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Purification and Characterization of an Avian Myeloblastosis and Human Immunodeficiency Virus Reverse Transcriptase Inhibitor, Sulfated Polysaccharides Extracted from Sea Algae

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A new reverse transcriptase (RT) inhibitor was extracted and purified from the red alga *Schizymenia pacifica*. The chromatographic behavior and chemical properties of this sea algal extract (SAE) suggest that it is a sulfated polysaccharide having a molecular weight of approximately 2,000,000. SAE is composed of galactose (73%), sulfonate (20%), and 3,6-anhydrogalactose (0.65%). SAE is a member of the λ-carrageenan family, based on its infrared spectrum and products of hydrolysis. SAE selectively inhibited human immunodeficiency virus (HIV) RT and replication in vitro. When MT-4 cells were treated with more than 10^4 inhibitory units (IU) of SAE per ml after HIV infection, significant inhibition of viral antigen synthesis was observed. Furthermore, more than 90% of cells were viable in the cultures exposed to 4×10^4 to 8×10^4 IU of SAE per ml, while almost all the MT-4 cells in the control culture had died by 10 days after HIV infection. The inhibitory effect of SAE on HIV replication was confirmed by plaque reduction assays. The 50% inhibitory dose of SAE was 9.5×10^3 IU/ml. Chondroitin sulfate A, dermatan sulfate, heparan sulfate, keratan polysulfate, and heparin also inhibited the RT of avian myeloblastosis virus. SAE immediately inhibited RT activity when added to an assay mixture after the start of the reaction.

Retroviruses are reverse transcriptase (RT)-containing RNA viruses that cause neoplasms and immunosuppression in various species of vertebrates. Since RT reverse-transcribes the RNA of the virus into double-stranded DNA, an agent that inhibits this transcription process may prevent the viral DNA from becoming integrated into the genome of the host cell, thereby inhibiting viral replication (5). It has recently been shown that human T-cell leukemia virus type I causes adult T-cell leukemia (25), a disease that has a high frequency in southwestern Japan (10) and the Caribbean basin (20) but occurs worldwide. Another retrovirus, formerly called human T-cell lymphotropic virus type III/lymphadenopathy-associated virus/acquired immune deficiency syndrome-associated retrovirus (1, 12, 17) and now referred to as human immunodeficiency virus (HIV) (3), causes acquired immune deficiency syndrome and the complex of diseases related to it. Although some drugs that inhibit the activity of RT are being tested for possible therapeutic use in these diseases, there are as yet no effective ways to cure them. Moreover, the drugs tested to date have relatively severe side effects (4, 13, 14, 19, 21).

In another report (H. Nakashima, Y. Kido, N. Kobayashi, Y. Motoki, M. Neushul, and N. Yamamoto, J. Cancer Res. Clin. Oncol., in press), we reported that a sea algal extract (SAE) from *Schizymenia pacifica* inhibited the RT of avian myeloblastosis virus (AMV) while having almost no cytotoxicity for cells. The present report describes the purification and preliminary characterization of the active ingredient in SAE and its inhibitory effect on AMV RT and HIV replication.

MATERIALS AND METHODS

SEA. *S. pacifica* was collected from Montaña de Oro State Park on the central California coast in the summer and stored at −80°C until use. The alga was homogenized in citrate-phosphate buffer (0.07 M citric acid, 0.15 M Na_2HPO_4 [pH 7.4]) at a concentration of 20% (wet wt/vol). The homogenate was maintained at 4°C for 16 h and centrifuged at 1,500 × *g* for 10 min. The resulting supernatant was designated as SAE as described elsewhere (Nakashima et al., in press).

Cells. The HTLV-I-carrying cell line, MT-4, and the HIV-producing cell line, MOLT-4/$HIV_{HTLV\text{-}IIIB}$, were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum, 100 U of penicillin per ml, and 100 μg of streptomycin per ml at 37°C in a CO_2 incubator. Viable cells were counted by the trypan blue dye exclusion method.

Virus. HTLV-IIIB, one of the HIV strains, was obtained from the culture supernatant of MOLT-4/$HIV_{HTLV\text{-}IIIB}$ cells as described elsewhere (11). The titer of the virus preparation was 6×10^4 PFU/ml (9). Fluids from 4-day-old MOLT-4/$HIV_{HTLV\text{-}IIIB}$ cells were partially purified by sucrose gradient ultracentrifugation (15 to 60%) and suspended in buffer consisting of 5 mM Tris hydrochloride (pH 8.1), 0.5 M KCl, 0.1 mM dithiothreitol, and 0.1% Triton X-100. This disrupted HIV was used for the RT inhibition assay.

RT inhibition assay. AMV RT (P-L Biochemicals, Inc., Milwaukee, Wis.) was assayed as described elsewhere (Nakashima et al., in press). Briefly, 1 U of AMV RT was

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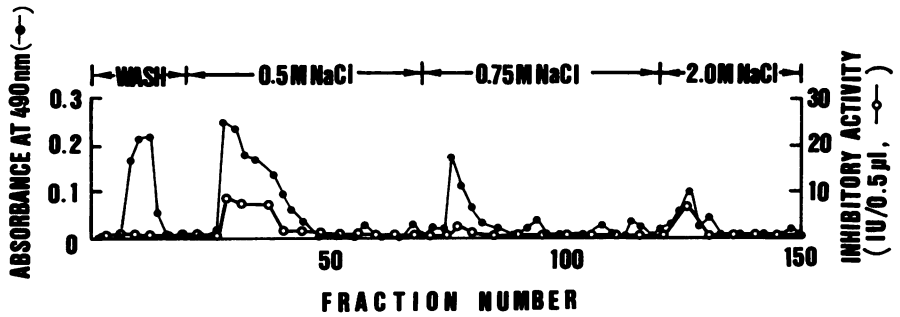

FIG. 1. DEAE-cellulose chromatography of citrate buffer-extracted SAE. SAE bound to DEAE-cellulose was eluted with stepwise concentrations of NaCl. Fractions of 17 ml each were collected, and 5 μl of each fraction was assayed for the inhibition of RT.

mixed with various concentrations of SAE or other compounds in a reaction mixture (50 μl) consisting of 50 mM Tris hydrochloride (pH 8.4), 2 mM dithiothreitol, 100 mM KCl, 10 mM $MgCl_2$, 0.1% Triton X-100, 50 μg of poly(rA)-oligo(dT) (P-L Biochemicals) per ml, and 1.25 μCi of [^{3}H] dTTP (57 Ci/mmol; Amersham, Buckinghamshire, England). The reaction mixture was incubated at 37°C for 30 min, and the reaction was stopped by the addition of 200 μl of 5% trichloroacetic acid. Precipitates were collected on glass fiber filters, and the radioactivity was counted in a liquid scintillation counter. All experiments were carried out in duplicate. One inhibitory unit (IU) of SAE was defined as the activity that caused 50% inhibition of 1 U of AMV RT.

The inhibition assay for RT from disrupted HIV was performed in the same manner, except that the reaction mixture was incubated for 60 min. The disrupted HIV RT sample was obtained from 0.5 ml of MOLT-4/$HIV_{HTLV-IIIB}$ cell culture supernatant (titer, 3×10^4 PFU/ml).

Purification of SAE. Crude SAE solution from 1.5 g of *S. pacifica* was lyophilized, and the sample (198 mg) was dissolved in 100 ml of distilled water and applied to a DEAE-cellulose (Wako Junyaku Co., Osaka, Japan) column (3 by 17.5 cm) which had been equilibrated with 0.01 M acetate buffer (pH 7.0). The bound sample was then eluted by stepwise increases in the NaCl concentration (0.5, 0.75, and 2.0 M) in 0.01 M citrate buffer. Chromatography was carried out at 4°C. The active fractions that inhibited AMV RT were pooled and precipitated by the addition of 3 volumes of ethanol. The precipitates were suspended in 2.0 ml of 0.01 M citrate buffer (pH 7.0) containing 2.0 M NaCl and separated in a Sepharose CL-4B (Pharmacia, Uppsala, Sweden) column (1.5 by 88 cm).

Polysaccharides. λ-, κ-, and ι-carrageenans were purchased from Sigma Chemical Co., St. Louis, Mo. Alginic acid was obtained from Kimitsu Kagaku Kogyo Co., Tokyo, Japan. Chondroitin, chondroitin sulfate A, dermatan sulfate, heparan sulfate, keratan sulfate, keratan polysulfate, and hyaluronic acid were obtained from Seikagaku Kogyo Co., Tokyo, Japan. Heparin was purchased from Wako Junyaku Co., Osaka, Japan.

Analysis of polysaccharides. Infrared (IR) spectra were recorded with a KBr disk and a Hitachi model 260-10 IR spectrophotometer. UV absorbance was measured with a Hitachi model 220A spectrophotometer.

Neutral sugar was analyzed with a phenol-sulfuric acid reagent and with galactose as a standard. 3,6-Anhydrogalactose was determined by the method of Yaphe and Arsenault with fructose as a standard (24). Sulfate was determined by the method of Terho and Hartiala (22).

HIV infection and virus-induced cytopathic effects. In vitro HIV infection was studied with the HTLV-I-carrying cell line, MT-4, as described elsewhere (9, 15, 16). MT-4 cells were exposed to HIV at a multiplicity of infection of 0.002 and incubated for 60 min at 37°C. After virus adsorption, the cells were washed and suspended in fresh medium to make a concentration of 3×10^5 cells per ml. This infected cell suspension was then cultured in the presence of various concentrations of purified SAE in a CO_2 incubator. HIV-induced cytopathic effects were determined by the trypan blue dye exclusion method.

Assay for HIV-specific antigen expression. The number of HIV-specific antigen-expressing cells was counted by the indirect immunofluorescence (IF) method. Briefly, methanol-fixed cells were reacted first with 1:1,000-diluted anti-HIV human serum (IF titer, 1:4,096) for 30 min and then with fluorescein isothiocyanate-conjugated rabbit anti-human immunoglobulin G (Dakoppatts A/S, Copenhagen, Denmark) for 30 min at 37°C. More than 500 cells were counted under a fluorescence microscope, and the percentage of IF-positive cells was calculated.

Plaque assay. The suppressive effect of SAE on plaque formation by HIV in MT-4 cells was measured by a plaque assay described previously (9, 15). MT-4 cells were infected with a fixed number of infectious virus particles (120 PFU per dish) and cultured in an agarose overlay medium with various concentrations of purified SAE. All experiments were carried out in triplicate.

RESULTS

Purification of SAE. Crude SAE was fractionated by DEAE-cellulose column chromatography (Fig. 1). The main fractions that inhibited AMV RT were eluted from the column by 0.5 M NaCl. Over half of the inhibitory activity was recovered in these fractions. The active fractions were separated by using the Sepharose CL-4B column (Fig. 2). A single peak of inhibitory activity was detected in the same fractions in which dextran, with an average molecular weight of 2,000,000, was eluted.

Composition of SAE. We analyzed the sugar components of the SAE purified in the Sepharose CL-4B column. The composition of SAE was similar to that of λ-carrageenan, except that λ-carrageenan contains slightly more sulfate. No protein was detectable in this fraction (Table 1). The IR spectra of purified SAE (Fig. 3) were very similar to those of λ-carrageenan but less similar to those of κ-carrageenan.

Inhibitory effects of SAE on the RT activity of HIV. The inhibitory effect of purified SAE on the RT activity of HIV was studied. HIV RT activity alone produced 32.8×10^4 cpm/0.5 ml and decreased in a dose-dependent fashion (Fig.

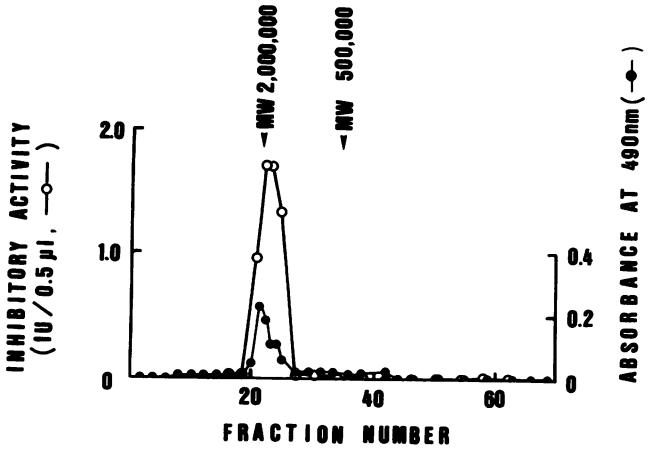

FIG. 2. Sepharose CL-4B gel filtration of SAE purified by DEAE-cellulose column chromatography. Fractions (3 ml) were collected and assayed for the inhibition of AMV RT. MW, Molecular weight.

4). When 4×10^4 IU of SAE per ml was added to the various RT preparations, RT activity decreased with decreasing RT concentrations.

Inhibition of virus-specific antigen expression and virus-induced cytopathic effects in HIV-infected MT-4 cells. When MT-4 cells were infected with HIV, 54 and 90% of the cells became positive for HIV antigen 3 and 5 days after infection, respectively. At both times, the inhibitory effects of graded doses of SAE were essentially linear up to a dose of 2×10^4 IU (Table 2). Furthermore, although almost all HIV-infected MT-4 cells had died 10 days after being infected, more than 90% of the cells were viable in the cultures exposed to 4×10^4 and 8×10^4 IU of SAE per ml. However, there was a 20% inhibition of cell growth when 8×10^4 IU of SAE per ml was used. Growth inhibition was negligible at lower doses.

Suppression of HIV replication by SAE in HIV-infected MT-4 cells. Plaque assays were done to directly assess the effects of graded doses of SAE on HIV replication. A total of 127 plaques per dish were counted in the control dishes, and 100, 60, 11, and 1 plaques per dish were counted in the dishes containing 0.5×10^4, 1×10^4, 2×10^4, and 4×10^4 IU of SAE per ml, respectively. When 8×10^4 IU of SAE per ml was used in the agarose, no plaques were detected. These data indicated that the 50% inhibitory dose of SAE was 9.5×10^3 IU/ml; that of 3'-azido-3'-deoxythymidine was 0.023 µM, as previously reported (15).

Inhibition of RT by polysaccharides. The RT-inhibiting activity of SAE was compared with that of several acidic polysaccharides of seaweeds as well as heparin and other polysaccharides. λ-Carrageenan had much stronger activity than heparin, while the activity of SAE was comparable to that of κ-carrageenan and ι-carrageenan (Table 3). Chondroitin sulfate A, dermatan sulfate, heparan sulfate, and keratan polysulfate also inhibited RT activity but at much lower levels than the carrageenans. All of the three carrageenans tested suppressed HIV infection in MT-4 cells, inhibiting cytopathic effects and HIV-specific antigen expression (data not shown).

TABLE 1. Physicochemical properties of SAE

Compound	Appearance	%		
		Galactose	Sulfate (as $NaSO_3$)	Anhydro-galactose
SAE	Solid	73	20	0.65
λ-Carrageenan	Solid	54	39	1.60
κ-Carrageenan	Solid	50	20	26.70

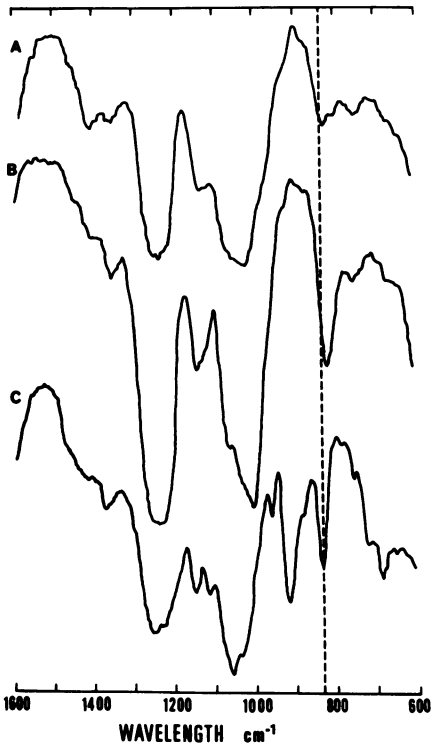

FIG. 3. IR spectra of purified SAE (A), λ-carrageenan (B), and κ-carrageenan (C). The dashed line at 830 cm^{-1} represents absorption caused by hydroxyl groups.

Mode of action of SAE. The mechanisms of the inhibitory activity of SAE on purified AMV RT were studied under various conditions. When 4×10^4 or 8×10^4 IU of SAE per ml was added to the reaction mixture 5 or 10 min after the reaction was started, SAE immediately inhibited the ongoing RT reaction (Fig. 5).

DISCUSSION

This paper summarizes the biochemical features of an extract (SAE) made from *S. pacifica* that selectively inhibited the RT of AMV and the replication of HIV. The purified active ingredient has an estimated molecular weight of 2,000,000 and consists of galactose (73%), 3,6-anhydrogalactose (0.65%), and sulfonate (20%). No protein was

TABLE 2. Inhibitory effect of SAE on the expression of virus-specific antigens in HIV-infected MT-4 cells

Dose of SAE (10^4 IU/ml)	% of IF-positive cells on day[a]:	
	3	5
0	54 ± 18	90 ± 10
0.5	44 ± 17	82 ± 17
1	22 ± 11	34 ± 20
2	8 ± 5	12 ± 5
4	4 ± 6	9 ± 5
8	1 ± 0.5	4 ± 1

[a] Results represent the mean ± standard deviation of two experiments.

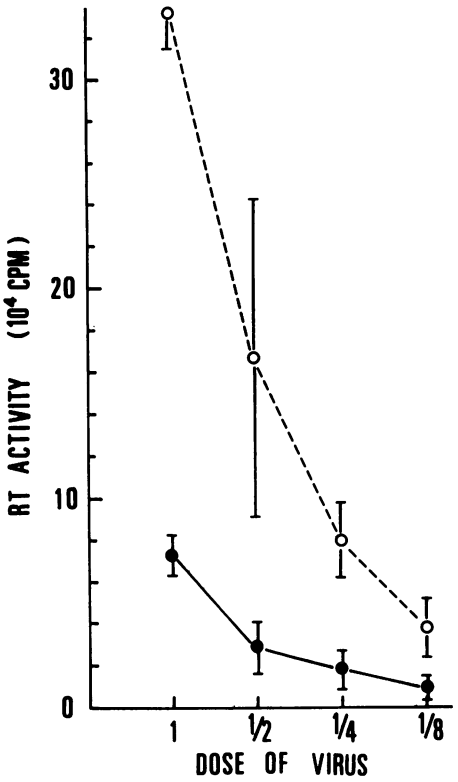

FIG. 4. RT activity of various concentrations of a disrupted HIV preparation (○) and the inhibitory effect of 4×10^4 IU of SAE per ml (●). One dose of virus corresponded to 0.5 ml of culture fluid, which contained 3×10^4 PFU of virus. Data were pooled from three replicate experiments; the bars represent the standard deviations from mean values.

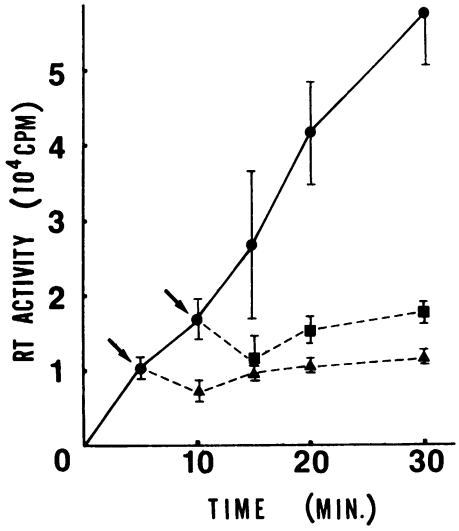

FIG. 5. Inhibition of ongoing AMV RT activity. SAE at 4×10^4 (▲) or 8×10^4 (■) IU/ml was added 5 or 10 min after the reaction was started with 1 U of RT. The reaction was terminated at the indicated times by the addition of trichloroacetic acid. ●, Control: no SAE was added. Data were pooled from three replicate experiments; the bars represent the standard deviations from mean values.

detected in purified SAE. Thus, we assume that SAE is a sulfated polysaccharide. These data confirm our observations that the inhibitory activity of SAE could not be inactivated by pronase digestion or boiling at 100°C, whereas boiling in the presence of 0.6 N HCl reduced this activity (Nakashima et al., in press). Moreover, we demonstrated here that purified SAE effectively suppressed HIV infection in vitro. Our data in this report clearly suggest that the active substance of SAE is one of the carrageenans, especially a member of the λ-carrageenan family. Carrageenans are sulfated polysaccharides that have a common structural backbone of D-galactose residues and are grouped into three families: the λ family (λ-, π-, and ξ-carrageenans); the β family (β- and γ-carrageenans), and the κ family (μ-, ν-, κ-, and ι-carrageenans) (2, 23). Alkaline treatment of purified SAE indicated that it had a low content of galactose 3- or 6-sulfate, which could be converted into 3,6-anhydride. The IR spectrum in the region of 800 to 1,400 cm^{-1} revealed an absorption band which was thought to indicate the presence of sulfate esters linking secondary hydroxyl groups (830 cm^{-1}) together to produce the absorption band characteristic of the sulfate group (1,240 cm^{-1}). λ-, ι-, and κ-carrageenans also have inhibitory activity against AMV RT and a suppressive effect on HIV infection in vitro.

TABLE 3. Inhibition of RT activity by polysaccharides

Polysaccharide	IU/mg[a]
SAE	30,000 ± 7,000
λ-Carrageenan	69,600 ± 13,050
ι-Carrageenan	34,600 ± 8,500
κ-Carrageenan	30,000 ± 7,230
Alginic acid	0
Chondroitin	0
Chondroitin sulfate A	20 ± 12
Dermatan sulfate	140 ± 80
Heparan sulfate	40 ± 23
Keratan sulfate	0
Keratan polysulfate	30 ± 17
Hyaluronic acid	0
Heparin	840 ± 326

[a] Determined by using AMV RT. Results represent the mean ± standard deviation of three experiments, each performed in duplicate.

The acidic polysaccharides (carrageenans, heparin, dermatan sulfate, heparan sulfate, and keratan polysulfate), that inhibit RT activity in vitro also have sulfate residues. Thus, it seems that sulfate residues may play a key role in the inhibition of RT. It was reported that polyvinyl sulfate inhibited RT by virtue of competition between the sulfated side chains of the molecule and the template primer for a site on the enzyme (8).

It was also reported that adsorption or penetration or both of some viruses could be inhibited by polyacrylic acid, polymethacrylic acid, polyvinyl sulfate, dextran sulfate, and polyphloroglucinol phosphate (6, 7, 18). Thus, the inhibition of HIV infection by SAE and purified carrageenans may not be entirely due to the inhibition of RT. Sulfated polysaccharides, including SAE and other carrageenans, may suppress HIV infection by interfering with virus adsorption as well as by inhibiting RT. In this regard, SAE markedly inhibited RT but had little effect on DNAalpha-polymerase and RNA polymerase (Nakashima et al., in press). Such selective activity provides a basis for designing drugs with inhibitory activity for HIV replication. Further work on the characterization of SAE and other related substances and their potency as antiviral drugs is in progress.

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ADDENDUM IN PROOF

In accordance with the hypothesis that sulfate residues might play a key role in RT inhibition, we synthesized glycyrrhizin sulfate and demonstrated that the addition of sulfate residues to glycyrrhizin apparently resulted in the endowment with RT inhibitory activity of the substance (Nakashima et al., Jpn. J. Cancer Res. **78**:767–771, 1987). Using the same approach, we were also able to convert several nonsulfated polysaccharides (e.g., dextran, xylofranan, ribofuranan, lentinan) into effective anti-HIV substances (Nakashima et al., submitted for publication). Thus, the present strategy of chemical modification appears to be very promising for the further development of antiretroviral drugs.

LITERATURE CITED

1. **Barre-Sinoussi, F., J. C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Dauguet, C. Axler-Blin, F. Vezinet-Brun, C. Rouzioux, W. Rozenbaum, and L. Montagnier.** 1983. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science **220**: 868–871.
2. **Carlos, A. S., and S. C. Cerzo.** 1986. The potassium chloride-soluble carrageenans of the red seaweed Iridaea undulosa B. Carbohydrate Res. **145**:219–235.
3. **Coffin, J., A. Haase, J. A. Levy, L. Montagnier, S. Oroszlan, N. Teich, H. Temin, K. Toyoshima, H. Varmus, P. Vogt, and R. Weiss.** 1986. Human immunodeficiency viruses. Science **232**: 697.
4. **De Clercq, E.** 1979. Suramin: a potent inhibitor of the reverse transcriptase of RNA tumor viruses. Cancer Lett. **8**:9–22.
5. **De Clercq, E.** 1985. Inhibitors of reverse transcriptase and retrovirus replication. Drug Res. **35**:1007–1008.
6. **De Somer, P., E. De Clercq, A. Billiau, E. Schonne, and M. Claesen.** 1968. Antiviral activity of polyacrylic and polymethacrylic acids. I. Mode of action in vitro. J. Virol. **2**:878–885.
7. **De Somer, P., E. De Clercq, A. Billiau, E. Schonne, and M. Claesen.** 1968. Antiviral activity of polyacrylic and polymethacrylic acids. II. Mode of action in vivo. J. Virol. **2**:886–893.
8. **Hallinan, F. M., S. H. S. Lee, and K. R. Rozee.** 1981. Inhibition of reverse transcriptase by polyvinyl sulfate (PVS). Cancer Biochem. Biophys. **98**:97–101.
9. **Harada, S., Y. Koyanagi, and N. Yamamoto.** 1985. Infection of human T-lymphotropic virus type-I (HTLV-I)-bearing MT-4 cells with HTLV-III (AIDS virus); chronological studies of early events. Virology **146**:272–281.
10. **Hinuma, Y., H. Komoda, T. Chosa, T. Kondo, M. Kohakura, T. Takenaka, M. Kikucki, M. Ichimaru, K. Yunoki, I. Sato, R. Matsuo, Y. Takiuchi, H. Uchino, and M. Hanaoka.** 1982. Antibodies to adult T-cell leukemia-virus-associated antigen (ATLA) in sera from patients with ATL and controls in Japan: a nation-wide seroepidemiologic study. Int. J. Cancer **29**:631–635.
11. **Koyanagi, Y., S. Harada, and N. Yamamoto.** 1986. Establishment of a high production system for AIDS retroviruses with a human T-leukemic cell line, Molt-4. Cancer Lett. **30**:299–310.
12. **Levy, J. A., A. D. Hoffman, S. M. Dramer, J. A. Landis, J. M. Shimabukuro, and L. S. Oshiro.** 1984. Isolation of lymphocytopathic retroviruses from San Francisco patients with AIDS. Science **225**:840–842.
13. **Mitsuya, H., and S. Broder.** 1986. Inhibition of the in vitro infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphadenopathy-associated virus by 2′,3′-dideoxynucleosides. Proc. Natl. Acad. Sci. USA **83**:1911–1915.
14. **Mitsuya, H., K. J. Weinhold, P. A. Furman, M. H. S. Clair, S. N. Lehrman, R. C. Gallo, D. Bolognesi, D. W. Barry, and S. Broder.** 1985. 3′-Azido-3′-deoxythymidine (BWA509U): 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**:7096–7100.
15. **Nakashima, H., T. Matsui, S. Harada, N. Kobayashi, A. Matsuda, T. Ueda, and N. Yamamoto.** 1986. Inhibition of replication and cytopathic effect of human T cell lymphotropic virus type III/lymphadenopathy-associated virus by 3′-azido-3′-deoxythymidine in vitro. Antimicrob. Agents Chemother. **30**: 933–937.
16. **Nakashima, H., T. Yoshida, S. Harada, and N. Yamamoto.** 1986. Recombinant human interferon gamma suppresses HTLV-III replication in vitro. Int. J. Cancer **38**:433–436.
17. **Popovic, M., M. G. Sarngadharan, E. Read, and R. C. Gallo.** 1984. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science **224**:497–500.
18. **Rouhandeh, H., L. L. Sells, and M. Chapin.** 1966. Effect of L-cystine and sulfated polysaccharides on replication of echovirus type 32 in monkey kidney cells. Proc. Soc. Exp. Biol. Med. **123**:246–249.
19. **Rozenbaum, W., D. Dormont, B. Spire, E. Vilmer, M. Gentilini, C. Griscelli, L. Montagnier, F. Barre-Sinoussi, and J. C. Chermann.** 1985. Antimoniotungstate (HPA23) treatment of three patients with AIDS and one with prodrome. Lancet **i**: 450–451.
20. **Schupbach, J., V. S. Kalyanaraman, M. G. Sarngadharan, W. A. Blattner, and R. C. Gallo.** 1983. Antibodies against three purified proteins of the human type C retrovirus, human T-cell leukemia-lymphoma virus, in adult T-cell leukemia-lymphoma patients and healthy blacks from the Caribbean. Cancer Res. **43**:886–891.
21. **Sundquist, B., and B. Oberg.** 1979. Phosphonoformate inhibits reverse transcriptase. J. Gen. Virol. **45**:273–381.
22. **Terho, T. T., and K. Hartiala.** 1971. Method for determination of the sulfate content of glycosaminoglycans. Anal. Biochem. **41**:471–476.
23. **Thomson, A. W., and E. F. Fowler.** 1981. Carrageenan: a review of its effects on the immune system. Agents Actions **11**:265–272.
24. **Yaphe, W., and G. P. C. Arsenault.** 1965. Improved resorcinol reagent for the determination of fructose and of 3,6-anhydrogalactose in polysaccharides. Anal. Biochem. **13**:143–148.
25. **Yoshida, M., I. Miyoshi, and Y. Hinuma.** 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. USA **79**:2031–2035.