Ecotoxicology and Environmental Safety 45, 208–227 (2000)
Environmental Research, Section B
doi:10.1006/eesa.1999.1862, available online at http://www.idealibrary.com on IDEAL®

REVIEW

Anti-HIV Activity of Extracts and Compounds from Algae and Cyanobacteria

David J. Schaeffer[1] and Victor S. Krylov

Department of Veterinary Biosciences, University of Illinois, 2001 South Lincoln Avenue, Urbana, Illinois 61802

Received June 8, 1999

1. EXECUTIVE SUMMARY

1.1. Abstract

The human immunodeficiency virus (HIV) is the retrovirus that causes the acquired immune deficiency disease syndrome (AIDS). This review discusses the anti-HIV activity of extracts and compounds isolated from freshwater and marine algae, and cyanobacteria (formerly called "blue-green algae"). Compounds and extracts with anti-HIV activity are also active against other retroviruses such as herpes simplex virus (HSV), but the amount of antiviral activity varies with the compound and the virus. Most of the research has focused on sulfated homopolysaccharides and heteropolysaccharides. Sulfoglycolipids, carrageenans, fucoidan, sesquiterpene hydroquinones, and other classes of compounds with anti-HIV activity that have been isolated from algae have received less attention. Most studies have used *in vitro* test systems, but a few *in vivo* studies have been carried out using compounds isolated from algae or analogs produced synthetically or isolated from other natural sources. Sulfated homopolysaccharides are more potent than sulfated heteropolysaccharides. The presence of the sulfate group is necessary for anti-HIV activity, and potency increases with the degree of sulfation. Studies using nonsulfated and sulfated homo- and heteropolysaccharides isolated from algae or other natural sources, or synthesized, have revealed the mechanisms of binding of drugs to the virion, and the mechanisms of viral binding to host cells. However, given the few classes of compounds investigated, most of the pharmacopeia of compounds in algae and cyanobacteria with antiretroviral activity is probably not known.

Key Words: HIV; AIDS; antiretroviral activity; algae; seaweed; cyanobacteria; sulfated polysaccharides; fucoidan; carrageenan; sesquiterpene hydroquinones; sulfogylcolipids.

[1] To whom correspondence should be addressed.

0147-6513/00 $35.00

1.2. Introduction

In the 20 years since the "acquired immune deficiency syndrome" (AIDS)[2] was first recognized, over 40 million people have become infected by the human immunodeficiency virus (HIV). UNAIDS estimates that each day 16,000 people become infected by the HIV (Piot, 1998). The process by which the virus evades the immune system is not fully elucidated. Many studies have investigated the mechanism of the virus's action, or the pharmacological activity of compounds of diverse structures. A glycoprotein on the virus's outer coat, gp120, latches onto CD_4 receptors on the surface of T lymphocytes, the immune cells that are the virus's primary target. X-ray crystallography has revealed that structural features of the gp120–CD_4 complex block antibody access (Rizzuto *et al.*, 1998), so the "immune system essentially cannot 'see' these prime targets and generate a response to them" (Balter, 1998). A toxicological problem is to identify compounds that target the virus but not affect the host cell. Natural products derived from numerous species have been found to have antiviral activity against the HIV. One of first sources of natural compounds with *in vitro* anti-HIV activity, micro- and macroalgae, are the focus of this review.

Algae include a wide variety of plants that range from diatoms, which are microscopic, unicellular organisms, to seaweeds extending over 30 m. Unlike land plants, algae do not have true roots, stem, and leaves. Algae are grouped into six main classes, mainly on the basis of their color (Fogg, 1953). Most algae are found in fresh or salt water, but a few of the Chrysophyta and Cyanophyta[3] may be terrestrial. The unicellular algae are placed in the kingdom Protista and are classified as euglenoids (phylum Euglenophyta), dinoflagellates (phylum Pyrrophyta), and diatoms (phylum Chrysophyta). All have chloroplasts and carry out photosynthesis similar to that of plants. Multicellular green algae (division Chlorophyta), red algae (division Rhodophyta), and brown algae (division Phaeophyta) are all seaweeds. Seaweeds are widely distributed in the ocean, occurring from the tide level to considerable depths, free-floating or anchored, and include kelp, dulse, rockweed, and sea lettuce. Many are of economic importance as food, fertilizer, agar, potash, or source of iodine.

The antibacterial activity of an aquatic microalgae was first reported for *Chlorella vulgaris* (Pratt and Fong, 1940). An early report of the antimicrobial properties of seaweed extracts was published a decade later (Pratt *et al.*, 1951), and several other papers appeared in the next two decades (Burkholder and Sharma, 1969). The antiviral effects of polysaccharides from marine algae toward mumps virus and influenza B virus were reported by Gerber *et al.* (1958). Subsequently, polysaccharides fractions from extracts of red algae were found to inhibit herpes simplex virus (HSV) and other viruses (Burkholder and Sharma, 1969; Deig, 1974; Ehresmann *et al.*, 1977; Richards *et al.*, 1978). These observations did not generate much interest because the antiviral action of the compounds was considered to be largely nonspecific (Witvrouw and De Clercq, 1997).

Isolation from algae of sulfated polysaccharides and other compounds with antiviral activity against enveloped viruses increased the interest in algae as a source of antiviral compounds. Enveloped viruses have nucleic acid within either a helical or a polyhedral core surrounded by an envelope. Enveloped viruses include HIV, herpes simplex virus type 1 and type 2 (HSV-1 and HSV-2), influenza A virus, RSV, simian immunodeficiency virus (SIV), pseudorabies virus, bovine herpes virus, and human cytomegalovirus (HCMV).

[2]Abbreviations used: AIDS, acquired immune deficiency syndrome; AMV, avian myeloblastosis virus (A species of avian type C retroviruses.); APTT, activated partial thromboplastin time; ARC, AIDS-related complex; ATA, aurintricarboxylic acid; AZT, azidothymidine (3′-azido-2′,3′-dideoxythymidine); Ca-SP, calcium spirulan; CV-N, cyanovirin-N; COSY, correlation spectroscopy; DEAE dextran, diethylaminoethyldextran; DGG, digalactopyranosyl glycerols; DNA, deoxyribonucleic acid; DNTP, deoxyribonucleoside 5′-triphosphate; DS, dextran sulfate; GC, gas chromotography; GS, galactan sulfate; HCMV, human cytomegalovirus. (HCMV is a widely occurring intracellular herpesvirus characterized by narrow host specificity and slow reproduction (latency), that causes AIDS-related oculopathy in humans.); HIV, human immunodeficiency virus; HSV, herpes simplex virus. (Herpesviridae is a family of enveloped DNA viruses that occurs in man, cold-blooded vertebrates, and invertebrates.); HTLV, human T-cell lymphotropic virus or human T-cell leukemia virus; IC_{50}, inhibitory concentration 50%; IU, inhibitory unit; iv, intravenous; LC, Langerhans cells; mAb, monoclonal antibody; MGG, monogalactopyranosyl glycerol; MS, mass spectrometry; NCI, National Cancer Institute (U.S.); NMR, Nuclear magnetic resonance; PAS, polyanethole sulfonate; PBL, peripheral blood lymphocyte; PBMC, peripheral blood mononuclear cells; PHA, phytohemagglutinin; pol α, polymerase alpha; pol β, polymerase beta; PVS, polyvinyl sulfate; RNA, ribonucleic acid; RNase H, ribonuclease H; RSV, respiratory syncytial virus; RT, reverse transcriptase; SAE, sea algal extract; SI, selectivity index; sCD_4, soluble CD_4; SIV, simian immunodeficiency virus; SP, sulfated polyanions; SQG, sulfoquinovopyransoyl glycerols; TNF-α, tumor necrosis factor-α; TNF-α, tumor necrosis factor-α; VSV, vesicular stomatis virus.

[3]A subgroup of the oxygenic photosynthetic bacteria comprising unicellular to multicellular bacteria possessing chlorophyll a and carrying out oxygenic photosynthesis. Cyanobacteria are the only known organisms capable of fixing both carbon dioxide (in the presence of light) and nitrogen. Formerly called blue-green algae, cyanobacteria were traditionally treated as algae. By the late 19th century, however, it was realized that the blue-green algae were unique and lacked the traditional nucleus and chloroplasts of the green and other algae. The comparison of nucleotide base sequence data from 16S and 5S rRNA indicates that cyanobacteria represent a moderately deep phylogenetic unit within the gram-negative bacteria (National Library of Medicine, IGM Metathesaurus Information Screen).

2. ANTI-HIV ACTIVITY OF MARINE AND FRESHWATER MICRO- AND MACROALGAE

2.1. Scope of This Review

Compounds extracted from algae have *in vitro* or *in vivo* activity against a wide range of retroviruses, including herpes viruses (HSV-1, HSV-2, HCMV), togaviruses (Sindbis virus, Semliki Forest virus), paramyxoviruses (RSV), rhabdoviruses (vesicular stomatitis virus [VSV]), and human immune deficiency viruses (SIV, HIV). Compounds isolated from algae that have been tested against HIV include steroids and sulfoglycolipids, but most of the research has used natural and synthetic sulfated polysaccharides. "Since 1988, the activity spectrum of the sulfated polysaccharides has been found to extend to various enveloped viruses, including viruses that emerge as opportunistic pathogens [e.g., HSV and HCMV] in immunosuppressed (e.g., AIDS) patients ... [S]ulfated polysaccharides ... are able to block HIV replication in cell culture at concentrations as low as 0.1 to 0.01 μg/ml without toxicity to the host cells at concentrations up to 2.5 μg/ml" (Witvrouw and De Clercq, 1997). The first step in the replicative cycle of HIV-1, the binding of the virions to the cellular CD4 receptor, is an important target for chemotherapeutic agents against AIDS (Schols *et al.*, 1989). This explains some of the molecular basis for the anti-HIV activity of sulfated polysaccharides (Schols *et al.*, 1989; Batinic and Robey, 1992; Jagodzinski *et al.*, 1994; Damonte, 1996).

This article reviews the research on the anti-HIV activity of compounds present in marine and freshwater micro- and macroalgae, and cyanobacteria. Studies of the anti-HIV activity of green, red, and brown algae are summarized in Table 1, and cyanobacteria studies are in Table 5. Research that used purchased compounds previously isolated from algae are included in this review. Studies of the antiretroviral activity of algal extracts that did not include assays against the HIV are selectively cited if they (1) expand data or conclusions from similar studies with HIV, or (2) identify species, extracts, or compounds that have not been tested using HIV.

2.2. Anti-HIV Activity of Marine Algae Other Than Cyanobacteria

Most of the research on the anti-HIV activity of marine algae has used red and brown macroalgae. The initial studies using these algae isolated sulfated polysaccharides with antiviral activity and later investigators continued interest in this class of compounds. However, other classes of compounds with anti-HIV activity have been identified (Table 1). The research is reviewed in this section.

2.2.1. Polysaccharides, sulfated polysaccharides, fucoidan, carrageenans. Polysaccharides and sulfated polysaccharides are generally extractable by hot water, dilute acid, or dilute alkali. Some natural polysulfates isolated from algae, as well as some synthetic polysulfates, exhibit a differential inhibitory activity against different HIV strains, which suggests differences in the target molecules with which these compounds interact (Witvrouw and De Clercq, 1997). They inhibit the cytopathic effect of HIV and also prevent HIV-induced syncytium (giant cell) formation. Antiviral activity increases with increasing molecular weight and degree of sulfation, as indicated by using dextran sulfate[4] (DS) of increasing molecular weight and sulfated cyclodextrins of different degrees of sulfation.[5]

2.2.1.1. Extracts of the marine red alga, Schizymenia pacifica. Nakashima *et al.* (1987b) prepared a citrate buffer extract of the marine red alga *S. pacifica*. A cell-free system was used to test the effect of the extract on reverse transcriptase (RT) from avian myelblastosis virus (AMV), and Rauscher murine leukemia virus. The extract inhibited RT from both these retroviruses over a wide range of dilutions, down to $1:10^4$. Defining an inhibitory unit (IU) of activity as the amount inhibiting 50% of the control reverse transcriptase of AMV, the activity of the crude extract was 8.4×10^5 IU/mL. The extract had a small stimulatory effect on the activity of cellular DNA polymerase alpha and a small inhibitory effect on RNA polymerase II *in vitro*. The specificity of extract was demonstrated by its lack of adverse affect on [^{3}H]thymidine and [^{3}H]uridine incorporation into cultured human T cells. The inhibitory activity of the extract was stable over the pH range 1–11. The active principle had a molecular weight in excess of 105 Da. Because inhibitory activity was not lost after pronase digestion but was lost after boiling at 100°C in 0.67 N HCl, or treatment with 100 mM $NaIO_4$ (which breaks down carbohydrates), the active compound was postulated to be a polysaccharide.

Subsequently, Nakashima *et al.* (1987a) purified the new reverse transcriptase inhibitor from *S. pacifica*. The compound, termed "sea algal extract" (SAE), is a sulfated polysaccharide having a molecular weight of approximately 2×10^6. SAE is composed to galactose (73%), sulfonate (20%), and

[4] Dextran is a high-molecular-weight polysaccharide produced from sucrose by several bacteria. Its predominant backbone consists of α(1 → 6) linked D-glucose units. Dextrans of differing chain length and degree of branching (at α(1 → 3) and α(1 → 4) branch points) are produced. Partial hydrolysis and sulfation produces dextran sulfate fractions of different average molecular weight (Witvrouw and De Clercq, 1997). α-Dextrin is a short polysaccharide derived from starch and is made up of several glucose units joined by an α-1,6 linkage in addition to α-1,4 linkages. More than 85% of dextrin molecules have molecular masses between 1640 and 45,000, with a claimed-average molecular mass of approximately 20,000.

[5] "A sugar backbone is not strictly needed for the anti-HIV activity of polysulfates because sulfated polymers composed of a carbon–carbon backbone have also proved to be highly efficient anti-HIV agents *in vitro*. Other, yet to be defined, structural features may also play an important role" (Witvrouw and De Clercq, 1997).

TABLE 1
Anti-HIV Activity of Extracts of, and Compounds Isolated from, Green, Red, and Brown Algae

Reference	Species[a]	Compounds isolated	Other compounds tested
Nakashima *et al.* (1987b)	*Schizymenia pacifica* (red seaweed)	Extract, polysachharide MW, 10^2 kDa	
Nakashima *et al.* (1987a)	*Schizymenia pacifica* (red seaweed)	SAE MW, 2×10^3 kDa	Chondroitin sulfate A, dermatan sulfate, heparin sulfate, and keratan polysulfate inhibited RT of avian myeloblastosis virus
Beress *et al.* (1993)	*Fucus vesiculosus* (brown seaweed)	Polysachharides, fucoidan, polyphenols	AZT
Moen and Clark (1993)	*Fucus vesiculosus* (brown seaweed)	Fucoidan and water-soluble, noncarbohydrate component of fucoidan	
Hoshino *et al.* (1998)	*Sargassum horneri* (brown seaweed)	Sulfated polysaccharide with fucose as the main component sugar	Dextran sulfate (50 kDa)
Witvrouw and De Clercq (1997)	Marine algae (review); Red seaweeds: *Nothogenia fastigiata, Agardhiella tenera, Eucheuma cottonii, Gigartina aciculaire, Gigartina pistillata*	Xylomannan, Galactan sulfate, κ-, λ-Carrageenan	Dextran sulfate, pentosan sulfate, dermatan sulfate
Damonte (1996)	Alga	Not directly relevant	
Hasui *et al.* (1995)	*Cochlodinium polykrikoides*	Sulfated polysachharides	
Damonte *et al.* (1994)	*Nothogenia fastigiata* (red seaweed)	Sulphated xylomannan	
Baba *et al.* (1988b, 1990)	Purchased compounds	Fucoidan, κ-, λ-Carrageenan	Dextran sulfate (10^3 to 500 kDa), pentosan polysulfate (3.1 kDa), dermatin sulfate (31 kDa), heparin (30 kDa), mannan sulfate (30 kDa), suramin (1.4 kDa), PAA (27 kDa). COAM (150 kDa)
Sugawara *et al.* (1989); Mizumoto *et al.* (1988)		Fucoidan	Dextran sulfate, DEAE dextran (diethyla-minoethyldextran)
Parish *et al.* (1990)		Fucoidan, κ-, λ-, ι-Carrageenan	Chondroitin 4-sulfate, chondroitin 6-sulfate heparin, dextran sulfate (5, 500 kDa), polyvinyl sulfate, polyanethole sulfonate, pentosan sulfate
McClure *et al.* (1992)		Fucoidan	Dextrin sulfate, dextran sulfate
Ohta *et al.* (1998)	*Gigartina tenella* (red seaweed)	Sulfoquinovosyldiacylglycerol	
Loya *et al.* (1995)	*Peyssonelia* sp. (red seaweed)	Peyssonol A. Peyssonol B (sesquiterpene hydroquinones)	
Bagasra *et al.* (1989)		Carrageenan, fucoidan	Dextran sulfate, heparin, heparin sulfate, chondriotin sulfate, pentosan polysulfate, D-glucosamine 2-sulfate, D-glucosamine 6-sulfate, D-glucosamine 2,6-disulfate, inositol hexasulfate
Elias *et al.* (1997)		ι-Carrageenan	

[a]When "Species" column is blank, compounds were purchased rather than isolated.

3,6-anhydrogalactose (0.65%), and its infrared spectrum and hydrolysis products suggest that it is a λ-carrageenan.[6] SAE selectively inhibited HIV RT and replication *in vitro*. More than 92% of the RT activity of the control was inhibited by 2×10^3 IU/mL of SAE. This concentration stimulated the activity of DNA polymerase *in vitro*, but had no adverse effect on the cellular incorporation of either [^{3}H]thymidine or [^{3}H]thymidine or [^{3}H]uridine into cultured human T cells. Consequently, the inhibitor was determined to be specific for reverse transcriptase, and is not likely to have adverse effects on cell growth.

Treatment of HTLV-1[7]-carrying MT-4 cells with 4×10^4 and 8×10^4 IU of SAE per milliliter after HIV infection with HTLV-IIIB[8] resulted in up to 20% inhibition of viral

[6]Carrageenans are mixtures of sulfated polysaccharides extracted from different species of red seaweed that have a common structural backbone of D-galactose residues. The three families of carrageenans are the λ family (λ-, π-, and ε-); the β family (β- and γ-); and the κ family (μ-, ν-, κ-, and ι-). "The κ-form is characterized by a repeating unit of 4-sulfate-β-D-galactopyranosyl(1 → 4)-3,6-anhydro-α-D-galactose-linked (1 → 3). The λ form is characterized by a repeating (1 → 3)-linked disaccharide of 2-sulfate-β-D-galactopyranosyl (1 → 4)-α-D-galactose-2,6-sulfated. The degree of sulfation for the κ and λ form are 25% and 35%, respectively" (Witvrouw and De Clercq, 1997).

[7]Human T-cell leukemia virus type I, which causes adult T-cell leukemia.

[8]An HIV strain from the HIV-producing cell line Molt-4/HIVHTLV-IIIB.

antigen synthesis (Nakashima *et al.*, 1987b). Most of the MT-4 cells in the control culture died by 10 days after HIV infection but more than 90% of cells were viable in the cultures exposed to 4×10^4 to 8×10^4 IU of SAE/mL. The inhibitory effect of SAE on HIV replication was confirmed by assays for inhibition of plaque formation by HIV in MT-4 cells. Reverse transcription activity was immediately inhibited when the compound was added to an assay mixture. The 50% inhibitory dose (IC_{50}) was 9.5×10^3 IU/mL. The activity of SAE was similar to that of κ-carrageenan (30,000 ± 7230 IU/mg) and ι-carrageenan (34,600 ± 8500 IU/mg), and less than that for λ-carrageenan (69,600 ± 13,050 IU/mg). The sulfate residues were hypothesized to play a key role in RT inhibition; this hypothesis was supported by inducing activity in polysaccharides derivatized as the sulfate (Nakashima *et al.*, 1987b). Another study reported an IC_{50} of 12 μg/mL for κ-carrageenan against HIV-1 (III_B) and 1.9 μg/mL for λ-carrageenan against HIV-2 (ROD) (Witvrouw and De Clercq, 1997).

2.2.1.2. Extracts of the brown seaweed Fucus vesiculosus. Anti-HIV-active polysaccharides and polyphenols were isolated from the brown seaweed *Fucus vesiculosus* (Beress *et al.*, 1993; Moen and Clark, 1993). Some of the fractions inhibited both HIV-induced syncytium formation and HIV RT enzyme activity at concentrations that were not cytotoxic. Beress *et al.* (1993) reported that most fractions inhibited both. For these fractions, syncytium formation was inhibited at lower concentrations than RT enzyme activity; e.g., fractions 57 and 58 inhibited syncytium formation at 1 and 2.0 μg/mL, respectively, but only caused "modest" RT enzyme inhbition at 50 μg/mL. The fractions that did not inhibit HIV syncytium formation but did inhibit the HIV RT enzyme "may have inhibited syncytium formation by mechanisms that did not involve the virus RT enzyme" (Beress *et al.*, 1993; Moen and Clark, 1993).

A water-soluble, noncarbohydrate component of fucoidan isolated from *F. vesiculosus* inhibits HIV RT *in vitro* (Moen and Clark, 1993). Kinetic analysis of the interactions of crude fucoidan with HIV RT indicated mixed inhibition, which could be due to more than one anti-HIV RT component in the crude fucoidan. However, kinetic analysis of the noncarbohydrate component indicated noncompetitive inhibition with respect to nucleotide, and competitive inhibition with respect to primer-template. "The simple inhibition patterns suggest that this component is a single compound which inhibits HIV-RT by competing with the nucleic acid substrate" (Moen and Clark, 1993).

2.2.1.3. Extracts of a brown alga, Sargassum horneri (Turner) C. Agardh. A sulfated polysaccharide, with fucose as the main component sugar, was isolated from the hot water extract of a brown alga, *S. horneri* (Turner) C. Agardh (Hoshino *et al.*, 1998). This compound demonstrated potent antiviral activity against HSV-1, human cytomegalovirus, and HIV-1 (Molt-4/HTLV-IIIB cells). The anti-HIV activity of the sodium salt (Na-HOR) and the desulfated polysaccharide (DS-HOR) are given in Table 2. Time-of-addition experiments indicated that the compound(s) inhibited both the initial stages of viral infection, such as attachment to and penetration into host cells, and also later replication stages after virus penetration. However, the inhibition mechanism of Na-HOR and DS-HOR were different, at least in part. Both sulfated polysaccharides suppressed HSV-1 replication when they were present during infection. Neither had an antiviral effect when virus particles were pretreated, nor when host cells were treated with the compounds for 3 h before infection. The IC_{50} value of Na-HOR against HSV-1 "immediately after treatment and 2 h after infection was 20- [*sic* 2-] or 50- [*sic* 36-] fold lower than for DS, respectively." They concluded "that binding of the polysaccharides to virion or host cells could not be an inhibitory factor of virus replication" (Hoshino *et al.*, 1998).

TABLE 2
Anti-HIV-1 Activity of Polysachharides Isolated from *Sargassum horneri* (Hoshino *et al.*, 1998)

Sample	Cytotoxicity (CC_{50})[a]	Anti-HIV activity IC_{50}[b] / IC_{90}[c]				Selectivity index CC_{50}/IC_{50}	
		A[d]	B[e]	A	B	A	B
Na-HOR	2350	1.2	5.4	4.0	12	1958	435
DS-HOR	2440	2.8	8.4	4.9	40	871	290

[a]Concentration (μg/mL) required to reduce the growth of MT-4 cells by 50%.
[b]Concentration (μg/mL) required to reduce viral replication by 50%
[c]Concentration (μg/mL) required to reduce viral replication by 90%.
[d]Each compound was added to the medium at the same time as the viral infection for 1 h and throughout the incubation thereafter.
[e]Each compound was added to the medium immediately after viral infection.

2.2.1.4. Extracts of the red seaweed Agardhiella tenera. Galactan sulfate (GS), a polysaccharide isolated from the red seaweed *A. tenera*, had an IC_{50} of 0.5–0.6 μg/mL against HIV-1 (III_B) and HIV-2 (ROD) (Witvrouw *et al.*, 1994). GS inhibited the cytopathic effect of HIV-1 and HIV-2 in MT-4 cells, with IC_{50} values of 0.5 and 0.05 μg/L, respectively. These IC_{50} values are 10-fold higher than those required for inhibition by 5 kDa DS. Syncytium formation between Molt-4 cells and HIV-1- or HIV-2-infected HUT-78 cells was inhibited at GS concentrations >5 μg/L. GS, and the synthetic compounds DS and aurintricarboxylic acid (ATA), inhibited the binding of HIV-1 to the cells and also the binding of anti-gp 120 mAb to HIV-1 gp120. GS, DS, and ATA were active against other enveloped viruses, i.e., herpes viruses, togaviruses, arenaviruses RSV, and others.

2.2.1.5. Extracts of the marine microalgae Cochlodinium polykrikoides. Extracellular sulfated polysaccharides A1 (1.3×10^6 Da) and A2 (6.3×10^5 Da) were isolated and purified from *C. polykrikoides*, a marine microalgae (Hasui *et al.*, 1995). These polysaccharides were homogeneous using ultracentrifugal and electrophoretic analyses. They were composed of mannose, galactose, glucose, and uronic acid, together with sulfate groups ($S = 7$–8% w/w). A1 and A2 inhibited the cytopathic effect of influenza virus types A and B grown on MDCK cells, and RSV types A and B grown on Hep-2 cells. Both A1 and A2 had an $IC_{50} = 1.7$ μg/mL for anti-HIV-1 activity (in MT-4 cells). However, except for A1 against HSV-1 and A2 against parainfluenza virus type 2 (both grown in HMV-2 cells), these polysaccharides had no antiviral activity against parainfluenza virus types 2 and 3, measles virus, mumps virus, or HSV-1 (all grown in HMV-2 cells). A1 and A2 were not cytotoxic to host cells at a concentration of 100 μg/mL. The concentrations that inhibited various viruses had weak (10%) inhibitory effects on blood coagulation.

2.2.1.6. Extracts of the red seaweed Nothogenia fastigiata. Polysaccharide fractions obtained from water extracts of the red seaweed *N. fastigiata* had antiviral activity (Damonte *et al.*, 1994). Fraction F6, containing a sulfated xylomann,[9] inhibited the antiviral activity of replication of HSV-1. F6 was only weakly active ($IC_{50} \approx 13.5$ μg/mL) compared to DS ($IC_{50} \leq 0.1$ μg/mL) against HIV-1 (III_B) and HIV-2 (ROD). However, F6 selectively inhibited the replication of several other enveloped viruses, including HSV-2, HCMV, RSV, influenza A and B virus, Junin and Tacaribe virus, and simian immunodeficiency virus. The mode of action of F6 against HSV-1 and HCMV was due to an inhibitory effect on virus adsorption. Additional studies of anti-HIV activity have been reported for fraction F6 (Pujol *et al.*, 1995), fractions F1 and F7 (Damonte *et al.*, 1996), and for two new compounds (Kolender *et al.*, 1997).

2.2.1.7. Anti-HIV activity of carrageenans isolated from the red seaweeds Gigartina skottsbergii and Cryptopleura ramosa. Carrageenans and their cyclized derivatives isolated from the red seaweed *G. skottsbergii* were potent inhibitors of herpes viruses (Carlucci *et al.*, 1997a). λ-Carrageenans, and the partially cyclized μν-carrageenan, inhibited acyclovir-resistant variants and clinical isolates of herpes viruses ($IC_{50} < 1$ μg/mL). "Antiherpetic activity was directly correlated to the amount of α-D-galactose 2,6-disulfate residues in the natural carrageenans" (Carlucci *et al.*, 1997a). Cyclization of the α-D-galactose 6-sulfate and 2,6-disulfate units into 3,6-anhydro-α-D-galactose and 3,6-anhydro-α-D-galactose 2-sulfate residues lowered the antiherpetic activity. The anticoagulant activity of some carrageenans was much higher than the IC_{50}, and others had no anticoagulant activity. Based on the anti-HIV activity of other carrageenans, similar relationships are expected for the anti-HIV activity of these compounds.

A low-molecular-weight (2800 Da) sulfated galactan isolated from *C. ramosa* was a selective inhibitor of HSV-1 and HSV-2 replication Vero cells, with IC_{50} in the range 1.6–4.2 μg/mL and 50% cytotoxic concentration of 476 μg/mL (Carlucci *et al.*, 1997b). The compound did not inhibit blood coagulation at concentrations much higher than the IC_{50}. The mode of action was "ascribed to an inhibitory action on virus adsorption." Analogous to Witvrouw *et al.* (1994), this sulfated galactan should also inhibit HIV and other retroviruses.

2.2.2. Studies using purchased compounds. The alga products fucoidan and κ- and λ-carrageenan were inhibitory to the replication of the DNA viruses, herpes virus[10] (HSV-1, HSV-2, TK⁻HS-1), and human cytomegalovirus (Baba *et al.*, 1988b). The compounds were active against the RNA viruses: VSV, Sinbis virus, and HIV-1. They were inactive against coxsackievirus, poliovirus, and parainfluenza virus. κ- and λ-Carrageenan disturbed the normal cell morphology of Vero cells[11] at a concentration of 40 μg/mL, respectively. The IC_{50} concentrations for these alga compounds are compared with the IC_{50} for dextran sulfate (10 kDA) in Table 3. The results indicate that "sulfated polysaccharides have a broad-spectrum antiviral activity against enveloped viruses" (Baba *et al.*, 1988b).

A subsequent article gave IC_{50} data (μg/mL) for giant cell formation, HIV-1 replication, and host cell viability for synthetic and natural sulfated polysaccharides: DS (1 to 500 kDa) dermatin sulfate (31 kDa), pentosan polysulfate

[9] Structures of F1–F7 were determined by Matulewicz and Cerezo (1987).

[10] Gonzalez *et al.* (1987) reported that carrageenan was active against some other enveloped viruses (HSV-2, Semliki Forest, vaccinia, swine fever) but not against others (vesicular stomatitis virus, measles virus). Activity against HIV was not tested. At 5 μg mL, carrageenan prevented the cell monolayer from destruction by HSV-1 growth. At 10 μg mL, carrageenan reduced the formation of new infectious HSV-1 virions by almost 10^5. No cytotoxic effects were detected with up to 200 μg/mL of carrageenan. In these studies. The antiviral index for carrageenan against HSV-1, defined as the IC_{50} for inhibition of cell growth (> 200 μg/mL) divided by the concentration conferring 50% protection of the cytopathic effect (< 2 μg/mL), is > 100. Viral protein synthesis is prevented by carrageenan only when the compound is present during virus entry. Studies with labeled virion particles show that carrageenan does not inhibit virus attachment at concentrations 10 times greater than those needed to block viral replication.

[11] Vero cells are derived from the kidney of the African green (vervet) monkey *Cercopithecus aethiops* and are used in virus replication studies and plaque assays (National Library of Medicine, IGM Metathesaurus Information Screen).

TABLE 3
Inhibitory Effect of Polysaccharides on Replication of DNA and RNA Viruses (Baba *et al.*, 1988b)

Virus	Cell culture	IC_{50} (μg/mL)			
		Dextran sulfate	Fucoidan	κ-Carrageenan	λ-Carrageenan
		DNA viruses			
HSV-1	PRK	2	1.7	3.7	1.6
HSV-2	PRK	0.5	1.1	2	1.5
TK⁻ HSV-1	PRK	1.3	1.5	15	4.5
CMV	HEL	0.5	2	2.8	0.3
		RNA viruses			
HIV-1	MT-4	0.5	2.8	12	1.9
Sindbis virus	Vero	3.5	7	7	2
VSV	PRK	0.3	0.3	0.3	0.2
VSV	HeLA	0.5	11	7	4

(3.1 kDa), fucoidan, heparin (11 Da), mannan sulfate[12] (30 kDa), ι-, κ-, and λ-carrageenan (Baba *et al.*, 1990). Comparative data were obtained for polyacrylic acid (PAA; >27 kDa), chlorite-oxidized oxyamylose (COAM; 150 kDa), suramin (1.4 kDa), and azidothymidine (267 Da). Sulfated polysaccharides, but not azidothymidine and other dideoxynucloside analogs, blocked giant cell formation and protected uninfected CD_4^+ Molt-4 cells against destruction by HIV-1-infected HUT-78 cells (Baba *et al.*, 1990). Some of the structural requirements that sulfated polysaccharides have to fulfill can be deduced from these experiments. For example, Fig. 1 indicates that, for dextran sulfate, the ratio of virus replication to giant cell formation is low at low molecular weights, rises rapidly between 3 and 40 kDa, and is then level. For dextran sulfate "the optimal molecular size should be in excess of 5,000 [Da]" (Baba *et al.*, 1990). Other structural features for activity include a least two sulfate groups/monosaccharides unit and the density of sulfate groups. Differences in the anionic groups (e.g., sulfonate vs sulfate) might explain the activity of suramin, and the inactivity of 1 kDa DS, in giant cell formation.

Giant cell formation and virus replication both depend on the interaction between gp120 and CD_4 (Baba *et al.*, 1990). However, the gp120 molecules in the former are in the viral envelope and in the latter are in the outer cell membrane. The consequence is that inhibition of giant cell formation is more difficult to achieve than inhibition of virus replication, presumably because it is more difficult block the gp120 molecules located on the cell surface than on the virus particle (Baba *et al.*, 1990). The newly determined structure of the gp120–CD_4 complex explains the topological features giving rise to this differential activity (Balter, 1998; Rizzuto *et al.*, 1998).

The anti-HIV-1 activities of various sulfated mono- and polysaccharides, including fucoidan (0.5–14 kDa) and λ-carrageenan (40 kDa), were measured by (1) HIV-1-induced

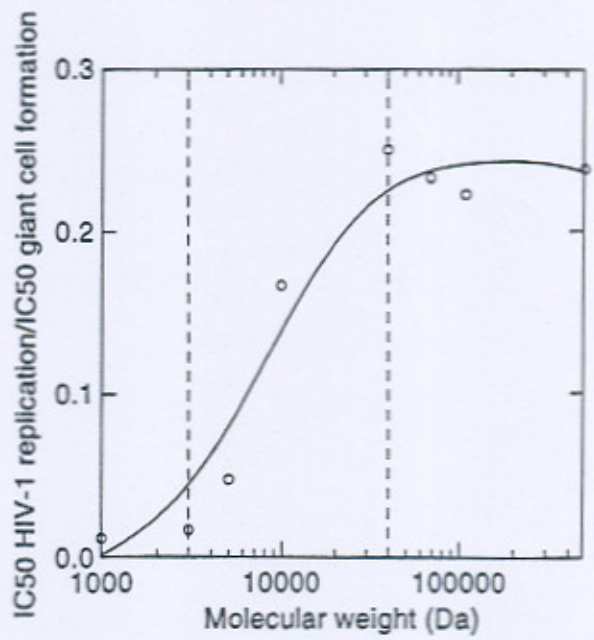

FIG. 1. Ratio of IC_{50} for HIV-1 replication to IC_{50} for giant cell formation, for dextran sulfates of molecular weights between 1000 and 500,000 Da. The plotted ratio is the inverse of that in Baba *et al.* (1990).

[12] Ito *et al.* (1989) reported that mannan sulfate (4 μg/mL) completely inhibited HIV-1-induced cell destruction and viral antigen expression in HIV-1-infected Molt-4 (clone 8) cells. The antiviral IC_{50} to Molt-4 (clone 8) cells and in MT-4 cells were 1.5 and 9.3 4 μg/mL, respectively. There was no toxicity for Molt-4 (clone 8) cells or MT-4 cells using 4000 and 2500 μg/mL, respectively. Mannan sulfate inhibited other enveloped viruses, i.e., HSV-1 and HSV-2, vaccinia (smallpox) virus, and vesicular stomatitis virus. In an effort to develop selective inhibitors of different classes of viruses, De Clercq (1990) investigated mannan sulfate, heparin, DS, and pentosan polysulfate as inhibitors of retroviruses.

syncytium formation; (2) infectivity of cell-free HIV-1 after preincubation with the compound; (3) protective ability of the agents for target CD_4^+ cells; and (4) anti-reverse transcriptase activity (Bagasra *et al.*, 1991). The toxicity of the compounds was measured by their effects on cellular proliferation, cytotoxicity and effects on coagulation processes. Sulfated polysaccharides and significant anti-HIV-1 activity. All of the sulfated polysaccharides inhibited HIV RT (25–99%) at 50 μg/mL. H9/HTLV-III_B-induced syncytium formation was inhibited by 6 μg/mL of DS, carrageenan, fucoidan, and pentosan polysulfate. [^{3}H]Thymidine incorporation into SUP-T1 cells, eosin Y exclusion assays, and preincubation studies, revealed that inhibition was not due to killing of target cells. Preexposure of cell-free virus to 200 μg/mL of fucoidan, carrageenan, dextran sulfates, and pentosan polysulfate caused a 100% reduction in the amount of HIV-1 p24 antigen released. The compounds prevented progression of syncytium formation after the initial HIV-1 infection. Preincubation of target cells with fucoidan and pentosan polysulfate, and less effectively, DS, protected the target cells from infection HIV-1. All of the sulfated polysaccharides at 50 μg/mL had anti-RT activity. All sulfated polysaccharides had significant anticoagulant activity, but fucoidan and carrageenan were less active than DS, heparin sulfate, and pentosan polysulfate. Fucoidan and carrageenan "seem to have the combination of anti-HIV-1 activities and low anticoagulation properties that may warrant clinical trials for their potential oral absorption" (Bagasra *et al.*, 1991).

The activity of fucoidan was compared with the activity of dextran sulfate, dextrin sulfate,[13] and AZT (McClure *et al.*, 1992). The polysaccharides were "potent inhibitors of diverse strains of HIV-1 in a variety of human cell lines and in peripheral blood lymphocytes (PBL) using a range of assays, including cell-free and cell-to-cell spread of infection" (McClure *et al.*, 1992). The polysaccharides did not adversely affect cell proliferation or protein metabolism of PBL. These compounds interact with target cells to inhibit virus entry, but do not neutralize virions directly. The sulfated polysaccharides inhibited infection of both CD_4^+ and CD_4^- cell lines by HIV, HTLV-1 and the simian retrovirus MPMV, which use receptors other than CD_4, were also inhibited. Radiolabeled fucoidan bound to an 18-kDa protein on the surface of human T cells, "but its significance is not yet clear" (McClure *et al.*, 1992).

Sulfated homopolysaccharides (fucoidan, DS, κ-carrageenan), neutral homopolysaccharides, and heteropolysaccharides (heparin, heparan sulfate) were cultivated with Molt-4 and supernatant from human T-cell lymphotropic virus type III (HTLV-III)-infected TALL-1 (Mizumoto *et al.*, 1988). The most potent anti-HTL-III activities occurred at mitogenic doses (cytopathic effects assay, fluorescence antibody assay, RT assay, cell proliferation assay). Neutral homopolysaccharides and sulfated heteropolysaccharides had little or no effect on anti-HTLV-III activities. The presence of a sulfate group is important in inhibiting growth of HTLV-III, but the greater potency of homopolysaccharides than heteropolysaccharides shows that the structure of the polysaccharides is also important.

Extending the study of Mizumoto *et al.* (1988), the activity against peripheral mononuclear cells of fucoidan, DS, and diethylaminoethyldextran (DEAE dextran, as the negative control) were tested alone or in combination with AZT (Sugawara *et al.*, 1989). Dextran sulfate (100 μg/mL), but not DEAE dextran, inhibited the RT activity of a coculture of mononuclear cells from people with AIDS and PHA-stimulated lymphocytes from healthy volunteers. Molt-4 cells were pretreated for 24 h with 100 μg/mL of fucoidan or DS. Dextran sulfate, but not fucoidan, reduced survival of HIV, which suggests that DS, but not fucoidan, was taken up in the cytoplasm. Molt-4 cells were also cultured in the presence of HIV for 2, 6, 12, or 24 h, washed to remove the remaining HIV, and cultured in the presence of fucoidan or DS for 10 days. Pretreatment with either compound for up to 12 h resulted in almost no RT activity. Evidently, HIV invades the cytoplasm of the target cells slowly enough that fucoidan and DS can react with the HIV in the cell membranes. Additional studies suggested that the compounds bind to the *env* protein of HIV. In another series of experiments, fucoidan was found to synergize with AZT (Fig. 2), and the authors suggested that this synergism might permit using much lower therapeutic doses of AZT.

2.2.3. Molecular mechanism of action of sulfated polysaccharides. Parish *et al.* (1990) determined the effects of

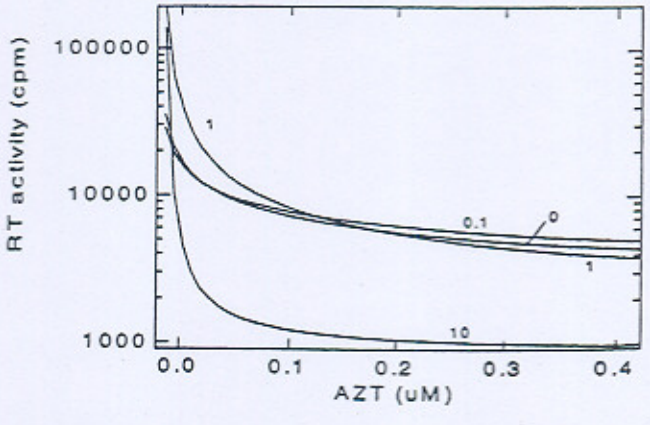

FIG. 2. Synergistic anti-HIV activity induced by fucoidan (0, 0.1, 1, or 10 μg/mL) and AZT (0, 0.005, 0.014, 0.042, 0.125, or 0.375 μM).

[13] Dextrin 2-sulfate (CAS RN 142985-55-5), a synthetic derivative of dextrin, should not be confused with dextran sulfate (CAS RN 9042-14-2). The parent compounds are dextrin (CAS RN 9004-53-9) and dextran (CAS RN 9004-54-0).

TABLE 4
Effect of Natural and Synthetic Sulfated Polyanions on HIV Infection and Anti-CD_4 mAb Binding (Parish *et al.*, 1990)

Compound	Mass (kDa)	Inhibition of anti-CD_4 binding (μg/mL)	Inhibition of HIV infection (μg/mL)		CD_4/HIV ratio
			Normal serum	Dextran sulfate-treated serum	
		Compounds found in algae			
κ-Carrageenan	400	400	> 1000	100	4
λ-Carrageenan	400	400	> 1000	> 100	< 4
ι-Carrageenan	400	> 800	100	100	8
Fucoidan	100	50	100	10	5
		Compounds from other natural sources			
Chondroitin-4-sulfate	40	> 1000	> 1000	> 1000	
Chondroitin-6-sulfate	60	> 1000	> 1000	> 1000	
Heparin	10	> 1000	> 1000	100	> 10
		Synthetic sulfated polyanions			
Dextran sulfate, 5 kDa	5	800	100	10	5
Dextran sulfate, 500 kDa	500	0.4	10	100	8
Polyvinyl sulfate	200	0.4	100	1	0.4
Polyanethole sulfonate	10	6	10	1	0.4
Pentosan sulfate	3	> 1000	1	1	> 1000

several natural and synthetic sulfated polyanions (SP) to inhibit HIV infection and block anti-CD_4 monoclonal antibody (mAb) binding (Table 4). Dextran sulfate (500 kDa), polyvinyl sulfate (PVS), and polyanethole sulfonate (PAS) strongly blocked the binding of the 11 mAb known to interact with the two amino-terminal Ig-like domains of CD_4. Dextran sulfate "exhibited an hierarchy of inhibition of anti-CD_4 mAb which suggests that SP bind to a conformational site incorporating the first two Ig-like domains of CD_4. This SP binding site is clearly distinct but closely associated with the gp120 binding region of CD_4" (Parish *et al.*, 1990). The anti-HIV activity of the SP was not at the virion level as evidenced by the lack of binding of rgp120 to immobilized SP and "preincubation of virions with SP did not affect infectivity." Based on anti-CD_4 mAb blocking studies and binding of soluble CD_4 to immobilized SP, many of the compounds exhibited some affinity for CD_4. The anti-HIV activity of DS and PVS, the most active compounds, "could be entirely due to disruption of the CD_4-gp120 interaction." However, the anti-HIV activity of heparin, fucoidan, the carrageenans, and PAS was not entirely due to CD_4 blocking and "some other anti-viral mechanism is also operating." Although the anti-HIV activity of pentosan sulfate was comparable to that of DS and PVS, it indicated little or no reactivity with CD_4 and consequently "must inhibit HIV infection by a totally CD_4-independent mechanism."

2.2.4. Other compounds isolated from marine algae with anti-HIV activity. 6-Sulfo-α-D-quinovopyranosyl-(1 → 3')-1',2'-diacylglycerol, a new sulfolipid from the marine red algae *Gigartina tenella*, is a potent inhibitor of eukaryotic DNA polymerases and HIV-RT-1 (Ohta *et al.*, 1998). Inhibition is dose-dependent. More than 90% inhibition of DNA polymerase alpha (pol α), DNA polymerase beta (pol β), and HIV RT-1 occurred at respective concentrations of 5, 10, and 30 μM; the respective IC_{50} values are 0.25, 3.6, and 11.2 μM (Ohta *et al.*, 1998).

Two sesquiterpene hydroquinones, peyssonol A and peyssonol B, were isolated from the Red Sea algae *Peyssonelia* species (Loya *et al.*, 1995). These compounds are potent inhibitors of the RNA-directed DNA synthesis of the RTs of HIV-1 and HIV-2. DNA-dependent DNA polymerase activity is inhibited to a lesser extent, and ribonuclease H (RNase H) activity is unaffected. The nature of the template primers used does not affect inhibition of DNA polymerase activity. Because the mode of inhibition by peyssonol A is noncompetitive with respect to both deoxyribonucleoside 5'-triphosphates (dNTPs) and template primer, peyssonol A is thought to bind the RT at a site distinct from those occupied by the substrates of the RNA-directed DNA synthesis. Peyssonol B is competitive with respect to the template primers, but is noncompetitive with respect to dNTP. Peyssonol B and the template primer bear no apparent structural resemblance. The simplest explanation of the activity of peyssonol B is that the competitive pattern of inhibition is due to indirect steric hindrance. Another explanation is that inhibition is due to the overlap of distinct binding sites of the enzyme for the inhibitor and the substrate. Last, the binding of the inhibitor to a distinct

TABLE 5
Anti-HIV Activity of Extracts of, and Compounds Isolated from, Cyanobacteria

Reference	Species	Compounds isolated	Other compounds tested
Gustafson *et al.* (1989)	*Lyngbya lagerheimii*, *Phormidium tenue* strain, CN-2-1 *Phormidium cebennse*, *Oscillatoria raciborskii*, *Scytonema burmanicum*, *Calothrix elenkinii*, *Anabaena variabilis*	Extracts containing sulfolipids (sulfoquinovosyl diacylglycerols)	
Reshef *et al.* (1997)	*Scytonema*, spp., *Oscillatoria* spp.	Sulfoglycolipids, (*Scytonema*), acylated diglycolipids (*Oscillatoria*)	
Loya *et al.* (1998)	*Oscillatoria raoi* (TAU IL-76-12), *Syctonema* spp. (TAU SL-30-1-4), *Oscillatoria trichoides* (TAU IL104-3-2) *Phormidium tenue* (TAU IL-144-1), *Oscillatoria limnetica* Lemmermann (TAU NG-4-1-2)	Sulfolipids, sulfoglycolipids, hydrolysis products, synthetic derivative	See Reshef *et al.* (1997) for previous work from this group
Ayehunie *et al.* (1998)	*Spirulina platensis* (now *Arthrospira platensis*)	Extract, activity in polysaccharide fraction	
Chamorro *et al.* (1996)	*Spirulina*		
Hayashi *et al.* (1996)	*Spirulina platensis* (marine)	Calcium spirulan (sulfated polysaccharide)	Calcium-free spirulan, desulfated compound from Ca-SP
Hayashi *et al.* (1996)	*Spirulina platensis* (marine)	Calcium spirulan, Ca-SP (sulfated polysaccharide)	Dextran sulfate
Lau *et al.* (1993)	900 strains	Lipophilic and hydrophilic extracts	AZT
Cardellina *et al.* (1993)	Review of natural products	Extracts	
Boyd *et al.* (1996)	*Nostoc ellipsosporum*	Cyanovirin-N (11-kDa antiviral protein)	
Gustafson *et al.* (1996)	*Nostoc ellipsosporum*	Cyanovirin-N (11-kDa antiviral protein)	
Nowotny *et al.* (1997)	*Microcystis aeruginosa*	Aqueous extract showed antiviral activity against influenza A virus	

site might induce conformational changes that distort the binding of the template primer. Both peyssonol A and peyssonol B interfere with the direct binding of the RT to the template primer, which might explain the mechanism of the enzyme inhibition. The authors concluded that the "insensitivity of DNA polymerase beta and the poor response of DNA polymerase alpha to peyssonol A make this inhibitor more attractive for the future development of a potent anti-HIV-RT drug" (Loya *et al.*, 1995).

2.3. *Cyanobacteria (Blue-Green Bacteria)*

Several studies have used extracts of freshwater and marine cyanobacteria. These are summarized in Table 5 and in the following discussion.

2.3.1. Glycolipids and sulfoglycolipids. Like other algae (Ohta *et al.*, 1998), a number of cyanobacteria species have been found to produce highly anti-HIV active sulfoglycolipids (Gustafson *et al.*, 1989; Reshef *et al.*, 1997; Loya *et al.*, 1998). In addition to their activity, the sulfoglycolipids constitute part of the chloroplast membrane and are therefore abundant (Harwood, 1987). Loya *et al.* (1998) concluded that together these facts argue "in favor of further development of these compounds as anti-AIDS agents."

Gustafson *et al.* (1989) used a tetrazolium-based microculture to screen extracts of cultured marine cyanobacteria, *Lyngbya lagerheimii* and *Phormidium tenue* (strain CN-2-1), for inhibition of HIV-1. This led to the discovery of sulfonic acid-containing glycolipids as a new class of HIV-1-inhibitory compounds. Four compounds were isolated and their structures characterized. The compounds have an α-linked hexapyranose backbone. The substitution pattern and relative stereochemistry were demonstrated by NMR proton decoupling and correlation spectroscopy (COSY) experiments to be similar to those of glucose. ^{13}C = NMR indicated the sulfonic acid moiety on the 6′ carbon of the sugar. The optical rotation of the cyclohexylammonium salt from one of the deacylated compounds revealed the absolute stereochemistry of the D-sulfoquinovosyl glycerol to be 2*S*. Mass spectral fragments from cleavage between C-1 and C-2 of the glycerol subunit were used to assign the position of the acyl groups. Linolenic acid was recovered from the hydrolysate of this compound. Sulfolipids "are structural components of chloroplast membranes and occur widely in higher plants, algae, and photosynthetic microorganisms."

The two active compounds isolated from *L. lagerheimii* had 18:3, 16.0 or 18:2, 16:0 fatty acid side chains (Gustafson *et al.*, 1989). The two active compounds isolated from *P. tenue* had 18:1, 16:0 or 16:1, 16:0 fatty acid side chains.

The pure compounds isolated from the algae cultures were active against HIV-1 in cultured human lymphoblastoid CEM, MT-2, LDV-7, and c3-44 cell lines in the tetrazolium assay, p24 vial protein assay, and syncytium formation assays of Nakashima *et al.* (1988). Other cyanobacteria, *Phormidium cebennse*, *Oscillatoria raciborskii*, *Scytonema burmanicum*, *Calothrix elenkinii*, and *Anabaena variabilis*, gave extracts that inhibited HIV-1, and gave positive tests for the presence of sulfolipids. The individual sulfolipids generally had comparable activity, "suggesting that acyl chain length and degree of unsaturation, at least over the small variation examined, do not critically affect potency. However, the concentrations resulting in inhibition ranged from 1 to 100 μg/mL, and depended on the cell line and the mode of infection. Structurally related acyl glycerols, complex lipids, detergents, and simple sulfonic acid derivatives did not protect against HIV-1 infection in the assays (Gustafson *et al.*, 1989).

Five new diacylated sulfoglycolipids were isolated from the cyanobacteria *Scytonema* spp. (TAU strain SL-30-1-4) and four new acylated diglycolipids were isolated from the cyanobacterium *Oscillatoria raoi* (TAU strain IL-76-1-2) (Reshef *et al.*, 1997). Related known glycolipids were isolated from these two and three other strains of cyanobacteria, *Phormidium tenue* (TAU strain IL-144-1), *O. trichoides* (TAU strain IL-104-3-2), and *O. limnetica* (TAU strain NG-4-1-2). Structures were assigned based on the selective hydrolysis of the glycerol ester moieties, GC-MS analysis of the methyl ester derivatives of the liberated fatty acids, homo- and heteronuclear-2D-NMR techniques, and MS. These compounds inhibited HIV-1 RT polymerase activity to different extents. Four sulfoglycolipids were potent inhibitors, and at a final concentration of 10 μM inhibited the initial enzymatic activity by almost 100%. Four glycolipids were moderate to weak inhibitors of RT DNA polymerase activity (65, 42, 33, and 8%). These patterns of activity were similar to the cell-culture results of sulfoglycolipids from *L. lagerheimii* (strain DN-7-1) and *P. tenue* (strain CN-2-1) reported by Gustafson *et al.* (1989).

Lipophilic extracts from several species of cyanobacteria produce potent *in vitro* inhibition of the enzymatic activity of the HIV-1 RT (Loya *et al.*, 1998). Compounds[14] **1–4, 9** are natural sulfoquinovopyranosyl glycerols (SQG); **5, 6, 8, 10** are natural monogalactopyranosyl glycerols (MGG); **7, 11** are natural digalactopyranosyl glycerols (DGG), and synthetic compound **12** is a monogalactopyranosyl glycerol (MGG). Activity was primarily attributed to the sulfoquinovosylpranosyl lipids. The four tested sulfoglycolipids efficiently and selectively inhibited the DNA polymerase activity of HIV-1 RT and not the ribonuclease H function. The IC_{50} concentrations were (**1**) 24, (**2**) 77, (**3**) 112, and (**4**)2950 nm. Natural substitution of the two hydroxy groups on the sugar moiety by palmitoyl residues resulted in a significant decrease in the maximal inhibition capacity. "It is possible, therefore, that the contribution of acylated groups to the molecule at these positions interferes with inhibition, possibly, by steric hindrance. Both the sulfonic acid moiety and the fatty acid ester side chain have a substantial effect in potentiating the extent of inhibition" (Loya *et al.*, 1998). Without the sulfonic acid moiety, the inhibitory effects of the four natural glycolipids tested were reduced, and hydrolysis of the fatty acid side chain removed most of the inhibition of HIV RT. For example, the IC_{50} of the unsulfonated galactosyl version (**6**) of the most potent sulfoglycolipid (**1**) was completely inactive against the DNA polymerase activity ($IC_{50} > 100$ μM). They postulated that "the lipophilic groups interact with the hydrophobic core of the enzyme, whereas the negatively charged sulfonic moiety may interact with the positively charged side chains on the enzyme" (Loya *et al.*, 1998). The sulfoglycolipids are inhibitors of HIV-1 RT and also HIV-2 RT.

2.3.2. Polysaccharides. It had previously been found that aqueous extracts from *Arthrospira platensis* (previously called *Spirulina platensis*) inhibited HSV-1 (Hayashi *et al.*, 1992). Subsequently, Ayehunie *et al.* (1998) determined that an aqueous extract of *A. platensis*, at concentrations that were nontoxic to human cells, inhibited syncytium formation and HIV-1 replication in human T-cell lines, peripheral blood mononuclear cells (PBMC), and Langerhans cells (LC). The EC_{50} of the extract was 0.01–0.10 μg/mL in the p24 gag antigen assay for inhibition of HIV-1 (strain HIV-IIIB) in Jurkat cells. Extract concentrations of 0.3 to 1.2 μg/mL reduced viral production by around 50% in PBMCs. The IC_{50} of the extract for PBMC growth varied between 0.8 and 3.1 mg/mL. The XTT assay[15] (Weislow *et al.*, 1989) was used to determine simultaneously cell growth inhibition and viral replication; the EC_{50} was 24 μg/mL. Langerhans cells pretreated for 4 h with the extract were pulsed with HIV-1 in the presence of extract. After extensive washing, the virus-exposed LC were cocultured with mitogen-activated, uninfected human T cells in the absence of extract. Cocultures with tumor necrosis factor-α (TNF-α) demonstrated that the algae extract had no significant effect on postintegration steps in the virus life cycle. "The brief treatment of the LC/HIV-1 mixture with extracted prevented virus transmission to susceptible T-cells; cytotoxicity

[14]Compound numbers *per* Loya *et al.* (1998), which should be consulted for structures. Reshef *et al.* (1997) should be consulted for the isolation procedure and structure determination.

[15]Metabolic reduction of XTT, the tetrazolium dye [sodium,3'-(1-[phenylamino-carbonyl]-3,4-tetrazolium)-bis(4-methoxy-6-nitro) benzene-sulfonic acid hydrate, by live, uninfected cells or cells protected from HIV-1 lysis by the alga extract, produces an orange XTT-formazan product. The orange product is quantified at a test wavelength of 450 nm and a reference wavelength of 650 nm.

was negligible at these extract concentrations." Therapeutic indices varied with cell type and ranged between 200 and 6000. Fractionation produced an active polysaccharide precipitate that had an anti-HIV-1 activity (EC_{50}) of 40 μg/mL, and 100 and 200 μg/mL completely inhibited syncytium formation. The supernatant fraction, which was depleted of polysaccharides and tannins, also inhibited HIV-1 replication and syncytium formation. When this material was further fractionated using water–methanol mixtures, the water and water–methanol fractions, but not the two methanol fractions, had anti-HIV-1 activity. The authors concluded that aqueous *A. platensis* extracts have antiretroviral activity of potential clinical interest.

Calcium spirulan (Ca-SP), a sulfated polysaccharide, was isolated from a marine blue-green alga, *S. platensis* (Hayashi *et al.*, 1996a). The initial analyses indicated that Ca-SP was composed of rhamnose, ribose, mannose, fructose, galactose, xylose, glucose, glucuronic acid, galacturonic acid, sulfate, and calcium. Lee *et al.* (1998) found that the Ca-SP backbone was 1,3-linked rhamnose and 1,2-linked 3-*O*-methylrhamnose units, with some sulfate substitution at the 4-position. The polymer was terminated at the nonreducing end by 2,3-di-*O*-methyl-rhamnose and 3-*O*-methylxylose residues.

Hayashi *et al.* (1996a) determined the inhibitory effect of Ca-SP on viral replication for the following virus (cell culture) pairs: HSV-1 (HeLa), HCMV (HEL), measles (Vero), mumps (Vero), influenza (MDCK), polio (Vero), coxsackie (Vero), and HIV-1 (MT-4). The IC_{50} for the cytotoxicity of Ca-SP against MT-4 cells was 2900 μg/mL. The concentration required to reduce virus replication by 50% (ED_{50}) was 11.4 μg/mL at 0 h for Ca-SP added to the medium immediately after viral infection, and 2.3 μg/mL at − 3 h for Ca-SP added to the medium 3 h before viral infection. The selectivity index (IC_{50}/ED_{50}) was 254 at 0 h and 1261 at − 3 h. The selectivity index for HSV-1 was 479 at 0 h and 8587 at − 3 h, and indices for HCMV were 117 at 0 h and 578 at − 3 h. Selectivity indices for the other viruses were generally smaller than these values. A calcium-free spirulan and a desulfated compound from Ca-SP were strongly toxic to the growth of HeLa cells and weakly inhibited HSV-1 replication, resulting in selectivity indices of 3.3 and 6.1, respectively.

Hayashi *et al.* (1996b) compared the anti-HIV-1 and anti-HIV activities of Ca-SP with those of DS. Anti-HIV-1 activities were measured using three assays: (1) viability of acutely infected CD_4-positive cells, or a cytopathogy assay; (2) determination of HIV-1 p24 antigen released into culture supernatants; and (3) inhibition of HIV-induced syncytium formation. Anti-HSV-1 activity was assessed by plaque yield reduction. The effects of these compounds on the blood coagulation processes, and stability in the blood, were also determined. The bioassays indicated that Ca-SP is a potent antiviral agent against both HIV-1 and HSV-1. Ca-SP was subsequently found to inhibit replication of several enveloped viruses, including HSV-1, HCMV, measles virus, mumps virus, influenza A virus, and HIV-1, and selectively inhibited the penetration of virus into host cells. Retention of molecular conformation by chelation of calcium ion with sulfate groups was suggested to be indispensable to its antiviral effect.

> Furthermore, Ca-SP is quite promising as an anti-HIV agent because even at low concentrations of Ca-SP an enhancement of virus-induced syncytium formation was not observed. As was observed in DS-treated cultures, Ca-SP had very low anticoagulant activity, and showed a much longer half-life in the blood of mice when compared with that of DS. Thus, Ca-SP can be a candidate agent for an anti-HIV therapeutic drug that might overcome the disadvantages observed in many sulfated polysaccharides. When the role of chelation of calcium ion with sulfate groups was examined by removing calcium or its replacement by sodium, the presence of calcium ion in the molecule was shown to be essential for the dose-dependent inhibition of cytopathic effect and syncytium formation induced by HIV-1. (Hayashi *et al.*, 1996b).

2.3.3. Cyanovirin-N. Cyanovirin-N (CV-N), was initially isolated from an aqueous cellular extract of the cyanobacterium *Nostoc ellipsosporum* (Boyd *et al.*, 1996) using bioassay-guided fractionation (Parish *et al.*, 1990). Cyanovirin-N is an 11-kDa (Boyd *et al.*, 1996), 101 amino acid antiviral peptide (NSC 682999; $C_{467}H_{737}N_{133}O_{164}S_4$) (Gustafson *et al.*, 1997; Bewley *et al.*, 1998) and is unlike any other protein thus far characterized. "Low nanomolar concentrations of CV-N prevent the *in vitro* replication and cytopathicity of primate retroviruses, including SIV and diverse laboratory strains and clinical isolates of HIV-1 and HIV-2. CV-N mediates these antiviral effects through apparently conserved interactions with the viral envelope glycorotein that differ from gp120 interactions with either the cellular receptor derivative, sCD_4, or with antibodies directed against viral gp120 neutralizing determinants" (Boyd *et al.*, 1996). The U.S. National Cancer Institute (NCI) selected this novel anti-HIV agent for preclinical development as a potential prophylactic viricide (Boyd *et al.*, 1996). This intense effort has resulted in a large number of papers on the structure of the compound, and mechanism(s) of action (Shoemaker *et al.*, 1996; Mori *et al.*, 1996, 1997a,b, 1988; Boyd *et al.*, 1997; Gustafson *et al.*, 1997; Bewley *et al.*, 1998; Mariner *et al.*, 1998; Esser *et al.*, 1999; Yang *et al.*, 1999). It was first proposed that cyanovirin inactivates HIV by interacting with the virus's surface envelope glycoprotein gp120 (McMahon *et al.*, 1996). Mariner *et al.* (1998) found that CV-N does not block the binding of sCD_4-receptor to HIV-1 lysates nor the attachment of intact HIV-1 virions to several target T-cell lines. They concluded that the virucidal effects of CV-N result from interference with a step(s) in the fusion process subsequent to the initial binding of the virus to target cells.

2.3.4. Uncharacterized extracts. To find new sources of antiviral agents with different mechanisms of action,

TABLE 6
Inhibition of Reverse Transcriptase Activity by Blue-green Algae extracts[a]

Extract	Species	Inhibition of RT activity (% of Control)		Extract activities degrading:		
		HIV-RT[b]	AMV-RT[c]	RNA[d]	DNA[e]	Protein[f]
9190	*Nostoc* sp.	76.7 ± 9.9	50.7 ± 24.9	−	−	−
9238	*Phormidium corium*	89.1 ± 2.8	71.5 ± 12.1	−	−	−
9240	*Oscillatoria acutissma*	85.7 ± 1.8	66.4 ± 17.8	−	−	−
9400	*Chroococcus minor*	75.4 ± 2.4	64.7 ± 16.0	+	+ +	−
9472	*Oscillatoria animalis*	87.8 ± 2.6	80.6 ± 9.9	−	+ +	−
9490	*Phormidium valderianum*	88.1 ± 3.1	65.8 ± 20.2	+ +	−	+
9512	*Phoridium molle*	92.9 ± 2.4	86.0 ± 6.6	−	+	−
9611	*Phormidium facosum*	73.5 ± 8.6	66.4 ± 11.7	−	+ +	−
9931	*Schizothrix pallida*	90.3 ± 2.9	70.0 ± 7.2	−	+	−
9969	*Aphanocapsa pulchra*	85.2 ± 3.5	75.8 ± 7.5	−	+ +	+ +
10029	*Synechococcus elongatus*	84.5 ± 1.4	77.8 ± 10.4	−	+	+ +
10033	*Oscillatoria prolifica*	78.8 ± 2.1	73.9 ± 9.6	−	+	−
10207	*Aphanothece clathrata*	86.5 ± 3.6	71.9 ± 13.2	−	+ +	−
10214	*Apanothece nidulans*	91.3 ± 0.4	74.2 ± 10.7	−	+ +	+ +
10393	*Oscillatoria foreaui*	73.4 ± 11.5	76.1 ± 17.2	−	+ +	−
10539	*Synechooccus elongatus*	93.1 ± 1.3	88.7 ± 4.0	−	+ +	+ +
10546	*Xenococcus* sp.	75.1 ± 13.8	59.1 ± 16.4	−	+ +	−
10560	*Oscillatoria amoena*	90.2 ± 4.0	88.4 ± 5.9	−	+ +	−
	AZT[g]	90.4 ± 3.2	88.3 ± 7.3			

[a]Adapted from Tables 1 and 2 of Lan *et al.* (1993).
[b]Average values ± SEM, $n = 3$.
[c]Average values ± SEM, $n = 4$.
[d]Activity degrading ^{32}P-labeled RNA, measured by gel electrophoresis and autoradiography.
[e]Activity degrading ^{3}H-labeled DNA, measured by precipitation and retention on a filter.
[f]Activity degrading ^{35}S-labeled protein, measured as for DNA.[e]
[g]Inhibition of RT activity of 1 μM AZT.
Note. −, no activity detected; +, some degradation of substrate; + +, significant degradation of substrate.

Hayashi *et al.* (1996c) assayed extracts of 49 algae for anti-HSV and anti-HIV activities. Anti-HSV activity was found for 25 aqueous extracts, 4 of which were potent inhibitors (selective index > 1000). Anti-HIV replication activity was found for 8 aqueous extracts. The results suggest that algae extracts "are a promising source of antiviral agents which may act on different stages in virus replication cycle" (Hayashi *et al.*, 1996c).

Lipophilic and hydrophilic extracts of over 900 strains of cultured blue-green algae were examined *in vitro* for their ability to inhibit the reverse transcriptases of AMV and HIV-1 (Lau *et al.*, 1993). Eighteen (2.0%) aqueous extracts exhibited activity against AMV and HIV RTs (Table 6). The maximal level of RT inhibition (668 ng/mL) was equivalent to that measured for AZT. Examination of partially purified fractions revealed that the observed RT inhibition was not entirely due to the degradation of transcript DNA, template RNA, or enzyme protein in the reaction mixture. The authors concluded that the extracts contained RT inhibitors that "are at least novel among the blue-green algae" (Lau *et al.*, 1993).

Cardellina *et al.* (1993) reported that about 15% of aqueous extracts from terrestrial plants, cyanobacteria, and marine invertebrates and algae exhibited HIV-antiviral activity in the National Cancer Institute's primary AIDS-antiviral screen. A considerable number of extracts were eliminated after removing anionic polysaccharides. A chemical screening protocol, utilizing various solid-phase extraction cartridges, was devised for a second-stage dereplication and to assist in prioritization of these extracts for further investigations. Of 1754 cyanobacteria tested, 26 (1.5%) organic acid and 124 (7.1%) aqueous extracts were selected for follow-up. Following the initial precipitation procedure, 99 of these 150 (66%) were eliminated because they contained polysaccharides, and 43 (29%) active supernatants were retained for further investigation. The comparable numbers for "marine plants" are 1779 extracts tested, of which 15 (0.8%) organic and 307 (17.3%) aqueous were selected for initial follow-up.

Microcystis spp. produce "microcystins," a well-studied class of heptapeptide toxins currently numbering over 60 compounds (Codd, 1995). However, *Microcystis* spp. also produce compounds with anti-retroviral activity. A crude

aqueous extract of a field-collected strain of *Microcystis aeruginosa* (Kutz) exhibited antiviral activity against influenza A virus (Nowotny *et al.*, 1997). The concentration for 50% inhibition of virus replication in MDCK cells was 11 μg dry extract/mL. The virus-specific protein synthesis decreased if the extract was present over the whole time of replication. Protease inhibitory activity was estimated for the crude aqueous extract and subextracts. It is likely that the extract(s) will indicate activity against other retroviruses, but its anti-HIV activity is not reported.

3. HUMAN CLINICAL STUDIES

Natural and synthetic sulfated polysaccharides have been tested for their prophylactic properties, and some synthetic compounds have been tested for tolerance when administered to humans. Studies in humans of compounds covered in this review have been reported, including dextran sulfate, pentosan sulfate, and carrageenan. Five studies used dextran sulfate, one used pentosan sulfate. Although dextran sulfate and pentosan sulfate are not obtained from algae, their use as reference sulfated polysaccharides in the majority of the studies reviewed above warrants discussion here as model compounds to determine the clinical potential of sulfated polysaccharides derived from algae. No clinical studies were located for fucoidan, but some studies relevant to humans have been published.

3.1. Spermicidal Activity of Sulfated Polysaccharides, Fucoidan, and Carrageenan

Pearce-Pratt and Phillips (1996) used an *in vitro* model to simulate the mechanism of HIV-1 transmission during coitus. Sexual transmission of HIV-1 is a cell-mediated process involving, initially, adhesion of HIV-1-infected mononuclear cells to epithelial cells. Sulfated polysaccharides were used to test the hypothesis that blocking adhesion would prevent HIV-1 transmission. Cell–cell adhesion was inhibited by 140-kDa ι-carrageenan (IC_{50} 125 μg/ml) and 500-kDA DS (IC_{50} 25,000 μg/ml), while 300-kDA λ-carrageenan, 154-kDA κ-carrageenan, DEAE enhanced adhesion, and heparin, fucoidan, pentosan polysulfate, chondroitin sulfate, and heparan sulfate had no effect. However, excepting heparan sulfate, all of the compounds blocked infection. Carrageenan, DS, heparin, fucoidan, pentosan polysulfate, and other sulfated polysaccharides blocked "infection by mechanisms other than adhesion at concentrations of a thousand times lower than the dosages that are needed to block cell adhesion" (Pearce-Pratt and Phillips, 1996). ι-Carrageenan blocked infection of epithelia at concentrations of 1–2 μg and blocked "adhesion to a far greater extent than the other sulfated polysaccharides tested ... [so it] may be the best choice of the sulfated polysaccharides tested for use as a vaginal microbiocide" (Pearce-Pratt and Phillips, 1996). Related *in vitro* studies demonstrated that fucoidan, carrageenan, and other sulfated polysaccharides were active in a dose-dependent manner against HTLV-I (Zacharopoulos and Phillips, 1997) or provided "significant protection" against genital herpes (Zeitlin *et al.*, 1997).

A Phase-1 clinical trial tested the safety of ι-carrageenan in a vaginal formulation. Twenty-five women used a formulation containing ι-carrageenan (PC 213) (Elias *et al.*, 1997). Use of 5 mL of a 2% gel formulation of ι-carrageenan was associated with significant irritation of the vaginal epithelium when administered once daily in the absence of sexual intercourse. "Given the small number of participants in this initial study, careful observation for potential irritation must also be included in larger studies of this and other vaginal formulations" (Elias *et al.*, 1997).

O'Connor and Jeffries (1996) used existing and newly modified assays to determine the virucidal activity of several commonly used topical spermicides against cell-free and cell-associated virus. Fucoidan and dextrin 2-sulfate had selectively indices (SI) of 200. Dextrin 2-sulfate has undergone other human tests (Shaunak *et al.*, 1994, 1998; Stafford *et al.*, 1997).

3.2. Safety and Tolerance to Orally Administered Dextran Sulfate

Lorentsen *et al.*, (1989a,b) found that 1800 mg of orally administered dextran sulfate (7–8 kDa) was poorly absorbed in six volunteers. However, an iv infusion of 225 or 300 mg of DS 48 h later resulted in peak plasma concentrations of 26–35 μg/mL; recovery of 25 to 29% of the dose in the urine; increases in activated partial thromboplastin time (APTT) of 3.5–9.2 times the baseline value (median increase, 6.9 ×); and increases in plasma lipolytic activity by 185–548 times the baseline value (median increase, 438 ×) (Lorentsen *et al.*, 1989a). A dose–response study carried out in 12 people determined that an iv dose of 0.5 mg produced significant increases in the plasma lipolytic activity and that the iv dose–response curve between 0.5 and 50 mg is steep (Lorentsen *et al.*, 1989b).

Abrams *et al.* (1989a,b) carried out a two-part study using DS. First, a phase I/II dose-ranging trial determined the tolerance and safety of oral DS (7–8 kDa) in HIV p24 antigen-positive individuals (ACTG 060) (Abrams *et al.*, 1989a). Ten patients in each of 3 cohorts (AIDS, ARC[16] and

[16] AIDS-related complex (ARC). A prodromal phase of infection with the human immunodeficiency virus (HIV). Laboratory criteria separating AIDS-related complex from AIDS include elevated or hyperactive B-cell humoral immune responses, compared to depressed or normal antibody reactivity in AIDS; follicular or mixed hyperplasia in ARC lumph nodes, leading to lymphocyte degeneration and depletion more typical of AIDS; evolving succession of histopathological lesions such as localization of Kaposi's sarcoma, signaling the transition to the full-blown AIDS (National Library of Medicine, IGM Metathesaurus Information Screen).

asymptomatic) were given 2700 mg/day, and 10 other patients in each cohort were given 5400 mg/day. They concluded that oral dextran sulfate "is not without toxicity despite the current lack of conclusive evidence of systemic absorption" (Abrams *et al.*, 1989a). In their second study, DS was given orally three times daily for 8 weeks in total daily doses of 900 to 5400 mg (Abrams *et al.*, 1989b). There was no change in CD_4 lymphocyte numbers, β_2-microglobulin levels, or HIV antigen levels. There is no evidence of systemic absorption of the parent compound. However, they concluded that "in view of the promising *in vitro* effects and acceptable toxicity, oral dextran sulfate as a potential antiretroviral agent continues to be studied" (Abrams *et al.*, 1989b).

Flexner *et al.* (1991) administered the maximally tolerated dose of DS by continuous iv infusion to 10 subjects with symptomatic HIV infection for up to 14 days. Parenteral DS is an anticoagulant, so the infusion was adjusted to produce an acceptable activated partial thromboplastin time. Although plasma drug concentrations were up to 200-fold greater than the IC_{50} for free HIV infectivity *in vitro*, circulating HIV antigen (p24) levels increased 32–130% (median, 73.5%) in eight subjects ($P < 0.001$ vs untreated historical controls) who received the drug for more than 3 days. Plasma DS levels did not change as the infusion rate decreased, consistent with a decline in estimated drug clearance over time. "Continuous intravenous dextran sulfate was toxic, producing profound but reversible thrombocytopenia in all eight subjects who received drug for more than 3 days and extensive but reversible alopecia in five of these subjects. Because of its toxicity and lack of beneficial effect on surrogate markers, dextran sulfate is unlikely to have a practical role in the treatment of symptomatic HIV infection" (Flexner *et al.*, 1991).

Hiebert *et al.* (1999) found that previous methods used to quantify DS in the body were inadequate. They used an agarose gel electrophoresis technique with toluidine blue staining to determine absorption of orally administered hydrogenated DS (8 kDa) in HIV-positive subjects in a short-term (single dose, 4 g/day for 5 days, seven subjects) and following long-term study (1 g, 4 times per day for 29 to 335 days, eight subjects. The kinetics of the drug in plasma and circulating peripheral blood lymphocyte (PBL) were determined in the short-term study after the first day's dose and on plasma and PBL samples obtained 5 min after administration on 4 subsequent days. In the long-term study, samples were collected at monthly visits within 4 h of the last dose. The drug was found in all urine samples from all subjects in both studies. Thus, DS is absorbed after oral administration and they recommended "further studies on its efficacy, particularly in the early stages of the disease, along with additional observations on its toxicity" (Hiebert *et al.*, 1999).

3.3. Pentosan Polysulfate Administered to HIV-Seropositive Patients with Kaposi's Sarcoma

Sixteen HIV-seropositive patients with Kaposi's sarcoma received pentosan polysulfate via continuous venous infusion for 3–6 weeks (2, 3, or 4 mg/kg/d) and then received a subcutaneous dose three times/week (2, 3, or 4 mg/kg/day, respectively) (Pluda *et al.*, 1993). The respective group sizes were 6, 5, and 5 patients. Five of the patients also received injections of 1 mg of pentosan polysulfate into two different lesions two times a week for 3 weeks and then once weekly for another 3 weeks. The maximally tolerated dose of pentosan polysulfate that could be given by continuous venous infusion was 3 mg/kg per day.

> After receiving pentosan polysulfate for 6 weeks, patients were administered 100 mg zidovudine (AZT) orally every 4 hours in conjunction with pentosan polysulfate. No patient had an objective clinical antitumor response to either systemic or intralesional pentosan polysulfate administration: however, three patients had stable Kaposi's sarcoma for 3–27 weeks. No statistically significant effect on CD_4 cells or serum HIV p24 antigen was noted during pentosan polysulfate administration. Dose-limiting toxic effects were characterized by anticoagulation and thrombocytopenia and were reversible .... However, no objective tumor response or evidence of anti-HIV activity was noted; therefore, no claim of activity can be made in this trial. (Pluda *et al.*, 1993)

4. DISCUSSION

Compounds present in algae can affect HIV infectivity by various mechanisms, some of which have been found to be the basis for activity of selected alga extracts and others for which there is probable cause. As an example of the latter, lectins are proteins that can bind, through a highly specific molecular interaction, to oligosaccharide (glycan) side chains linked to a peptide backbone of glycoproteins *via* serine/thereonine or asparagine (Balzarini *et al.*, 1992). Lectins with specificity for different glycan structures bind to the glycans present in the gp120 molecule in the HIV envelope. This results in inhibition of both virus–cell fusion and HIV infectivity (Lifson *et al.*, 1986; Hansen *et al.*, 1989) and syncytium formation (Balzarini *et al.*, 1992). However, none of the papers in this review specifically studied algal lectins (Lopezrodas and Costas, 1997; Schussler *et al.*, 1997) as anti-HIV-active compounds. The absence of research using algal lectins seems to be a gap in the studies of antiviral compounds in algae rather than a conclusion that algal lectins are inactive. This omission may have a "historical" basis in the early emphasis placed on sulfated polysaccharides from algae. Filalimouhim and Hours (1995) state that sulfated oligo- and polysaccharides "have proved multiple activities," including antiviral activity.

Numerous studies have examined the mode of action of sulfated polyanions (SP) (Witvrouw and De Clercq, 1997). Anti-CD_4 mAb binding-inhibition studies identified a SP

binding site on the CD_4 molecule "which is closely associated with, but distinct from, the HIV-gap 120 binding region" (Parish *et al.*, 1990). Natural and synthetic sulfated polysaccharides and other SP bind to lymphocyte CD_4 and inhibit binding of monoclonal antibodies to the first two domains of CD_4. Lynch *et al.* (1994) characterized this interaction using soluble CD_4 (sCD_4) with four or two extracellular domains, and with other peptide analogues. Dextran sulfate (500 kDa), polyvinyl sulfate, fucoidan, or κ-carrageenan, immobilized on carboxymethyl cellulose fibers, bound strongly to both the two-domain and the four-domain recombinant CD_4 molecules. Binding to recombinants was similar to that using native CD_4, and differed from the poor binding of dextran sulfate (5 kDa), chondroitin 6-sulfate, and pentosan sulfate. Recombinant gp120 bound poorly (< 10%) to all of the immobilized polyanions, although there was some binding to pentosan sulfate (17%). There was 20–30% binding of radiolabeled V3 loop peptide to polyvinyl sulfate, dextran sulfate (500 kDa), and pentosan sulfate. Based on these results and competitive binding studies, they concluded that "disruption of the CD_4–gp120 interaction is probably responsible for most of the observed antiviral activity of [sulfated polyanions] toward HIV infection of lymphocytes. However, HIV infection and gp120 binding to monocytes was unaffected by [sulfated polyanions], probably because [these compounds] were unable to block the CD_4-gp 120 interaction in monocytes" (Lynch *et al.*, 1994). The anti-HIV activity of polysulfates is due to their "shielding off the positively charged sites in the V3 loop of the viral envelope glycoprotein (gp120). The V3 loop is necessary for virus attachment to cell surface heparin sulfate ... before more specific binding occurs to the CD_4 receptor of CD_4^+ cells. This general mechanism also explains the broad antiviral activity of polysulfates against enveloped viruses. Variations in the viral envelope glycoprotein region may result in differences in the susceptibility of different enveloped viruses to compounds that interact with their envelope glycoproteins" (Witvrouw and De Clercq, 1997).

Several papers have reported on synergism between azidothymidine (AZT) and natural or synthetic sulfated polysaccharides, e.g., fucoidan (Sugawara *et al.*, 1989), pentosan polysulfate (Anand *et al.*, 1989, 1990), cyclodextrin polysulfate (Anand *et al.*, 1990), and dextran sulfate (Ueno and Kuno, 1987a,b). The use of combinations of low-toxicity, synergistically acting antiviral agents that target different sites in the HIV replicative cycle could prevent the emergence of drug-resistant HIV mutants (Anand *et al.*, 1989; Witvrouw and De Clercq, 1997).

5. CONCLUSION

Sulfated polysaccharides have no immediate application in the treatment of HIV. However "comprehension of the mechanisms of action is a main strategy for the construction of specific inhibitors of the enveloped virus. The research of new sulfated polysaccharides in macroalgae, microalgae and cyanobacteria is a challenge for the future" (Filalimouhim and Hours, 1995).

> The efficacy of polysulfates in the therapy and/or prophylaxis of retroviral infections and opportunistic infections remains to be demonstrated both in animal models and humans. It is important to consider not only treatment of patients who are already infected with HIV, but also prophylaxis and protection from HIV and/or other virus infections. Because (i) sexual transmission is responsible for the large majority of HIV infections worldwide; (ii) this transmission is mostly mediated via mononuclear cells that infect epithelial cells of the genital tract; and because (iii) polysulfates effectively inhibit cell-cell adhesion, polysulfates may be considered as potentially effective in a vaginal formulation to protect against HIV infection. (Witvrouw and De Clercq, 1997)

Algae produce many other classes of compounds with known biochemical and cellular actions, and it might be worthwhile to investigate their anti-HIV activity. For example, algae produce compounds with potent antimitotic activity and specific antimicrotubule activity, e.g., microcystin-LR obtained from *Microcystis* spp. (Wickstrom *et al.*, 1995; Khan *et al.*, 1996). Some studies have investigated the effects on the human immunodeficiency virus of nonalga compounds having antimitotic or antimicrotubule activity (Sarin *et al.*, 1987; Grippo *et al.*, 1991; Husson *et al.*, 1997). For example, Itoh *et al.* (1990) studied the mitogenic activity and anti-HIV activity of polysaccharides of differing sulfur content produced by sulfating schizophyllan (sizofiran), a β-1,3-glycan produced by the fungus *Schizophyllum commune* Fries. Anti-mitogenic compounds derived from algae do not seem to have been similarly tested for anti-HIV activity.

Toxicologists need to perform studies of the anti-HIV activity of compounds from aquatic and terrestrial algae and cyanobacteria that have different modes of toxic action (e.g., antimicrotubule and antimitotic agents, steroids, and other classes) than those identified in this article. For example, chlorine-containing β-carboline alkaloids isolated from the terrestrial blue-green algae *Dichothrix baueriana* GO-25-2 (Larsen *et al.*, 1994) and indolocarbazoles isolated from *Nostoc sphaericum* EX-5-1 (Knubel *et al.*, 1990) had anti-HSV-2 activity but have not been tested for anti-HIV activity. The thiazolo-*iso*-indolinones is a new class of non-nucleoside inhibitors of HIV-1 reverse transcriptase (Maass *et al.*, 1993). Cyanobacteria produce indolines such as welwistatin (from *Hapalosiphon welwitschii*), which is a compound that circumvents multiple drug resistance that has been recommended for use in the chemotheraphy of drug-resistant tumors (Zhang and Smith, 1996). Another lead comes from patient records which suggest that cyanobacteria produce unidentified compounds that enhance the immune system or have anti-HIV activity. For example, the authors are undertaking human studies to confirm

physician reports that daily doses of the cyanobacterium *Aphanizomenon flos-aquae* enhance the immune system activity of patients with diseases, including HIV infections, that reduce immune system activity (Krylov *et al.*, 1999).

ACKNOWLEDGMENT

The authors thank Christian Drapeau (Cell Tech, Klamath Falls, OR) for providing unrestricted partial support of this research.

REFERENCES

Abrams, D., Pettinelli, C., Power, M., Kubacki, V. B., Grieco, M. H., and Henry, W. K. (1989a). A phase I/II dose ranging trial of oral dextran sulfate in HIV p24 antigen positive individuals (ACTG 060): Results of a safety and efficacy trial. *Int. Conf. AIDS* **5**, 404.

Abrams, D. I., Kuno, S., Wong, R., Jeffords, K., Nash, M., Molaghan, J. B., Gorter, R., and Ueno, R. (1989b). Oral dextran sulfate (UA001) in the treatment of the acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. *Ann. Intern. Med.* **110**, 183–188.

Anand, R., Nayyar, S., Galvin, T. A., Merril, C. R., and Bigelow, L. B. (1989). Sodium pentosan polysulfate (SP-54), an anti-HIV agent also exhibits lymphocyte proliferative activity and synergism with AZT. *Int. Conf. AIDS* **5**, 635.

Anand, R., Nayyar, S., Galvin, T. A., Merril, C. R., and Bigelow, L. B. (1990). Sodium pentosan polysulfate (PPS), an anti-HIV agent also exhibits synergism with AZT, lymphoproliferative activity, and virus enhancement. *AIDS Res. Hum. Retroviruses* **6**, 679–689.

Anand, R., Nayyar, S., and Pitha, J. (1990). The sulfated sugar alpha-cyclodextrin sulfate (α-CDS) is a potent anti-HIV agent, has marked synergism with AZT, and exhibits lymphoproliferative activity. *Int. Conf. AIDS* **6**, 178.

Ayehunie, S., Belay, A., Baba, T. W., and Ruprecht, R. M. (1998). Inhibition of HIV-1 replication by an aqueous extract of *Spirulina platensis* (*Arthrospira platensis*). *J. Acquir. Immune. Defic. Syndr. Hum. Retrovirol.* **18**, 7–12.

Baba, M., Nakajima, M., Schols, D., Pauwels, R., Balzarini, J., and De Clercq, E. (1988a). Pentosan polysulfate, a sulfated oligosaccharide, is a potent and selective anti-HIV agent *in vitro*. *Antiviral Res.* **9**, 335–343.

Baba, M., Snoeck, R., Pauwels, R., and De Clercq, E. (1988b). Sulfated polysaccharides are potent and selective inhibitors of various enveloped viruses, including herpes simplex virus, cytomegalovirus, vesicular stomatitis virus, and human immunodeficiency virus. *Antimicrob. Agents Chemother.* **32**, 1742–1745.

Baba, M., Schols, D., Pauwels, R., Nakashima, J., Balzarini, E., and De-Clereq, E. (1989). Comparative inhibitory effects of sulfated polysaccharides on giant cell formation induced by co-cultivation of CD_4-positive cells with persistently HIV-1-infected cells. *Int. Conf. AIDS* **5**, 554–554.

Baba, M., Schols, D., Pauwels, R., Nakashima, H., and De Clercq, E. (1990). Sulfated polysaccharides as potent inhibitors of HIV-induced syncytium formation: A new strategy towards AIDS chemotherapy. *J. Acquir. Immune. Defic. Syndr.* **3**, 493–499.

Bagasra, O., Lischner, H. W., Halligen, G. E., and Heins, B. (1989). Anti-HIV-1 activities of sulfated monosaccharides (SMS) and polysaccharides (SPS): Therapeutic indices (TI). *Int. Conf. AIDS* **5**, 660.

Bagasra, O., Whittle, P., Heins, B., and Pomerantz, R. J. (1991). Anti-human immunodeficiency virus type 1 activity of sulfated monosaccharides: comparison with sulfated polysaccharides and other polyions. *J. Infect. Dis.* **164**, 1082–1090.

Balter, M. (1998). Revealing HIV's T cell passkey. *Science* **280**, 1833–1834.

Balzarini, J., Neyts, J., Schols, D., Hosoya, M., Van Damme, E., Peumans, W., and De Clercq, E. (1992). The mannose-specific plant lectins from *Cymbidium* hybrid and *Epipactis helleborine* and the (*N*-acetylglucosamine)$_n$-specific plant lectin from *Urtica dioica* are potentent and selective inhibitors of human immunodeficiency virus and cytomegalovirus replication *in vitro*. *Antiviral Res.* **18**, 191–207.

Batinic, D., and Robey, F. A. (1992). The V3 region of the envelope glycoprotein of human immunodeficiency virus type 1 binds sulfated polysaccharides and CD_4-derived synthetic peptides. *J. Biol. Chem.* **267**, 6664–6671.

Beress, A., Wassermann, O., Tahhan, S., Bruhn, T., Beress, L., Kraiselburd, E. N., Gonzalez, L. V., de Motta, G. E., and Chavez, P. I. (1993). A new procedure for the isolation of anti-HIV compounds (polysaccharides and polyphenols) from the marine alga *Fucus vesiculosus*. *J. Nat. Prod.* **56**, 478–488. [Erratum appears in *J. Nat. Prod.* (1996). **59**(5), 552]

Bewley, C. A., Gustafson, K. R., Boyd, M. R., Covell, D. G., Bax, A., Clore, G. M., and Gronenborn, A. M. (1998). Solution structure of cyanovirin-N, a potent HIV-inactivating protein. *Nat. Struct. Biol.* **5**, 571–578.

Boyd, M. R., Gustafson, K., McMahon, J., and Shoemaker, R. (1996). Discovery of cyanovirin-N, a novel HIV-inactivating protein from *Nostoc ellipsosporum* that targets viral gp120. *Int. Conf. AIDS* **11**, 71–71.

Boyd, M. R., Gustafson, K. R., McMahon, J. B., Shoemaker, R. H., O'Keefe, B. R., Mori, T., Gulakowski, R. J., Wu, L., Rivera, M. I., Laurencot, C. M., Currens, M. J., Cardellina, J. H., Buckheit, R. W. J., Nara, P. L., Pannell, L. K., Sowder, R. C., and Henderson, L. E. (1997). Discovery of cyanovirin-N, a novel human immunodeficiency virus-inactivating protein that binds viral surface envelope glycoprotein gp120: Potential applications to microbicide development. *Antimicrob. Agents Chemother.* **41**, 1521–1530.

Burkholder, P. R., and Sharma, G. M. (1969). Antimicrobial agents from the sea. *Lloydia* **32**, 466–483.

Cardellina, J. H., Munro, M. H., Fuller, R. W., Manfredi, K. P., McKee, T. C., Tischler, M., Bokesch, H. R., Gustafson, K. R., Beutler, J. A., and Boyd, M. R. (1993). A chemical screening strategy for the dereplication and prioritization of HIV-inhibitory aqueous natural products extracts. *J. Nat. Prod.* **56**, 1123–1129.

Carlucci, M. J., Pujol, C. A., Ciancia, M., Noseda, M. D., Matulewicz, M. C., Damonte, E. B., and Cerezo, A. S. (1997a). Antiherpetic and anticoagulant properties of carrageenans from the red seaweed *Gigartina skottsbergii* and their cyclized derivatives: Correlation between structure and biological activity. *Int. J. Biol. Macromol.* **20**, 97–105.

Carlucci, M. J., Scolaro, L. A., Errea, M. I., Matulewicz, M. C., and Damonte, E. B. (1997b). Antiviral activity of natural sulphated galactans on herpes virus multiplication in cell culture. *Planta Med.* **63**, 429–432.

Chamorro, G., Salazar, M., Favila, L., and Bourges, H. (1996). Pharmacology and toxicology of *Spirulina* alga. *Rev. Invest. Clin.* 389–399.

Codd, G. A. (1995). Cyanobacterial toxins: Occurrence properties and biological significance. *Water Sci. Technol.* **32**, 149–156.

Damonte, E. B. (1996). Antiviral agents that act in the early phases of the viral cycle. *Rev. Argent. Microbiol.* **28**, 204–216.

Damonte, E. B., Matulewicz, M. C., Cerezo, A. S., and Coto, C. E. (1996). Herpes simplex virus-inhibitory sulfated xylogalactans from the red seaweed *Nothogenia fastigiata*. *Chemotheraphy* **42**, 57–64.

Damonte, E. B., Neyts, J., Pujol, C. A., Snoeck, R., Andrei, G., Ikeda, S., Witvrouw, M., Reymen, D., Haines, H., and Matulewicz, M. C. (1994). Antiviral activity of a sulfated polysaccharide from the red seaweed *Nothogenia fastigiata*. *Biochem. Pharmacol.* **47**, 2187–2192.

De Clercq, E. (1990). Selective virus inhibitors. *Microbiologica* **13**, 165–178.

Deig, E. F. (1974). Inhibition of herpes virus replication by marine algae extracts. *Antimicrob. Agents Chemother.* **6**, 524–525.

Ehresmann, D. W., Dieg, E. F., Hatch, M. T., DiSalvo, L. H., and Vedros, N. A. (1977). Antiviral substances from California marine algae. *J. Phycol.* **13,** 37–40.

Elias, C. J., Coggins, C., Alvarez, F., Brache, V., Fraser, I. S., Lacarra, M., Lahteenmaki, P., Massai, R., Mishell, D. R., Jr., Phillips, D. M., and Salvatierra, A. M. (1997). Colposcopic evaluation of a vaginal gel formulation of iota-carrageenan. *Contraception* **56,** 387–389.

Esser, M. T., Mori, T., Mondor, I., Sattentau, Q. J., Dey, B., Berger, E. A., Boyd, M. R., and Lifson, J. D. (1999). Cyanovirin-N binds to gp120 to interfere with CD_4-dependent human immunodeficiency virus type 1 virion binding, fusion, and infectivity but does not affect the CD_4 binding site on gp120 or soluble CD_4-induced conformational changes in gp120. *J. Virol.* **73,** 4360–4371.

Filalimouhim, R., and Hours, M. (1995). Antiviral activities of sulfated polysaccharides. *Acta Botanica Gallica* **142,** 125–130.

Flexner, C., Braditch-Crovo, P. A., Kornhauser, D. M., Farzadegan, H., Nerhood, L. J., Chaisson, R. E., Bell, K. M., Lorentsen, K. J., Hendrix, C. W., and Petty, B. G. (1991). Pharmacokinetics, toxicity, and activity of intravenous dextran sulfate in human immunodeficiency virus infection. *Antimicrob. Agents Chemother.* **35,** 2544–2550.

Fogg, G. E. (1953). *The Metabolism of Algae.* Methuen, London.

Gerber, P., Dutcher, J. D., Adams, E. V., and Sherman, J. H. (1958). Protective effect of seaweed extracts for chicken embryos infected with influenza B or mumps. *Proc. Soc. Exp. Biol. Med.* **99,** 590–593.

Gonzalez, M. E., Alarcon, B., and Carrasco, L. (1987). Polysaccharides as antiviral agents: Antiviral activity of carrageenan. *Antimicrob. Agents Chemother.* **31,** 1388–1393.

Grippo, P., Orlando, P., Carretta, F., Pastore, M., Strazzullo, G., Manzari, V., Sinibaldi, P., and Silvestri, I. (1991). Inhibition of HTLV1 replication by Kelletinin A. *Int. Conf. AIDS* **7,** 103.

Gustafson, K. R., Cardellina, J. H., Fuller, R. W., Weislow, O. S., Kiser, R. F., Snader, K. M., Patterson, G. M., and Boyd, M. R. (1989). AIDS-antiviral sulfolipids from cyanobacteria (blue-green algae). *J. Natl. Cancer Inst.* **81,** 1254–1258.

Gustafson, K. R., Sowder, R. C., Henderson, L. E., Cardellina, J. H., McMahon, J. B., Rajamani, U., Pannell, L. K., and Boyd, M. R. (1997). Isolation, primary sequence determination, and disulfide bond structure of cyanovirin-N, an anti-HIV (human immunodeficiency virus) protein from the cyanobacterium *Nostoc ellipsosporum*. *Biochem. Biophys. Res. Commun.* **238,** 223–228.

Gustafson, K. R., Sowder, R., Henderson, L., Pannel, L., Cardellina, J., McMahon, J., Shoemaker, R., and Boyd, M. (1996). Sequence determination and chemical characteristics of a novel anti-HIV protein, cyanovirin-N, isolated from the cyanobacterium *Nostoc ellipsosporum*. *Int. Cont. AIDS* **11,** 70.

Hansen, J. E., Nielsen, C. M., Nielsen, C., Heegaard, P., Mathiesen, L. R., and Nielsen, J. O. (1989). Correlation between carbohydrate structures on the envelope glycoprotein gp120 of HIV-1 and HIV-2 and syncytium inhibition with lectins. *AIDS* **3,** 635–641.

Harwood, J. (1987). Sulfolipids. In P. K. Stumpf (Ed.), Vol. 9, pp. 275–289. *The Biochemistry of Plants.* Academic Press, New York.

Hasui, M., Matsuda, M., Okutani, K., and Shigeta, S. (1995). *In vitro* antiviral activities of sulfated polysaccharides from a marine microalga (*Cochlodinium polykrikoides*) against human immunodeficiency virus and other enveloped viruses. *Int. J. Biol. Macromol.* **17,** 293–297.

Hayashi, K., Hayashi, T., and Morita, M. (1992). An extract of *Spirulina platensis* is a selective inhibitor of herpes simplex virus type-1 penetration into HeLa cells. *Phytother. Res.* **7,** 7680–7680.

Hayashi, T., Hayashi, K., Maeda, M., and Kojima, I. (1996a). Calcium spirulan, an inhibitor of enveloped virus replication, from a blue-green alga *Spirulina platensis*. *J. Nat. Prod.* **59,** 83–87.

Hayashi, K., Hayashi, T., and Kojima, I. (1996b). A natural sulfated polysaccharide, calcium spirulan, isolated from *Spirulina platensis*: *In vitro* and *ex vivo* evaluation of anti-herpes simplex virus and anti-human immunodeficiency virus activities. *AIDS Res. Hum. Retroviruses* **12,** 1463–1471.

Hayashi, K., Hamada, J., and Hayashi, T. (1996c). A screening strategy for selection of anti-HSV-1 and anti-HIV extracts from algae. *Phytother. Res.* **10,** 233–237.

Hiebert, L. M., Wice, S. M., Jaques, L. B., Williams, K. E., and Conly, J. M. (1999). Orally administered dextran sulfate is absorbed in HIV-positive individuals. *J. Lab. Clin. Med.* **133,** 161–170.

Hoshino, T., Hayashi, T., Hayashi, K., Hamada, J., Lee, J. B., and Sankawa, U. (1998). An antivirally active sulfated polysaccharide from *Sargassum horneri* (TURNER) C. AGARDH. *Biol. Pharm. Bull.* **21,** 730–734.

Husson, G. P., Subra, F., Lai-Kuen, R., and Vilagines, R. (1997). Antiviral activity of hydroalcoholic extract from *Haemanthus albiflos* on the Moloney murine leukemia virus and the human immunodeficiency virus. *C. R. Seances. Soc. Biol. Fil.* **191,** 473–485.

Ito, M., Baba, M., Hirabayashi, K., Matsumoto, T., Suzuki, M., Suzuki, S., Shigeta, S., and De Clercq, E. (1989). *In vitro* activity of mannan sulfate, a novel sulfated polysaccharide, against human immunodeficiency virus type 1 and other enveloped viruses. *Eur. J. Clin. Microbiol. Infect. Dis.* **8,** 171–173.

Itoh, W., Sugawara, I., Kimura, S., Tabata, K., Hirata, A., Kojima, T., Mori, S., and Shimada, K. (1990). Immunopharmacological study of sulfated schizophyllan (SPG). I. Its action as a mitogen and anti-HIV agent. *Int. J. Immunopharmacol.* **12,** 225–233.

Jagodzinski, P. P., Wiaderikiewicz, R., Kurzawski, G., Kloczewiak, M., Nakashima, H., Hyjek, E., Yamamoto, N., Uryu, T., Kaneko, Y., and Posner, M. R. (1994). Mechanism of the inhibitory effect of curdlan sulfate on HIV-1 infection *in vitro*. *Virology* **202,** 735–745.

Khan, S. A., Wickstrom, M. L., Haschek, W. M., Schaeffer, D. J., Ghosh, S., and Beasley, V. R. (1996). Microcystin-LR and kinetics of cytoskeletal reorganization in hepatocytes, kidney cells, and fibroblasts. *Nat. Toxins* **4,** 206–214.

Knubel, G., Larsen, L. K., Moore, R. E., Levine, I. A., and Patterson, G. M. (1990). Cytotoxic, antiviral indolocarbazoles from a blue-green alga belonging to the Nostocaceae. *J Antibiot. (Tokyo)* **43,** 1236–1239.

Kolender, A. A., Pujol, C. A., Damonte, E. B., Matulewicz, M. C., and Cerezo, A. S. (1997). The system of sulfated alpha-(1 → 3)-linked D-mannans from the red seaweed *Nothogenia fastigiata*: Structures, antiherpetic and anticoagulant properties. *Carbohydr. Res.* **304,** 53–60.

Krylov, V. S., C. Hassell, J. D., Strickland, J. W., and Schaeffer, D. J. (1999). Retrospective epidemiological study using medical records to determine which diseases are improved by *Aphanizomenon flos-aquae* supplements. *Med. Hypotheses.* (Manuscript submitted)

Larsen, L. K., Moore, R. E., and Patterson, G. M. (1994). beta-Carbolines from the blue-green alga *Dichothrix baueriana*. *J. Nat. Prod.* **57,** 419–421.

Lau, A. F., Siedlecki, J., Anleitner, J., Patterson, G. M., Caplan, F. R., and Moore, R. E. (1993). Inhibition of reverse transcriptase activity by extracts of cultured blue-green algae (cyanophyta). *Planta Med.* **59,** 148–151.

Lifson, J., Coutre, S., Huang, E., and Engleman, E. (1986). Role of envelope glycoprotein carbohydrate in human immunodeficiency virus (HIV) infectivity and virus-induced cell fusion. *J. Exp. Med.* **164,** 2101–2106.

Lopezrodas, V., and Costas, E. (1997). Characterization of morphospecies and strains of *Microcystis* (cyanobacteria) from natural populations and laboratory clones using cell probes (lectins and antibodies). *J. Phycol.* **33,** 446–454.

Lorentsen, K., Hendrix, C., Collins, J., Eckel, R., Petty, B., and Lietman, P. (1989a). Dextran sulfate is poorly absorbed after oral administration. *Int. Conf. AIDS* **5,** 336.

Lorentsen, K., Hendrix, C., Collins, J. M., Kornhauser, D. M., Petty, B. G., Klecker, R. W., Flexner, C., Eckel, R. H., and Lietman, P. S. (1989b). Dextran sulfate is poorly absorbed after oral administration. *Ann. Intern. Med.* **111,** 561–566.

Loya, S., Bakhanashvili, M., Kashman, Y., and Hizi, A. (1995). Peyssonols A and B, two novel inhibitors of the reverse transcriptases of human immunodeficiency virus types 1 and 2 *Arch. Biochem. Biophys.* **316,** 789–796.

Loya, S., Reshef, V., Mizrachi, E., Silberstein, C., Rachamim, Y., Carmeli, S., and Hizi, A. (1998). The inhibition of the reverse transcriptase of HIV-1 by the natural sulfoglycolipids from cyanobacteria: Contribution of different moieties to their high potency. *J. Nat. Prod.* **61,** 891–895.

Lynch, G., Low, L., Li, S., Sloane, A., Adams, S., Parish, C., Kemp, B., and Cunningham, A. L. (1994). Sulfated polyanions prevent HIV infection of lymphocytes by disruption of the CD_4–gp120 interaction, but do not inhibit monocyte infection. *J. Leukoc. Biol.* **56,** 266–272.

Maass, G., Immendoerfer, U., Koenig, B., Leser, U., Mueller, B., Goody, R., and Pfaff, E. (1993). Viral resistance to the thiazolo-iso-indolinones, a new class of nonnucleoside inhibitors of human immunodeficiency virus type 1 reverse transcriptase. *Antimicrob. Agents Chemother.* **37,** 2612–2617.

Mariner, J. M., McMahon, J. B., O'Keefe, B. R., Nagashima, K., and Boyd, M. R. (1998). The HIV-inactivating protein, cyanovirin-N, does not block gp120-mediated virus-to-cell binding. *Biochem. Biophys. Res. Commun.* **248,** 841–845.

Matulewicz, M. C., and Cerezo, A. S. (1987). Water-soluble sulfated polysaccharides from the red seaweed *Chaetangium fatigiatum*. Analysis of the system and structures of the α-D-(1 → 3)-linked mannans. *Carbohyd. Pol.* **7,** 121–132.

McClure, M. O., Moore, J. P., Blanc, D. F., Scotting, P., Cook, G. M., Keynes, R. J., Weber, J. N., Davies, D., and Weiss, R. A. (1992). Investigations into the mechanism by which sulfated polysaccharides inhibit HIV infection *in vitro*. *AIDS Res. Hum. Retroviruses* **8,** 19–26.

McClure, M. O., Whitby, D., Patience, C., Gooderham, N. J., Bradshaw, A., Cheingsong, P. R., Weber, J. N., Davies, D. S., and Cook, G. M.W. (1991). Dextrin sulphate and fucoidan are potent inhibitors of HIV infection *in vitro*. *Antiviral Chem. Chemother.* **2,** 149–156.

Mizumoto, K., Sugawara, I., Ito, W., Kodama, T., Hayami, M., and Mori, S. (1988). Sulfated homopolysaccharides with immunomodulating activities are more potent anti-HTLV-III agents than sulfated heteropolysaccharides. *Jpn. J. Exp. Med.* **58,** 145–151.

Moen, L. K., and Clark, G. F. (1993). A novel reverse transcriptase inhibitor from *Fucus vesiculosus*. *Int. Conf. AIDS* **9,** 145–161.

Mori, T., Gustafson, K. R., Pannell, L. K., Shoemaker, R. H., Wu, L., McMahon, J. B., and Boyd, M. R. (1998). Recombinant production of cyanovirin-N, a potent human immunodeficiency virus-inactivating protein derived from a cultured cyanobacterium. *Protein Expr. Purif.* **12,** 151–158.

Mori, T., Shoemaker, R. H., Gulakowski, R.J., Krepps, B. L., McMahon, J. B., Gustafson, K. R., Pannell, L. K., and Boyd, M. R. (1997a). Analysis of sequence requirements for biological activity of cyanovirin-N, a potent HIV (human immunodeficiency virus)-inactivating protein, *Biochem. Biophys. Res. Commun.* **238,** 218–222.

Mori, T., Shoemaker, R. H., McMahon, J. B., Gulakowski, R. J., Gustafson, K. R., and Boyd, M. R. (1997b). Construction and enhanced cytotoxicity of a [cyanovirin-N]-[*Pseudomonas* exotoxin] conjugate against human immunodeficiency virus-infected cells. *Biochem. Biophys. Res. Commun.* **239,** 884–888.

Mori, T., Shoemaker, R., McMahon, J., Gustafson, K., and Boyd, M. (1996). Analysis of sequence requirements for biological activity of recombinant cyanovirin-N, a novel anti-HIV virucidal protein. *Int. Conf. AIDS* **11,** 70.

Nakashima, H., Kido, Y., Kobayashi, N., Motoki, Y., Neushul, M., and Yamamoto, N. (1987a). Antiretroviral activity in a marine red alga: Reverse transcriptase inhibition by an aqueous extract of *Schizymenia pacifica*. *J. Cancer Res. Clin. Oncol.* **113,** 413–416.

Nakashima, H., Kido, Y., Kobayashi, N., Motoki, Y., Neushul, M., and Yamamoto, N. (1987b). Purification and characterization of an avian myeloblastosis and human immunodeficiency virus reverse transcriptase inhibitor, sulfated polysaccharides extracted from sea algae. *Antimicrob. Agents Chemother.* **31,** 1524–1528.

Nakashima, H., Tanabe, A., Tochikura, T. S., and Yamamoto, N. (1988). Rapid screening method with a cell multizier for inhibitors of human immunodeficiency virus-induced cell fusion *in vitro*. *J. Clin. Microbiol.* **26,** 1229–1232.

Nowotny, A., Mentel, R., Wegner, U., Mundt, S., and Lindequist, U. (1997). Antiviral activity of an aqueous extract of the cyanobacterium *Microcystis aeruginosa*. *Phytother. Res.* **11,** 93–96.

O'Connor, T. J., and Jeffries, D. J. (1996). Strategies for the inhibition of sexual transmission by topical antiviral agents. *Int. Conf. AIDS* **11,** 313. [abstract]

Ohta, K., Mizushina, Y., Hirata, N., Takemura, M., Sugawara, F., Matsukage, A., Yoshida, S., and Sakaguchi, K. (1998). Sulfoquinovosyldiacylglycerol, KM043, a new potent inhibitor of eukaryotic DNA polymerases and HIV-reverse transcriptase type 1 from a marine red alga, *Gigartina tenella*. *Chem. Pharm. Bull. (Tokyo)* **46,** 684–686.

Ohta, K., Mizushina, Y., Hirata, N., Takemura, M., Sugawara, F., Matsukage, A., Yoshida, S., and Sakaguchi, K. (1999). Action of a new mammalian DNA polymerase inhibitor, sulfoquinovosyldiacylglycerol. *Biol. Pharm. Bull.* **22,** 111–116.

Parish, C. R., Low, L., Warren, H. S., and Cunningham, A. L. (1990). A polyanion binding site on the CD_4 molecule. Proximity to the HIV-gp120 binding region. *J. Immunol.* **145,** 1188–1195.

Pearce-Pratt, R., and Phillips, D. M. (1996). Sulfated polysaccharides inhibit lymphocyte-to-epithelial transmission of human immunodeficiency virus-1. *Biol. Reprod.* **54,** 173–182.

Piot, P. (1998). The science of AIDS: A tale of two worlds. *Science* **280,** 1844–1845.

Pluda, J. M., Shay, L. E., Foli, A., Tannenbaum, S., Cohen, P. J., Goldspiel, B. R., Adamo, D., Cooper, M. R., Broder, S., and Yarchoan, R. (1993). Administration of pentosan polysulfate to patients with human immunodeficiency virus-associated Kaposi's sarcoma. *J. Natl. Cancer Inst.* **85,** 1585–1592.

Pratt, R., and Fong, J. (1940). Studies on *Chlorella vulgaris*. Further evidence that chlorella cells form a growth inhibiting substance. *Am. J. Bot.* **27,** 431–436.

Pratt, R., Mautner, R. H., Gardner, G. M., Sha, Y., and Dufenoy, J. (1951). Report on antibiotic activity of seaweed extracts. *J. Am. Pharm. Assoc.* **40,** 575–579.

Pujol, C. A., Coto, C. E., and Damonte, E. B. (1995). Determination of the antiviral activity of a naturally occurring sulfated xylomannan under various experimental conditions. *Rev. Argent. Microbiol.* **27,** 91–98.

Reshef, V., Mizrachi, E., Maretzki, T., Silberstein, C., Loya, S., Hizi, A., and Carmeli, S. (1997). New acylated sulfoglycolipids and digalactolipids and related known glycolipids from cyanobacteria with a potential to inhibit the reverse transcriptase of HIV-1. *J. Nat. Prod.* **60,** 1251–1260.

Richards, J. T. K. E. R., Glasgow, L. A., Overall, J. C., Jr., Deig, E. F., and Hatch, M. T. (1978). Antiviral activity of extracts from marine algae. *Antimicrob. Agents Chemother.* **14,** 24–30.

Rizzuto, C. D., Wyatt, R., Hernández-Ramos, N., Sun, Y., Kwong, P. D., Hendrickson, W. A., and Sodroski, J. (1998). A conserved HIV gp120 glycoprotein structure involved in chemokine receptor binding. *Science* **280**, 1949–1953.

Sarin, P. S., Sun, D., Thornton, A., and Muller, W. E. (1987). Inhibition of replication of the etiologic agent of acquired immune deficiency syndrome (human T-lymphotropic retrovirus/lymphadenopathy-associated virus) by avarol and avarone. *J. Natl. Cancer Inst.* **78**, 663–666.

Schols, D., Baba, M., Pauwels, R., and De Clercq, E. (1989). Flow cytometric method to demonstrate whether anti-HIV-1 agents inhibit virion binding to $T4^+$ cells. *J. Acquir. Immune Defic. Syndr.* **2**, 10–15.

Schols, D., Baba, M., Pauwels, R., Desmyter, J., and De Clercq, E. (1989). Specific interaction of aurintricarboxylic acid with the human immunodeficiency virus/CD_4 cell receptor. *Proc. Natl. Acad. Sci. USA* **86**, 3322–3326.

Schussler, A., Meyer, T., Gehrig, H., and Kluge, M. (1997). Variations of lectin binding sites in extracellular glycoconjugates during the life cycle of *Nostoc punctiforme*, a potentially endosymbiotic cyanobacterium. *Eur. J. Phycol.* **32**, 233–239.

Shaunak, S., Gooderham, N. J., Edwards, R. J., Payvandi, N., Javan, C. M., Baggett, N., MacDermot, J., Weber, J. N., and Davies, D. S. (1994). Infection by HIV-1 blocked by binding of dextrin 2-sulfate to the cell surface of activated human peripheral blood mononuclear cells and cultured T-cells. *Br. J. Pharmacol.* **113**, 151–158.

Shaunak, S., Thornton, M., John, S., Teo, I., Peers, E., Mason, P., Krausz, T., and Davies, D. S. (1998). Reduction of the viral load of HIV-1 after the intraperitoneal administration of dextrin 2-sulfate in patients with AIDS. *AIDS* **12**, 399–409.

Shoemaker, R. H., Wu, L., Smythe, A., Gulakowski, R., McMahon, J., Gustafson, K., and Boyd, M. (1996). Construction and expression in *E. coli* of a synthetic gene coding for cyanovirin-N, a novel anti-HIV protein originally isolated from a cultured cyanobacterium. *Int. Conf. AIDS* **11**, 70.

Stafford, M. K., Cain, D., Rosenstein, I., Fontaine, E. A., McClure, M., Flanagan, A. M., Smith, J. R., Taylor-Robinson, D., Weber, J., and Kitchen, V. S. (1997). A placebo-controlled, double-blind prospective study in healthy female volunteers of dextrin sulfate gel: A novel potential intravaginal virucide. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* **14**, 213–218.

Sugawara, I., Itoh, W., Kimura, S., Mori, S., and Shimada, K. (1989). Further characterization of sulfated homopolysaccharides as anti-HIV agents. *Experientia* **45**, 996–998.

Ueno, R., and Kuno, S. (1987a). Anti-HIV synergism between dextran sulphate and zidovudine [letter]. *Lancet* **2**, 796–797.

Ueno, R., and Kuno, S. (1987b). Dextran sulphate, a potent anti-HIV agent *in vitro* having synergism with zidovudine [letter]. *Lancet* **1**, 1379.

Weislow, O. S., Kiser, R., Fine, D. L., Bader, J., Shoemaker, R. H., and Boyd, M. R. (1989). New soluble formazan assay for HIV cytopathic effect: applications for high flux screening of synthetic and natural products for AIDS-antiviral activity. *J. Nat. Cancer Inst.* **81**, 577–586.

Wickstorm, M. L., Khan, S. A., Haschek, W. M., Wyman, J. F., Eriksson, J. E., Schaeffer, D. J., and Beasley, V. R. (1995). Alterations in microtubules, intermediate filaments, and microfilaments induced by microcystin-LR in cultured cells. *Toxicol. Pathol.* **23**, 326–337.

Witvrouw, M., and De Clercq, E. (1997). Sulfated polysaccharides extracted from sea algae as potential antiviral drugs. *Gen. Pharmacol.* **29**, 497–511.

Witvrouw, M., Este, J. A., Quinones Mateu, M. E., Reymen, D., Andrei, G., Snoeck, R., Ikeda, S., Pauwels, R., Vittori Bianchini, N., Desmyter, J., and De Clercq, E. (1994). Antiviral activity of a sulfated polysaccharide extracted from the red seaweed *Aghardhiella tenera* against human immunodeficiency virus and other enveloped viruses. *Antiviral Chem. Chemother.* **5**, 297–303.

Yang, F., Bewley, C. A., Louis, J. M., Gustafson, K. R., Boyd, M. R., Gronenborn, A. M., Clore, G. M., and Wlodawer, A. (1999). Crystal structure of cyanovirin-N, a potent HIV-inactivating protein, shows unexpected domain swapping. *J. Mol. Biol.* **288**, 403–412.

Zacharopoulos, V. R., and Phillips, D. M. (1997). Cell-mediated HTLV-I infection of a cervix-derived epithelial cell line. *Microb. Pathog.* **23**, 225–233.

Zeitlin, L., Whaley, K. J., Hegarty, T. A., Moench, T. R., and Cone, R. A. (1997). Tests of vaginal microbicides in the mouse genital herpes model. *Contraception* **56**, 329–335.

Zhang, X., and Smith, C. D. (1996). Microtubule effects of welwistatin, a cyanobacterial indolinone that circumvents multiple drug resistance. *Mol. Pharmacol.* **49**, 288–294.