drugs with their more selective and often safer enantiomers as well as provide a rapid, specific technique for pharmacological evaluation of racemic drugs. Computer modeling of x-ray crystal structures coupled with energy minimization calculations is a powerful technique for evaluating and understanding chiral interactions. Cyclodextrins are particularly amenable to this approach because of their well-defined and relatively static structure. However, we believe this method may be equally valuable in the evaluation and understanding of a variety of other chiral-separation systems (*3–17*).

REFERENCES AND NOTES

1. A. G. Wilson, O. G. Brooke, H. J. Lloyd, B. F. Robinson, *Br. Med. J.* **4**, 399 (1969).
2. M. Wuster, R. Schulz, A. Herz, *Biochem. Pharmacol.* **30**, 1983 (1981).
3. V. A. Davankov, in *Advances in Chromatography*, J. C. Giddings, E. Grushka, J. Cazes, P. R. Brown, Eds. (Dekker, New York, 1980), vol. 18, pp. 139–195.
4. V. A. Davankov, A. A. Kurganov, A. S. Bockkov, *ibid.*, vol. 22, pp. 71–116.
5. D. W. Armstrong, *J. Liq. Chromatogr.* 7, 353 (1984).
6. G. D. Y. Sogah and D. J. Cram, *J. Am. Chem. Soc.* **101**, 3035 (1979).
7. W. H. Pirkle, J. M. Finn, J. L. Schreiner, B. C. Hamper, *ibid.* **103**, 3964 (1981).
8. D. W. Armstrong and W. DeMond, *J. Chromatogr. Sci.* **22**, 411 (1984).
9. I. W. Wainer, T. D. Doyle, Z. Hamidzadeh, M. Aldridge, *J. Chromatogr.* **261**, 123 (1983).
10. I. W. Wainer and T. D. Doyle, *ibid.* **259**, 465 (1983).
11. ———, *Liq. Chromatogr.* **2**, 88 (1984).
12. Z.-Y. Yang, S. Barkan, C. Brunner, J. D. Weber, T. D. Doyle, I. W. Wainer, *J. Chromatogr.* **324**, 444 (1985).
13. S. Allenmark, *Liq. Chromatogr. Mag.* **3**, 348 (1985).
14. D. W. Armstrong, U.S. Patent No. 4,539,399 (1985).
15. ———, W. DeMond, B. P. Czech, *Anal. Chem.* **57**, 481 (1985).
16. T. E. Beesley, *Am. Lab.* **5**, 78 (1985).
17. D. W. Armstrong, T. J. Ward, A. Czech, B. P. Czech, R. A. Bartsch, *J. Org. Chem.* **50**, 5556 (1985).
18. M. L. Bender and M. Komiyama, *Cyclodextrin Chemistry* (Springer-Verlag, Berlin, 1978).
19. J. Szejtli, *Cyclodextrins and Their Inclusion Complexes* (Akademai Kiado, Budapest, 1982).
20. I. Tabushi, *Acc. Chem. Res.* **15**, 66 (1982).
21. R. Breslow, *Science* **218**, 532 (1982).
22. ———, G. Trainor, A. Veno, *J. Am. Chem. Soc.* **105**, 2739 (1981).
23. F. M. Menger and M. A. Dulany, *Tetrahedron Lett.* **26**, 267 (1985).
24. W. J. le Noble, S. Srivastava, R. Breslow, G. Trainor, *J. Am. Chem. Soc.* **105**, 2745 (1983).
25. W. L. Hinze, *Sep. Purif. Methods* **10**, 159 (1981).
26. A liquid chromatograph (Shimadzu LC-4A) with a variable wavelength detector (set at 254 nanometers) was used for all separations. High-coverage, stable β-cyclodextrin–bonded packing was prepared as reported (*8, 14*); columns were packed by Advanced Separation Technologies, Inc. Circular dichroism spectra were measured in a spectropolarimeter (Jasco 500A; cell pathlength, 1 cm).
27. E. J. Valente, W. F. Trager, L. H. Jensen, *Acta Crystallogr. Sect. B Struct. Sci.* **31**, 954 (1975).

28. Supported by Department of Energy grant DE-AS0584ER13159 and American Cancer Society grant CH-239. We thank N. Pattabiraman of the Computer Graphics Laboratory, University of California, San Francisco (R. Langridge, director; supported by NIH grant RR1081), for assistance with the computer graphics analysis and the figures.

15 October 1985; accepted 3 March 1986

Neutralization of HTLV-III/LAV Replication by Antiserum to Thymosin α_1

PREM S. SARIN, DAISY K. SUN, ARTHUR H. THORNTON, PAUL H. NAYLOR, ALLAN L. GOLDSTEIN

An antiserum prepared against thymosin α_1, a hormone secreted by the thymus gland, effectively neutralized the AIDS-associated virus [HTLV-III/LAV(clone BH-10)] and blocked its replication in H9 cells. Reverse transcriptase activity and expression of the HTLV-III/LAV antigens p15 and p24 were inhibited by purified immunoglobulin G preparations of antisera to thymosin α_1. The antiviral activity of the antiserum was found to be due to a region of homology between thymosin α_1 and p17, a product of the *gag* gene of HTLV-III/LAV. Comparison of the primary sequences of thymosin α_1 and the *gag* protein revealed a 44% to 50% homology in an 18–amino acid region, between positions 11 and 28 on thymosin α_1 and 92 and 109 on the *gag* protein. The effectiveness of the thymosin α_1 antiserum and of immunoglobulin G–enriched preparations in blocking replication of HTLV-III(BH-10) in H9 cells suggests a novel approach to the development of an AIDS vaccine. A vaccine directed against the *gag* protein might overcome the problem of genetic drift in the envelope region of the virus and be useful against all genetic variants of HTLV-III/LAV.

THE ACQUIRED IMMUNE DEFICIENCY syndrome (AIDS) is characterized by a decrease in the ratio of $T4^+$ to $T8^+$ lymphocytes, an increase in the incidence of opportunistic infections, and progressive paralysis of the immune system. The etiologic agent of AIDS has been identified as the human T-lymphotropic retrovirus HTLV-III/LAV (also termed ARV)(*1*). In many respects, the clinical symptoms of AIDS are indistinguishable from symptoms often seen in children with rare primary immunodeficiency diseases (PID's) associated with thymic aplasia or hypoplasia (*2*) and an increased susceptibility to opportunistic infections, including *Pneumocystis carinii* pneumonia. That the thymus gland, which plays a key role in the maturation and function of the lymphoid system, might be involved in the development of AIDS was first suggested by the detection of increased concentrations of peptides similar to thymosin α_1 in the blood of individuals with AIDS or belonging to the AIDS risk group (*3*).

Thymosin α_1 ($T\alpha_1$) was the first thymic hormone purified to homogeneity and sequenced from the partially purified thymosin fraction 5 (TF5) (*4*). It is an acidic polypeptide (*p*I 4.2) with a molecular weight of 3108, has many of the biological activities of TF5, and is a potent immunomodulator in vivo and in vitro (*5*). It acts primarily on helper T cells, and in humans and other animals enhances the expression of T-cell markers, stimulates the production of a number of lymphokines, including interleukin-2 (IL-2) and γ-interferon, and restores immune function and tumor immunity. $T\alpha_1$ has been found to restore helper T-cell function and prolong survival in phase II clinical trials in patients with lung cancer treated by radiotherapy (*6*).

Because of the clinical similarities between AIDS and PID's in children, we expected that $T\alpha_1$ levels would be depressed in patients with AIDS or AIDS-related complex (ARC). When we measured $T\alpha_1$ by radioimmunoassay (RIA) (*7*), however, we were surprised to find markedly elevated levels in the blood of patients with AIDS and ARC (*3*). For example, 44 of 72 individuals with Kaposi's sarcoma (61%) and 12 of 22 individuals with *P. carinii* pneumonia (55%) had $T\alpha_1$ levels above two standard deviations from the mean of normal individuals (*3*). Subsequent studies confirmed the initial findings for a nationwide sampling of patients with AIDS that included homosexuals and bisexuals, intravenous drug abusers, Haitians, and homosexuals with lymphadenopathy (*3*).

Three alternatives have been proposed to account for the elevation of $T\alpha_1$ in AIDS patients: (i) a defect in helper T cells resulting in increased levels of biologically active peptide, that is, end-organ failure; (ii) a viral invasion of the thymus epithelial cells with

P. S. Sarin, D. K. Sun, A. H. Thornton, Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, MD 20892.
P. H. Naylor and A. L. Goldstein, Department of Biochemistry, George Washington University School of Medicine and Health Sciences, Washington, DC 20037.

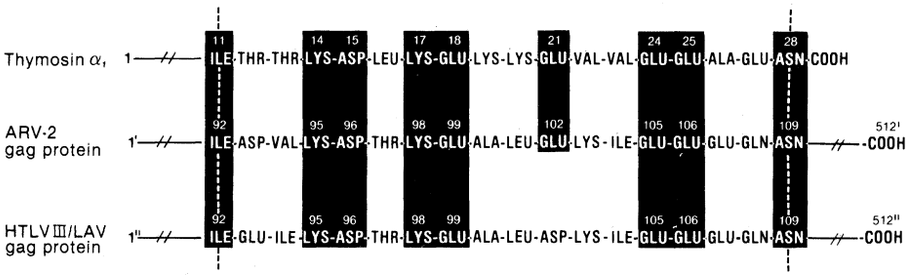

Fig. 1. Homology of p17 HTLV-III/LAV (clone BH-10) and ARV with thymosin α_1.

subsequent loss of control over hormone production or processing; or (iii) the presence of a biologically inactive but immunologically cross-reactive protein related to the AIDS virus. Further chemical and immunochemical characterization of the $T\alpha_1$-like peptide in the blood of homosexuals at high risk for AIDS indicated that this abnormally elevated peptide may be a cross-reacting molecule and that, in fact, authentic $T\alpha_1$ is often significantly decreased (*8*).

To identify the $T\alpha_1$ cross-reacting substances we began a comprehensive search for homologous peptides using computer-assisted analysis. We screened over 3500 protein sequences at the sequence bank of the National Biomedical Research Foundation in Washington D.C., and the catalog of available HTLV-III/LAV and other retroviral isolates from the laboratory of R. C. Gallo (National Institutes of Health). Although we did not find significant homologies with other thymic hormones or lymphokines, we did find (see Fig. 1) that $T\alpha_1$ and the *gag* proteins (p17) of HTLV-III/LAV (clone BH-10) are 44% to 50% homologous in an 18–amino acid region (positions 11 to 28 on $T\alpha_1$ and 92 to 109 on the p17 protein) of their primary sequences.

We therefore evaluated antibodies to $T\alpha_1$ for their ability to inhibit HTLV-III(BH-10) replication in the H9 permissive cell line (*9*). The presence of high titers of virus neutralizing antibodies in some patients with AIDS and ARC in the early stage of disease (*10*) suggests that these antibodies may provide a protective effect against virus infection. To detect the presence of neutralizing antibodies to HTLV-III(BH-10) in the serum of rabbits immunized with $T\alpha_1$, we used the HTLV-III(BH-10) replication system in H9 cells. Infection of the H9 cells was monitored by measuring reverse transcriptase (RT) activity in the culture medium and by using an indirect immunofluorescence assay for the expression of p15 and p24 of HTLV-III/LAV (*9*).

Analyses of ten different lots of heterologous antisera to $T\alpha_1$ prepared in rabbits (*11*) revealed neutralizing antibodies to HTLV-III(BH-10) (Table 1). In all cases, RT was significantly inhibited (53 ± 3%), but several of the crude antisera used at a dilution of 1:20 showed some cytotoxicity against the H9 cells or failed to inhibit expression of p15 and p24. We selected four lots of $T\alpha_1$ antisera and prepared from each of them an immunoglobulin (IgG)-enriched preparation to confirm that the antiviral activity was IgG-mediated.

As summarized in Table 2, the purified IgG preparations of antisera to $T\alpha_1$ were highly effective in neutralizing HTLV-III-(BH-10); they inhibited the expression of the viral proteins p15 and p24 and the activity of RT and were not cytotoxic against the H9 cells. The antisera from rabbits immunized against $T\alpha_1$ were as effective in their neutralizing activity as the sera of patients with ARC or AIDS who also exhibit neutralizing activity. Thus, the crude and IgG-enriched antisera to $T\alpha_1$ were directed toward a common epitope of $T\alpha_1$ and the *gag* protein of HTLV-III(BH-10) and compared favorably with neutralizing antibodies directed against the envelope protein that are present in the sera of some AIDS patients (*10*).

We also found (Fig. 2) that HTLV-III-(BH-10) proteins, but not Rauscher murine leukemia retroviral (RLV) proteins, could displace $T\alpha_1$ in a competitive RIA. The results demonstrated that large amounts of immunoreactive $T\alpha_1$-like material are present in an HTLV-III/LAV isolate but not in an RLV isolate. The displacement of $T\alpha_1$ in this RIA by proteins of HTLV-III/LAV but not of RLV suggests that the homologous sequences in HTLV-III/LAV and $T\alpha_1$ are not present in RLV. These observations support the neutralization studies since the same lot of antiserum used in the RIA was also effective in neutralizing the HTLV-III(BH-10) virus (Lot 1 in Table 1 and Fig. 2).

These studies strongly suggest that the homologies between $T\alpha_1$ and the *gag* protein of HTLV-III/LAV may be useful in developing a novel vaccine for AIDS. The data also suggest that the homologous region of the genome is critical to HTLV-III/LAV virus replication.

Table 1. Neutralization of HTLV-III/LAV by whole antiserum to thymosin α_1. The H9 cells were infected with HTLV-III(BH-10) as follows. The cells were treated with polybrene (2 μg/ml) for 30 minutes at 37°C, washed free of polybrene, and infected with 2×10^8 HTLV-III(BH-10) virus particles per 4×10^5 H9 cells. For the neutralizing antibody assays, the sera were heat-inactivated at 56°C for 30 minutes and filtered (0.45-μm filter). Antibodies at the appropriate dilutions were mixed with HTLV-III/(BH-10) (500 virus particles per cell) in 24-well flat bottom plates (2 ml). The plate was incubated at 4°C for 1 hour and at 20°C for 15 minutes. H9 cells were added to the wells to give a final concentration of 5×10^5 cells per milliliter. The cultures were incubated at 37°C in 5% CO_2 for 96 hours. After this period, a viable cell count was taken in the presence of trypan blue. The cell suspension was centrifuged at 500*g* for 10 minutes. The supernatant was processed for RT assays and the cells were mounted on toxoplasmosis slides, fixed, and analyzed with antibodies to p15 and p24 as described (*9*). The variation in response with each lot was addressed by repeating the experiments with partially purified preparations as shown in Table 2.

Experiment	Serum dilution	H9 cells (10^6 cells per milliliter)	Percent of control		
			p15	p24	RT
H9 cells (no virus)		1.3			
H9 cells infected with HTLV-III(BH-10)		0.96	100	100	100
Infected H9 cells plus serum from AIDS patients	1:100	1.4	25	33	13
Infected H9 cells plus:					
Normal rabbit serum	1:20	1.21	100	100	110
Antiserum to $T\alpha_1$					
Lot 1	1:20	0.33	100	33	43
Lot 2	1:20	1.03	50	33	42
Lot 3	1:20	0.33	50	66	60
Lot 4	1:20	0.19	25	33	45
Lot 5	1:20	0.14	25	33	59
Lot 6	1:20	0.29	100	133	62
Lot 7	1:20	0.71	125	166	72
Lot 8	1:20	0.14	125	100	58
Lot 9	1:20	0.16	50	33	50
Lot 10	1:20	0.31	50	66	42

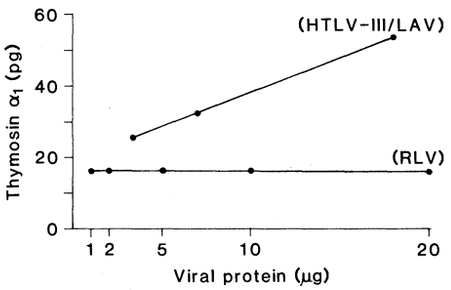

Fig. 2. Displacement of thymosin α_1 by retroviral proteins. Homogenates of the retroviruses were evaluated for proteins immunoreactive in the RIA for $T\alpha_1$. The human retrovirus HTLV-III(BH-10), but not the Rauscher murine leukemia retrovirus (RLV), was immunoreactive with $T\alpha_1$. The assays were performed in duplicate with standard errors, as previously reported (*7*), being less than 15%.

The finding of a homology between $T\alpha_1$ and the HTLV-III/LAV *gag* proteins may explain the lack of effective neutralizing antibodies in patients infected with HTLV-III/LAV. It is difficult to raise high titers of antibodies to an antigen or hormone that the body recognizes as self. Furthermore, if the host does not eliminate the virus by making neutralizing antibodies, the viremia may persist and the antibodies that do develop (because of the homologies to $T\alpha_1$) may initiate an autoimmune process that leads to damage or destruction of the epithelial hormone-producing cells of the thymus. That this indeed may occur is indicated by several recent reports revealing significant pathology of the thymus in children and adults that have died of AIDS (*12*). Thymus glands significantly smaller than normal and accompanied by severe epithelial destruction have been found. The pathological evidence documents that the thymus at autopsy has the appearance of a gland undergoing an autoimmune assault, characterized by architectural effacement, destruction of its epithelial microenvironment, infiltration of plasma cells, and reduction or calcification of Hassall's corpuscles.

A possible drawback to the use of $T\alpha_1$ or a synthetic *gag* protein as the immunogen in a vaccine would be the possibility of the vaccine damaging the thymus gland and its physiologic functions. However, we used rabbits for the preparation of the antisera used in our RIA and, although rabbit $T\alpha_1$ has not been sequenced and may not be identical to human $T\alpha_1$, we saw no signs of gross pathology, even when the antibody titers were high and when the rabbits were kept for up to 2 years.

Although the mechanism by which the antiserum to $T\alpha_1$ acts is not clear, a number of possibilities can be suggested: (i) the core proteins may be exposed as the virus enters the cell, (ii) *gag* proteins may be present on the envelope of the virus itself, or (iii) the antiserum may interfere with membrane assembly of the viral particles.

Most current strategies for the development of an AIDS vaccine are focusing on the envelope (*env*) gene and its products. However, considerable heterogeneity and antigenic variations have been observed among various HTLV-III/LAV isolates in the *env* gene, whereas the *gag* proteins are believed to be more conserved. Our data suggest that antigenic determinants common to the *gag* proteins of HTLV-III/LAV are accessible to the neutralizing effects of specific antibodies.

The effectiveness of antisera to $T\alpha_1$ and of IgG-enriched preparations in blocking the replication of HTLV-III/LAV suggests the possibility of developing novel vaccines based on immunization with thymosin peptides or analogs or with peptides derived from the *gag* region of the AIDS virus itself.

Table 2. Neutralization of HTLV-III/LAV and inhibition of viral replication with partially purified IgG antiserum to thymosin α_1. The H9 cells were treated with polybrene (2 μg/ml) for 30 minutes at 37°C, washed free of polybrene and infected with 2×10^8 HTLV-III(BH-10) virus particles per 4×10^5 H9 cells. The cultures were analyzed on day 4 after infection. Neutralizing antibody assays were carried out as described in Table 1. The IgG-enriched antisera were prepared by ammonium sulfate precipitation.

Experiment	Serum dilution	H9 cells (10^9 cells per milliliter)	Percent of control		
			RT	p15	p24
H9 cells (no virus)		1.56			
H9 cells infected with HTLV-III(BH-10)		0.26	100	100	100
Infected H9 cells plus:					
Normal rabbit IgG	1:20	0.32	100	100	100
Enriched antiserum to $T\alpha_1$					
Lot 2	1:20	1.00	31	18	21
Lot 2	1:50	0.98	38	26	36
Lot 5	1:20	1.48	46	21	15
Lot 5	1:50	1.34	77	58	43
Lot 7	1:20	1.26	36	15	18
Lot 7	1:50	1.32	62	33	25
Lot 9	1:20	1.18	42	18	18
Lot 9	1:50	1.02	57	33	28

REFERENCES AND NOTES

1. R. C. Gallo *et al.*, *Science* **224**, 500 (1984); A. S. Fauci *et al.*, *Ann. Intern. Med.* **100**, 92 (1984); N. Ciobanu *et al.*, *J. Clin. Immunol.* **3**, 332 (1983); H. W. Murray *et al.*, *N. Engl. J. Med.* **73**, 171 (1982); R. E. Stahl *et al.*, *Am. J. Med.* **73**, 171 (1982); P. S. Sarin and R. C. Gallo, *J. Clin. Immunol.* **4**, 415 (1984); F. Barré-Sinoussi *et al.*, *Science* **220**, 868 (1983); J. A. Levy *et al.*, *ibid.* **225**, 840 (1983).
2. J. M. Oleske *et al.*, *J. Am. Med. Assoc.* **249**, 2345 (1983); J. V. Joshi and J. M. Oleske, *Arch. Pathol. Lab. Med.* **109**, 143 (1985).
3. P. H. Naylor *et al.*, *NYAS Monograph on AIDS* **437**, 88 (1985); P. H. Naylor, A. Friedman-Kien, E. Hersh, M. Erdos, A. L. Goldstein, *Int. J. Immunopharmacol.*, in press; E. M. Hersh *et al.*, *N. Engl. J. Med.* **308**, 45 (1983); R. J. Biggar *et al.*, *ibid.* **309**, 49 (1983); J. K. Kreiss *et al.*, *Ann. Int. Med.* **100**, 178 (1984); C. M. Kessler *et al.*, *Br. J. Hematol.* **58**, 325 (1984).
4. A. L. Goldstein *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 725 (1977).
5. A. L. Goldstein, T. L. K. Low, M. M. Zatz, N. R. Hall, P. H. Naylor, *Clin. Immunol. Allergy* **3**, 116 (1983); A. L. Goldstein *et al.*, *Intervention in the Aging Process* (Liss, New York, 1983), p. 169; K. K. Oates and A. L. Goldstein, *Trends Pharmacol. Sci.* **4**, 347 (1984); M. M. Zatz and A. L. Goldstein, *Gerontology* **31**, 263 (1985).
6. R. S. Schulof *et al.*, *J. Biol. Response Modif.* **4**, 147 (1985).
7. J. E. McClure, N. Lameris, D. W. Wara, A. L. Goldstein, *J. Immunol.* **128**, 368 (1982).
8. P. H. Naylor *et al.*, *AIDS* (Liss, New York, 1985), p. 265.
9. P. S. Sarin *et al.*, *Biochem. Pharmacol.* **34**, 4075 (1985). Immunofluorescent assays were done on cells fixed at room temperature for 30 minutes in a mixture of methanol and acetone (1:1 by volume) and with monoclonal antibodies to HTLV-III/LAV p15 and p24. The HTLV-III(BH-10) infected cells with or without antibody treatment were fixed on toxoplasmosis slides and the slides were stored in sealed plastic bags at −20°C until used. The monoclonal antibodies were added to duplicate wells, and after incubation at room temperature in a humid chamber for 1 hour the cells were washed with phosphate-buffered saline (PBS) containing 0.25% Triton X-100 for 2 hours. The cells were then exposed to fluorescein (FITC)-conjugated goat antiserum to mouse IgG (Capell) for 1 hour and washed with PBS buffer containing 0.25% Triton X-100 overnight. The slides were mounted with 50% glycerol and cell fluorescence was observed under a Zeiss fluorescence microscope.
10. M. Robert-Guroff *et al.*, *Nature (London)* **316**, 72 (1985); R. A. Weiss *et al.*, *ibid.*, p. 69.
11. The antisera to $T\alpha_1$ (provided by Alpha 1 Biomedicals, Inc., Washington, DC) were prepared as previously described (*6*). In brief, synthetic $T\alpha_1$ was coupled via glutaraldehyde to keyhole limpet hemocyanin (KLH) (1:1 by weight) to give a final concentration of $T\alpha_1$ of 100 μg/ml. The conjugate (1 ml) was mixed with Freund's complete adjuvant (1 ml) and administered to New Zealand White rabbits intradermally in several sites. The rabbits received booster injections (100 μg of $T\alpha_1$ in Freund's complete adjuvant) at weekly intervals for 6 weeks. After 1 month of rest, the animals received booster injections monthly until maximum titers were achieved (2 to 4 months).
12. R. Elie *et al.*, *N. Engl. J. Med.* **308**, 841 (1983); S. Fligiel and F. Naeim, *Lab. Invest.* **48**, 25 (1983); C. M. Reichert *et al.*, *Am. J. Pathol.* **112**, 357 (1983); W. W. Grody *et al.*, *Am. J. Clin. Pathol.* **84**, 85 (1985).

13. This study was supported in part by an NCI grant CA 24974 and by gifts from Alpha 1 Biomedicals, Inc.

17 March 1986; accepted 21 April 1986