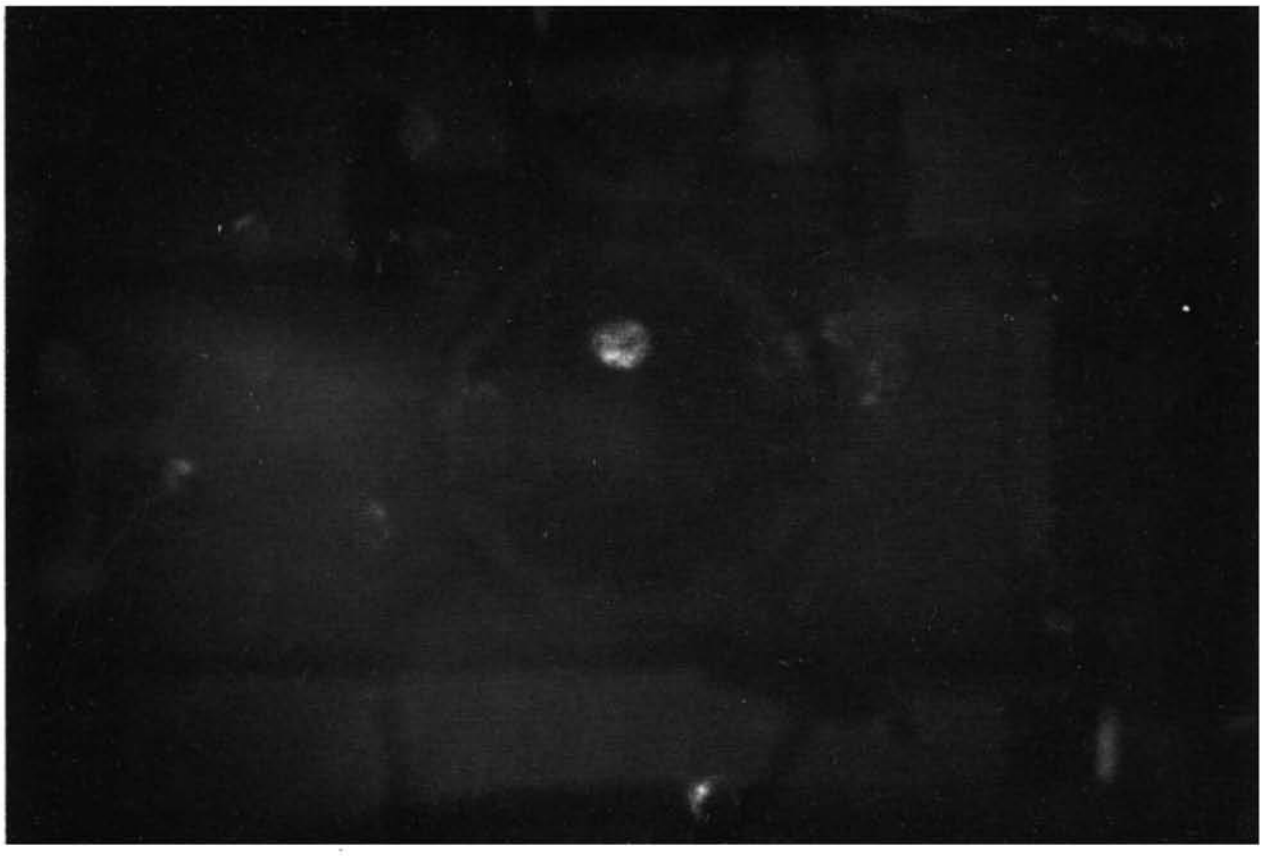

Fig. 4. Indirect immunofluorescence of freshly HIV-1-infected MT-2 cells treated with TPSg. The cells were prepared as described in "Material and Methods." They were labelled with an antiserum against HIV-1 and FITC-conjugated goat anti-human IgG. This micrograph shows freshly HIV-1-infected MT-2 cells treated with TPSg 625 µg/ml after 5 days of incubation. × 500

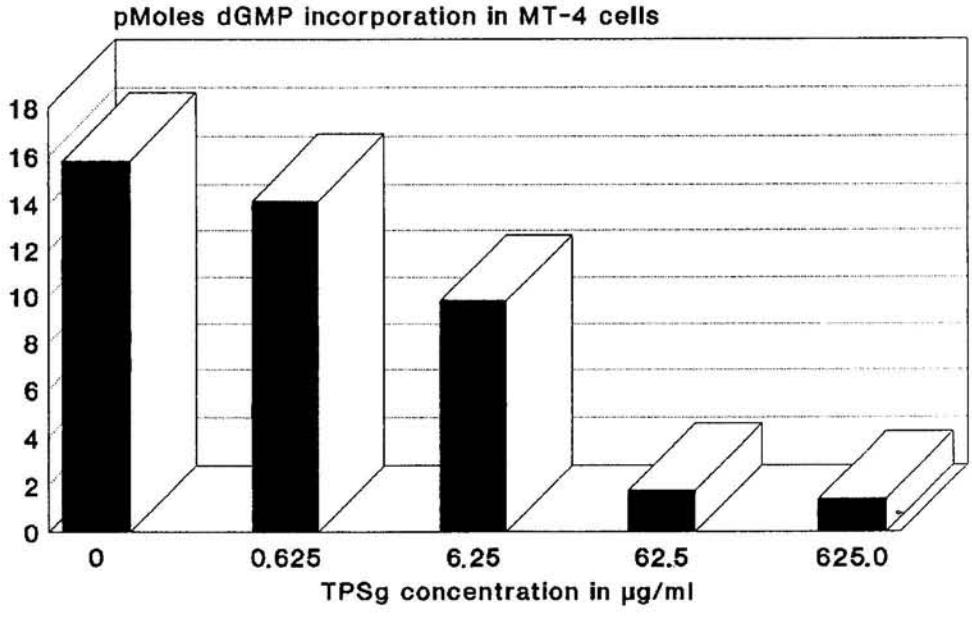

Fig. 5. RT activity in the supernatant of HIV-1-infected MT-4 cells treated with different concentrations of TPSg (median of three experiments). The cells were treated with final concentrations of TPSg of 625 ng/ml to 625 µg/ml. The RT activity is expressed in picomoles dGMP incorporated into DNA

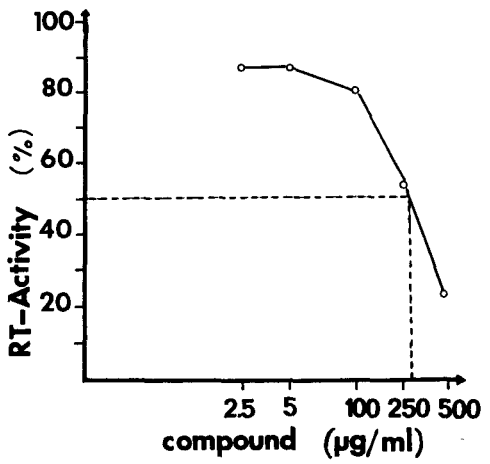

Fig. 6. Inhibition of the RT activity of an HIV-1 lysate expressed in %. The 50% inhibitory dose of TPSg (ID_{50}) is extrapolated from the curve

type 1 and some plant pathogenic viruses in vitro [34, 35].

TPSg, a high molecular weigth polysaccharide fraction isolated from *Thuja occidentalis*, was shown to be a compound with "immunomodulatory" properties. This compound was demonstrated to induce the proliferation of T-cells (CD^{4+}) of the human peripheral blood [1, 37, 48]. Furthermore, TPSg was shown to induce a different pattern of cytokines such as interleukin-1, interleukin-2, and interferon-γ [32].

In the BALB/c system, TPSg was found to cause a modification in terms of upregulation of natural killer cell activity against YAC-1 target cells [43].

These findings indicated possible antiviral properties of this compound. Hence, in this preliminary study, we have evaluated the antiretroviral potential of this compound.

TPSg was found to inhibit the HIV-1-dependent cell death of HIV-1-infected MT-4 cells at concentrations of 625 µg/ml. Additionally, it was shown to block the expression of HIV-1-specific proteins in freshly HIV-1-infected MT-2 cells in a dose-dependent manner, as judged by a 99.94% (99.6%) inhibition of the HIV-1-mediated specific immunofluorescences at a final concentrations of 625 µg/ml (62.5 µg/ml).

TPSg completely blocks HIV-1 release into the culture supernatant at concentrations up to 62.5 µg/ml, as demonstrated by the lack of RT activity in the supernatants of HIV-1-infected MT-4 cells. Furthermore, TPSg blocks the RT of disrupted virus particles with an IC_{50} of 300 µg/ml.

In the present paper, TPSg was demonstrated to be a compound with an inhibitory effect on both HIV-1 entry and HIV-1 absorption in both MT-2 and MT-4 cells. Even at high concentrations, it was shown to be nontoxic for MT-4 (Fig. 2) and MT-2 (data not shown) cells. Furthermore, it was demonstrated to be nontoxic for primary human leukocyte cultures (PBL), even at high concentrations [33].

In comparison with most of the plant extracts described above, TPSg therefore shows promising antiviral and immunomodulating properties.

Since TPSg is only a partially purified natural product, isolation of the active principle(s) is required. This work is in progress. First hints in this direction were given by Hans et al. [38], who described the monosaccharide composition of sprouts and wood of the Arborvitae.

Future investigations concerning this compound must rule out the possibility of its inducing autoimmune diseases and must show a lack of toxicity in vivo and mutagenicity in vitro. The present study might be a hint to further and more detailed investigations of the anti-HIV-1 properties of this compound. Whether TPSg might be of use in the therapy of primary and secondary immune deficiencies must be elucidated in further and more detailed investigations.

The present study exemplifies the necessity of synergy of pharmacognostic research with molecular biology, clinical research, and immunology, to obtain new substances with significant immunomodulatory and antiviral properties.

References

1. Gohla SH, Haubeck HD, Neth RD (1988) Mitogenic activity of high molecular polysaccharide fractions isolated from the cupressaceae *Thuja occidental* L. I. Macrophage-dependent induction of CD-4 positive T-helper (Th^+) lymphocytes. Leukemia 8:528
2. Sarin PS, Gallo RC (1986) The involvement of human T lymphotropic retroviruses in T cell leukemia and immune deficiency. Cancer Ref 1:1
3. Wong-Staal F, Gallo RC (1985) The family of human T-lymphotropic leukemia viruses: HTLV-1 as the cause of adult T-cell leukemia and HTLV-III as the cause of aquired immune deficiency syndrome. Blood 65:253
4. Popovic M, Sarngadharan MG, Read E et al (1984) Detection, isolation and continuous production of cytopathic retroviruses from patients with AIDS and pre AIDS. Science 224:497
5. Gallo RC, Salahuddin SZ, Popovic M et al (1984) Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and risk for AIDS. Science 224:500
6. Schüpbach J, Popovic M, Gilden RV et al (1984) Serological analysis of a subgroup of human T-lymphotropic retroviruses (HTLV-III) associated with AIDS. Science 224:503
7. Sarngadharan MG, Popovic M, Gilden RV et al (1984) Anitbodies reactive with human T-lymphotropic retroviruses (HTLV-III) in serum of patients with AIDS. Science 224:506
8. Barré-Sinoussi F, Chermann JC, Rey F et al (1983) Isolation of a T-lymphotropic retrovirus from a patient at risk for AIDS. Science 220:868
9. Klatzman D, Barré-Sinoussi F, Nugere MT et al (1984) Selective tropism of lymphadenopathy-associated virus (LAV) for helper/inducer lymphocytes. Science 225:59
10. Arndt R, Keeser D (1989) Impairment of the immunological system in HIV infection. Z Hautkr 64:353
11. Klatzman D, Gluckmann JC et al (1986) HIV infections: facts and hypotheses. Immunol today 7:291
12. Fahey IL, Khaitow D, Klatzmann D et al (1987) Immunology of HIV infection and AIDS: memorandum from a WHO/HUIS Meeting. Bull WHO 65:453
13. Pinching AJ (1986) The immunology of AIDS and HIV infection. Clin Immunol Allergy 6:645
14. Murray HW, Hillmann JK, Rubin BG et al (1985) Patients at risk for AIDS-related opportunistic infections: Clinical manifestations and impaired gamma interferon production. N Engl J Med 313:1504
15. Mitsuya H, Weinhold KJ, Furman PA et al (1985) 3′-Azido-3′-deoxythymidine (BW A509U): an antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphadenopathy-associated virus in vitro. Proc Natl Acad Sci USA 82:7096
16. McCormick JB, Getchell JP, Mitchell SW et al (1984) Ribavirin suppresses replication of lymphadenopathy-associated virus in cultures of human adult T-lymphocytes. Lancet 2:1367
17. De Clercq E (1985) A potent inhibitor of reverse transcriptase of RNA tumor viruses. Cancer Lett 8:9
18. Mitsuya H, Popovic M, Yarchoan R et al (1984) Suramin protection of T-cells in vitro against cytopathic effect of HTLV-III. Science 226:172
19. Sarin PS, Taguchi Y, Sun D et al (1984) Inhibition of HTLV-III/LAV replication by foscarnet. Biochem Pharmacol 34:4075
20. Rozenbaum W, Dormont D, Spire B et al (1985) Antimoniotungstate (HPA 23) treatment of three patients with AIDS and one with prodrome (letter). Lancet 1:450
21. Sen GC, Herz R, Davatelis V et al (1984) Antiviral and protein inducing activities of recombinant human leukocyte interferons and their hybrids. J Virol 50:445
22. Ho DD, Hartshorn KL, Rota TR et al (1985) Recombinant human interferon-alpha-A suppresses HTLV-III replication in vitro. Lancet 1:602
23. Sarin PS, Gallo RC, Scheer DI et al (1985) Effect of a novel compound (AL 721) on HTLV-III infectivity in vitro. N Engl J Med 313:1289
24. Chandra P, Sarin PS (1986) Selective inhibition of replication of the AIDS-associated virus HTLV-III/LAV by synthetic D-penicillamine. Arzneimittelforschung/Drug Res 36:184
25. Shaffner CP, Plescia OJ, Pontani D (1986) Antiviral activity of Amphotericin B methyl ester. Inhibition of HTLV-III replication in cell culture. Biochem Pharmacol 35:4110
26. Zamecnik PC, Goodchild J, Taguchi Y (1986) Inhibition of the replication and

expression of human T-cell lymphotropic virus type III in cultured cells by exogenous synthetic oligonucleotides complementary to viral RNA. Proc Natl Acad Sci USA 83:4143
27. Sarin PS, Sun D, Thornton A, Müller WEG (1987) Inhibition of replication of the etiologic agent of aquired immune deficiency syndrome (human T-lymphotropic retrovirus/lymphadenopathy-associated virus) by Avarol and Avarone. JNCI 78:663
28. Harada S, Koyanagi Y, Yamamoto N et al (1985) Infection of HTLV-III/LAV in HTLV-I-carrying cells MT-2 and MT-4 and application in a plaque assay. Science 229:563
29. Schmitz H, Grass F (1986) Specific enzyme immunoassay for the detection of antibody to HTLV-III using rheumatoid factor coated plates. J Immunol Meth 88:115
30. Weir DM (ed) (1978) Handbook of experimental immunology. Blackwell, Oxford (pp 15.18–15.21, 34.23–34.25, 34–36)
31. Kaerber G (1931) Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. Arch Exp Pathol Pharmakol 162:480
32. Gohla SH, Haubeck HD, Schrum S, Soltau H, Neth RD (1990) Immunmodulation am Beispiel der Cupressaceae *Thuja occidentalis* L. In: Albrecht H, Franz G (eds) Naturheilverfahren: Zum Stand der Forschung. Springer, Berlin Heidelberg New York, p 60
33. Gohla SH, Zeman RA, Gartner S, Popovic M, Jurkiewics E, Haubeck HD, Schrum S, Gallo RC, Neth RD, Hunsmann G (1990) Inhibition of the replication of HIV-1 by TPSg, a polysaccharide-fraction isolated from the cupressaceae *Thuja occidentalis* L. AIDS Res Hum Retroviruses 6:131
34. Beuscher N, Kopanski L (1986) Purification and biological characterization of antiviral substances from *Thuja occidentalis*. Planta Med (Suppl):75
35. Khurana P (1971) Effect of homeopathic drugs on plant viruses. Planta Med 20:142
36. Baba M, Pauwels R, Balzarini J, Arnout J, Desmyter J, De Clercq E (1988) Mechanism of inhibitory effect of dextrane-sulfate and heparin on replication of human immunodeficiency virus in vitro. Proc Natl Acad Sci USA 85:6132
37. Gohla SH, Haubeck HD, Soltau H, Schrum S, Neth RD (1989) Induction of CD4+ and Okt17+ T-cells by high molecular polysaccharide-fractions isolated from *Thuja occidentalis* L. In: Neth RD, Gallo RC, Greaves MF, Gaedicke G, Gohla SH, Mannweiler K, Ritter J (eds) Modern trends in human leukemia, vol 8. Springer, Berlin Heidelberg New York, p 268
38. Hans R (1970) Die Zusammensetzung nichtcellulosischer Polysaccharide in verschiedenen Altersstufen des Holzes von *Thuja occidentalis* und *Abies dablemensis*. Holzforschung 24:60
39. Harada S, Purtilo DT, Kojanagi Y, Sonnabend J, Yamamoto N (1986) Sensitive assay for neutralizing antibodies against AIDS-related viruses (HTLV-III/LAV). J Immunol Meth 92:177
40. Hoffmann AD, Banapour B, Levy JA (1985) Characterization of the AIDS-associated retrovirus reverse transcriptase and optimal conditions for its detection in virions. Virology 147:326
41. Wendler I, Jentsch KD, Schneider J, Hunsmann G (1987) Efficient replication of HTLV-II and STLV-IIImac in human Jurkat cells. Med Microbiol Immunol 176:273
42. Jurkiewics E, Panse P, Jentsch KD, Hartmann H, Hunsmann G (1989) In vitro anti-HIV-1 activity of chondroitin polysulfate. AIDS 3:423
43. Tabba HD, Chang RS, Smith KM (1989) Isolation, purification, and partial characterization of prunellin, an anti-HIV component of aqueous extracts of *Prunella vulgaris*. Antiviral Res 11:263
44. Zhang SM, He YS, Tabba HD, Smith KM (1989) Inhibitor against the human immunodeficiency virus in aqueous extracts of *Alternanthera philoxeroides*. Chin Med J 101:861
45. Ngan F, Chang Rs, Tabba HD, Smith KM (1988) Isolation, purification and partial characterization of an active anti-HIV compound from the Chinese medicinal herb *Viola yedoensis*. Antiviral Res 10: 107
46. Chang RS, Young HW (1988) Inhibition of growth of human immunodeficiency virus in vitro by crude extracts of Chinese medicinal herbs. Antiviral Res 9:163
47. Miyoshi I, Kubonishi I, Yoshimoto S, Akagi T, Ohtsuki Y, Shiraishi Y, Nagata

K, Hinuma Y (1981) Detection of type c viral particles in a cord T-cell line derived by cocultivation of normal human leukocytes and human leukemic T cells. Nature 294:770
48. Gohla SH (1988) In vivo- und in vitro Untersuchungen zur Immunmodulation des spezifischen und unspezifischen Immunsystems durch hochmolekulare Polysaccharidfraktionen der Cupressaceae *Thuja occidentalis* L. PhD thesis, University of Hamburg
49. Lai PK, Donovan J, Takayama H, Sakagami H, Tanaka A, Konno K, Nonoyama M (1990) Modification of human immunodeficiency viral replication by pine cone extracts. AIDS Res Hum Retroviruses 6:205
50. WHO (1989) In vitro screening of traditional medicines for anti-HIV activity: Memorandum from WHO meeting. Bull WHO 67:613
51. Hatano T, Yasuhara T, Fukuda T, Noro T, Okuda T (1989) Phenolic constituents of licorice. II. Structures of lipopyranocumarin, licoarylcoumarin and glisoflavone, and inhibitory effects of licorice phenolics on xanthine oxidase. Chem Pharm Bull (Tokyo) 37:3005