2′,5′-Oligoadenylate Synthetase from Cutaneous T-Cell Lymphoma: Biosynthesis, Identification, Quantitation, Molecular Size of the 2′,5′-Oligoadenylates, and Inhibition of Protein Synthesis†

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ABSTRACT: When cell-free extracts were made from peripheral blood mononuclear cells (PBMC) obtained from cutaneous T-cell lymphomas, there was a 10-fold increase in the 2′,5′-oligoadenylate synthetase activity compared with that of normal PBMC. The increased synthetase activity was based on the increased synthesis of 2′,5′-adenylates of molecular size of trimer or greater (dimer adenylate was not included because dimer adenylate does not inhibit protein synthesis). The 10-fold increase in 2′,5′-adenylates was determined by (i) the conversion of ATP to 2′,5′-oligoadenylate by cell-free extracts of PBMC and measurement of the 2′,5′-adenylates following displacement from a DEAE-cellulose column by 350 mM KCl, (ii) measurement of 2′,5′-adenylates synthesized following gradient separation and displacement from a DEAE-cellulose column, and (iii) synthesis of 2′,5′-adenylates with time of incubation. Whereas only two 2′,5′-adenylates that inhibit protein synthesis (i.e., trimer and tetramer) are synthesized by normal PBMC, trimer, tetramer, pentamer, and hexamer are synthesized by PBMC from cutaneous T-cell lymphomas. The significance of the synthesis of higher molecular weight 2′,5′-adenylates is that in vitro protein synthesis is inhibited 30% by a 2500-fold dilution of the 2′,5′-oligoadenylates synthesized by 2′,5′-oligoadenylate synthetase in PBMC cell-free extracts from cutaneous T-cell lymphoma, whereas a 2500-fold dilution of the 2′,5′-oligoadenylates from normal PBMC is not inhibitory. No appreciable difference in 2′,5′-oligoadenylate synthetase activity was found between T-cells and non-T-cells from cutaneous T-cell lymphomas. The response of PBMC after 24-h exposure to interferon, as measured by increased 2′,5′-oligoadenylate synthetase activity, showed no difference between cutaneous T-cell lymphomas and normals. These results demonstrate an increased activity of 2′,5′-oligoadenylate synthetase in PBMC from cutaneous T-cell lymphomas. The increased synthetase activity, in particular the increased synthesis of 2′,5′-adenylate tetramer, pentamer, and hexamer, suggests that a change in the biochemistry of cellular processes has occurred. This change might be attributable to a virus. Measuring increased synthetase activity by examination of the 2′,5′-adenylate profile may be a reliable method to substantiate viral etiology because there is a significant increase in 2′,5′-adenylate synthetase activity in all PBMC samples assayed, whereas virus particles cannot be isolated from most PBMC.

Although viral DNA is known to be incorporated into the human genome, there are many biochemical events in the cell that remain to be studied with respect to viral infection and/or DNA transformation. The double-stranded RNA (dsRNA)[1] dependent enzyme 2′,5′-oligoadenylate synthetase (2′,5′-A_n synthetase) varies with growth and hormone status and is known to be induced in response to interferon treatment (Baglioni, 1979; Stark et al., 1979). Production of interferon and subsequent induction of enzymes correlated with antiviral activity are natural defense responses of cells exposed to virus (Baglioni, 1979). Revel and co-workers have shown increased levels of 2′,5′-A_n synthetase from lymphocytes from patients with viral diseases but little if any increase in response to bacterial infections or noninfectious diseases (Schattner et al., 1981, 1982). Furthermore, in the case of persistent Epstein–Barr virus infection, where the illness is prolonged, higher than normal enzyme activity was measured many months after the onset of clinical symptoms when there was no evidence of an acute viral infection or any other disease, suggesting a continuing viral infection (Morag et al., 1982).

Because of our experience in protein synthesis and the relationship of protein synthesis to the antiviral state, we reasoned that many human diseases of unknown origin might be viral in origin. One of the diseases we selected to study was cutaneous T-cell lymphoma (mycosis fungoides). In 1982, we first reported the increased 2′,5′-oligoadenylate synthetase activity and increased 2′,5′-oligonucleotides in various stages of cutaneous T-cell lymphoma (Mosca et al., 1982). Gallo and co-workers had earlier isolated a type C retrovirus particle from PBMC obtained from a patient with cutaneous T-cell lymphoma (Poiesz et al., 1980). However, virus particles cannot be isolated from most PBMC obtained from these lymphomas nor are antibodies to virus present. Additional detailed studies on the type C virus particle from cutaneous T-cell lymphoma/leukemia (mycosis fungoides and Sézary syndrome) (HTLV), adult T-cell leukemia (ATL), and a T-cell variant of hairy-cell leukemia have appeared (Kalyanaraman et al., 1982; Miyoshi et al., 1981; Poiesz et al., 1981; Popovic et al., 1982; Seiki et al., 1982; Yoshida et al., 1982). Cell lines

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[1] Abbreviations: AMP, adenosine 5′-phosphate; 2′,5′-A_n, 2′,5′-linked oligomers of AMP with a 5′-triphosphate at the 5′-end; core, 5′-dephosphorylated 2′,5′-oligonucleotide; ATL, adult T-cell leukemia; ATLV, adult T-cell leukemia virus; HTLV, human T-cell lymphoma virus; BAP, bacterial alkaline phosphatase; dsRNA, double-stranded RNA; HBSS, Hanks' balanced salt solution; PBMC, peripheral blood mononuclear cells; PBS, phosphate-buffered saline; RBC, red blood cells; SRBC, sheep red blood cells; SVPD, snake venom phosphodiesterase; T2 RNase, ribonuclease T2; EDTA, ethylenediaminetetraacetic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DTT, dithiothreitol; Tris, tris(hydroxymethyl)aminomethane.

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established from HTLV-producing cells contained proviral sequences (Varmus, 1982). The finding of a type C virus particle and the known increase in the activities of the dsRNA-dependent 2′,5′-A_n synthetase point to either an important regulatory function of the increase in 2′,5′-A_n synthetase activity or to a possible viral etiology that would explain the increased synthesis of the 2′,5′-adenylates in cutaneous T-cell lymphoma. In this study, we examined and compared the activities of 2′,5′-A_n synthetase, the changes in the total amount of 2′,5′-adenylates synthesized, and the changes in the molecular size of the 2′,5′-adenylates synthesized in cell-free extracts from PBMC from cutaneous T-cell lymphomas with that of cell-free extracts from PBMC from normal blood samples.

Materials and Methods

Chemicals and Enzymes. [U-^{14}C]Leucine (330 mCi/mmol), [U-^{14}C]adenosine 5′-triphosphate (554 mCi/mmol), and [α-^{32}P]ATP (410 Ci/mmol) were obtained from Amersham; acid alumina WA-1, bacterial alkaline phosphatase [orthophosphoric-monoester phosphohydrolase (alkaline optimum), type III from *Escherichia coli*, BAP, EC 3.1.3.1], creatine phosphokinase (ATP:creatine *N*-phosphotransferase, EC 2.7.3.2, from rabbit muscle), Nonidet P-40, and ribonuclease T2 (ribonuclease 3′-oligonucleotidohydrolase, grade VI from *Aspergillus oryzae*, T2 RNase, EC 3.1.4.23) were from Sigma; snake venom phosphodiesterase I (venom exonuclease, from *Crotalus adamanteus* venom, SVPD, EC 3.1.4.1) was from Worthington; core 2′,5′-A_3, core 2′,5′-A_4, 2′,5′-p_3A_4, and poly(rI)·poly(rC)–agarose were from P-L Biochemicals; neuraminidase (mucopolysaccharide *N*-acetylneuraminylhydrolase, EC 3.2.1.1B, from *Vibrio cholerae*) was from Calbiochem; Ficoll and Percoll were from Pharmacia Fine Chemicals; Hypaque was from Winthrop Laboratories. Hanks' balanced salt solution (HBSS), RPMI 1640 medium, and fetal bovine serum were from GIBCO; cellulose thin-layer chromatograms, 20 × 20 cm, Chromagram 13254 with fluorescent indicator, were from Eastman. All other chemicals and reagents were of the highest purity available.

Blood Samples. Heparinized whole blood was obtained through Temple University Skin and Cancer Hospital from patients diagnosed as having cutaneous T-cell lymphoma (mycosis fungoides) or psoriasis and healthy age-matched volunteers.

Isolation of Peripheral Blood Mononuclear Cells (PBMC). Heparinized whole blood was diluted 1:1 with PBS. Two volumes of diluted blood was overlayered on 1 volume of Ficoll–Hypaque (Boyum, 1968) at a density of 1.080 and centrifuged at 20 °C, 30 min, 1000*g*. The PBMC layer was removed and washed once with 5 volumes of PBS. Isolated PBMC were resuspended in 5 mL of red blood cell (RBC) lysing buffer (155 mM NH_4Cl, 10 mM $NaHCO_3$, pH 7.4, 0.1 mM EDTA), kept on ice for 5 min, and washed twice with PBS. The cells were used for extract preparation, cultured, or further separated.

Separation of PBMC Subpopulations by Percoll Discontinuous Gradient Centrifugation. Isolated PBMC were dispersed and fractionated on a discontinuous gradient of Percoll as described (Gutierrez et al., 1979). Fraction 1 cells are enriched in non-T-cells, while fractions 2 and 3 are enriched in T-cells. Fractions 2 and 3 were combined. Each fraction was washed with 15 mL of PBS, and the cells were used for either lysate preparation or culture.

Separation of PBMC Subpopulations by Sheep Red Blood Cell Rosette Formation. One-day-old sheep red blood cells (SRBC), from Temple University, Department of Immunology and Serology, were treated with neuraminidase (Weiner et al., 1973). Treated SRBC were stored at 4 °C and used within 4 weeks. Isolated PBMC were resuspended in 2.5 mL of HBSS plus 10% fetal calf serum and enumerated via a hemacytometer. One-hundred-fold excess SRBC was added to the PBMC, incubated 15 min, 37 °C, and centrifuged (10 min, 200*g*), and the mixture was maintained at 4 °C overnight. The cells were then gently resuspended by mixing with a Pasteur pipet. By use of a hemacytometer, the number of rosetted cells (>3 SRBC bound) and nonrosetted cells was determined. Rosetted cells were separated from nonrosetted cells by Ficoll–Hypaque gradient centrifugation (40 min, 900*g*, 20 °C). Nonrosetted cells are washed twice with 10 mL of PBS. The rosetted cell pellet is treated with RBC lysing buffer (as above) and washed twice with PBS. Rosetted cells are designated as T-cells and the nonrosetted cells as non-T-cells (Weiner et al., 1973). Portions of rosetted and nonrosetted cells were run separately on Percoll gradients as described above. The recovery of rosetted cells in fractions 2 and 3 was 94% and of nonrosetted cells in fraction 1 was 75%. The rosette-forming fraction is typically greater than 60% of the total cells recovered.

Cell-Free Extract Preparation from PBMC, T-Cells, and Non-T-Cells. Cells isolated as described above were resuspended in 0.1 or 0.2 mL (approximately 10 times cell volume) of buffer B (20 mM Hepes, pH 7.5, 5 mM $MgCl_2$, 120 mM KCl, 10% glycerol, 1 mM DTT) containing 0.5% Nonidet P-40 and kept on ice 10 min to lyse the cells. Cytoplasmic extracts were obtained by centrifugation for 6 min, 8000*g*, 25 °C. Cell-free extracts were stored at −70 °C in either 25- or 50-μL aliquots.

Assay for 2′,5′-A_n Synthetase. 2′,5′-A_n synthetase [ATP:(2′,5′)-oligo(A) adenylyltransferase (EC 2.7.7.19)] was assayed as described (Merlin et al., 1981; Schattner et al., 1981). The reaction mixture (10 μL) contained 10 mM Hepes, pH 7.5, 2.5 mM $MgCl_2$, 60 mM KCl, 5% glycerol, 2.5 mM [α-^{32}P]ATP (0.12 Ci/mmol), 2.5 mM DTT, 3 units/mL creatine phosphokinase, and 10 mM creatine phosphate. After a 20-h incubation at 30 °C, the agarose was pelleted by centrifugation (3 min, 8000g, 25 °C). The mixture of 2′,5′-A_n in the supernatant was analyzed (see next section).

Bacterial Alkaline Phosphatase. Prior to acid alumina chromatography or high-voltage paper electrophoresis, 5 μL of unfractionated 2′,5′-A_n synthetase assays was hydrolyzed with BAP (0.25 units/mL, 0.08 M Tris, pH 7.5) for 60 min at 37 °C.

High-Voltage Paper Electrophoresis of Core 2′,5′-A_n. BAP-treated reaction products were spotted on Whatman 3MM paper and electrophoresed (Savant Instruments, Inc.) at 3000 V for 1.5 h in pyridine–acetic acid–water (1:10:98 v/v/v), pH 3.5 (Merlin et al., 1981), or for 1 h in 35 mM citric acid–15 mM sodium citrate–1 mM EDTA, pH 3.8 (Holmsen & Weiss, 1970). Authentic core 2′,5′-A_2, 2′,5′-A_3, and 2′,5′-A_4 were used as markers and detected by visualization under UV light. When the pyridine–acetic acid–H_2O system was used, it was necessary to saturate the paper with 1 M NH_4OH by spraying and then to dry thoroughly to visualize the oligonucleotides by UV light. ^{32}P-Labeled core 2′,5′-A_n oligomers were detected after autoradiography (Du Pont Cronex X-ray film). The radioactive spots equivalent to the core 2′,5′-A_n were cut and counted without scintillant (Cerenkof radiation) in the open channel of a Beckman LS-100C counter. Counts per minute were converted to picomoles of ATP incorporated into core 2′,5′-A_n per microgram of protein.

Determination of Structure of 2′,5′-A_n. [^{32}P]-2′,5′-A_n were treated with BAP, SVPD, and T2 RNase as described (Doetsch et al., 1981). Treated material and authentic markers were spotted on cellulose TLC, developed in solvent A (isobutyrate–NH_4OH–H_2O, 66:1:33 v/v/v) (Doetsch et al., 1981), and dried. The dried chromatogram was autoradiographed, and the R_f values of radioactive products were compared to R_f values of authentic nucleotides.

Isolation of Core 2′,5′-A_n by Acid Alumina Chromatography. Half of the BAP digest (7.5 μL) was diluted with 200 μL of 1 M glycine hydrochloride, pH 2, and applied to a 0.3-mL alumina column (0.5 × 1.5 cm) equilibrated with 1 M glycine hydrochloride, pH 2. The core 2′,5′-A_n were eluted with 4 mL of the same buffer (Merlin et al., 1981). One-milliliter fractions were collected, the radioactivity was determined, and the yield of 2′,5′-A_n was expressed as picomoles of ATP incorporated per microgram of protein.

Isolation of 2′,5′-A_n by DEAE-cellulose Column Chromatography. 2′,5′-A_n was isolated as described (Doetsch et al., 1981). Following displacement from the DEAE-cellulose column with 350 mM KCl buffer, the radioactivity in the 180-μL fractions was determined (Figure 1). Fraction 3 (150 μL) from lymphoma no. 42 (2.8 nmol of [^{32}P]-2′,5′-A_n, total dpm 578 000) and normal no. 44 (0.29 nmol of [^{32}P]-2′,5′-A_n, total dpm 58 000) were diluted to 700 μL by the addition of charge markers (AMP, ADP, ATP, and 2′,5′-$A_{3,4}$, 30 nmol each) in 50 mM NaCl, 50 mM Tris-HCl, pH 8.0, and 7 M urea. Samples were applied to DEAE-cellulose columns (0.5 × 17 cm) equilibrated in the same buffer. The nucleotides and 2′,5′-$A_{3,4}$ were displaced with a 50–150 mM linear gradient of NaCl (40 mL/40 mL) in 50 mM Tris-HCl, pH 8.0, in 7 M urea. The displacement of nucleotide charges was monitored on an LKB fraction collector Model 2070 with an Ultrorac II recording spectrophotometer. One-milliliter fractions were collected and assayed for radioactivity.

Translation Assays with 2′,5′-A_n Synthesized by Cell-Free Extracts of PBMC. 2′,5′-A_n displaced by 350 mM KCl buffer from the DEAE-cellulose column as described above were tested for inhibition of translation in rabbit reticulocyte cell-free systems (Doetsch et al., 1981). The concentration of 2′,5′-A_n was estimated from the specific activity of the [^{32}P]ATP in the 2′,5′-A_n synthetase reaction mix and based upon AMP equivalents. The 2′,5′-A_n samples used in the translation assays were from diluted, identical 350 mM KCl fractions obtained following 2′,5′-A_n synthetase assays of PBMC from lymphomas and normals (Figure 1). 2′,5′-A_n samples were preincubated for 20 min at 30 °C prior to the initiation of translation. Translation was assayed by measuring the incorporation of [U-^{14}C]leucine into trichloroacetic acid insoluble polypeptides.

Treatment of PBMC with Interferon. Cells isolated after Ficoll–Hypaque gradient centrifugation of T and non-T subpopulations were washed twice with PBS and resuspended to 10^6 cells/mL in RPMI 1640 medium with 20% fetal bovine serum. Aliquots of 0.5 mL were incubated 24 h (37 °C, 5% CO_2 in air) with or without 1000 units/mL human leukocyte interferon (HuIFN-α, 4 × 10^3 units/mg of protein, gift of Dr. S. Pestka). Following incubation, cells were washed with PBS and lysates prepared as described above.

Results

Isolation and Identification of 2′,5′-A_n by DEAE-cellulose Chromatography. The amount and molecular size of the 2′,5′-A_n synthesized by cell-free extracts of mammalian cells can be measured by DEAE-cellulose chromatography. The isolation of 2′,5′-A_n by displacement from DEAE-cellulose

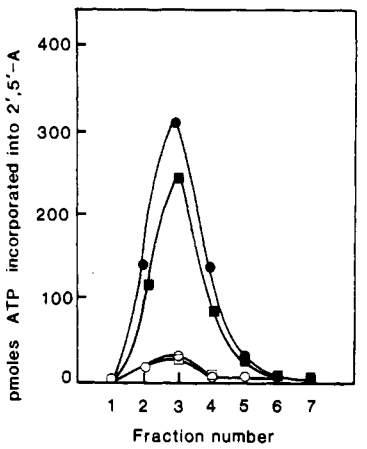

FIGURE 1: Biosynthesis of 2′,5′-adenylates in cell-free extracts of PBMC from cutaneous T-cell lymphomas and normals. [^{32}P]-2′,5′-A_n isolated following incubation of [α-^{32}P]ATP with PBMC cell-free extracts from cutaneous T-cell lymphomas and normals were displaced with 350 mM KCl buffer from DEAE-cellulose columns (see Materials and Methods). PBMC from T-cell lymphomas no. 42 (●) and no. 46 (■) and from normals no. 43 (□) and no. 44 (○). Fraction 3 of no. 42, 46, 43, and 44 are equivalent to 693 000, 549 000, 49 000, and 67 500 dpm, respectively. The amount of protein in assays 42, 46, 43, and 44 were 130, 142, 123, and 135 μg, respectively.

columns with 350 mM KCl showed a 10-fold increase in the picomoles of 2′,5′-A_n synthesized from [α-^{32}P]ATP in PBMC cell-free extracts from lymphoma blood samples when compared to that of cell-free extracts from normal PBMC (Figure 1). To quantitatively measure the 2′,5′-adenylates, gradient DEAE-cellulose chromatographs of the 2′,5′-A_n synthesized by the PBMC cell-free extracts from cutaneous T-cell lymphoma and normal blood were compared (Figure 2). From the cutaneous T-cell lymphoma assay, 2′,5′ trimer, tetramer, pentamer, and hexamer were displaced (charges 6–, 7–, 8–, and 9–) (Figure 2B). However the 2′,5′-A_n profile obtained from the normal assay showed only the synthesis of trimer and tetramer (charges 6– and 7–) (Figure 2A). There was a 10-fold increase in the total 2′,5′-adenylates isolated from the cutaneous T-cell lymphoma compared with the normal. These measurements did not include the 2′,5′-adenylate dimer. The percent distribution of ^{32}P for the 2′,5′-A_n displaced is 87% trimer and 13% tetramer for normal PBMC and 50% trimer, 40% tetramer, 9% pentamer, and 1.5% hexamer for the PBMC from cutaneous T-cell lymphoma.

Synthesis of 2′,5′-A_n by PBMC Cell-Free Extracts from Cutaneous T-Cell Lymphoma and Normals. A comparison of the 2′,5′-A_n synthesized with increasing time of incubation by PBMC cell-free extracts from a normal blood sample with that of cutaneous T-cell lymphoma was done. The enzymatic synthesis of total 2′,5′-A_n over a 28-h incubation by PBMC cell-free extract from cutaneous T-cell lymphoma was also 10-fold greater than that of normal (Figure 3). Furthermore, in the PBMC from lymphoma samples, the total synthesis of 2′,5′-A_n increased with increased time, whereas in the normal, the 2′,5′-A_n appears to decrease after 15 h.

Hydrolysis of 2′,5′-A_n by Bacterial Alkaline Phosphatase. 2′,5′-A_n synthesized from ATP by cell-free extracts of PBMC from cutaneous T-cell lymphoma and normals were treated with bacterial alkaline phosphatase. The core 2′,5′-A_n were either collected by alumina column chromatography or separated by high-voltage paper electrophoresis. The amount of core 2′,5′-A_n (n = 2–6) eluted from alumina was also 10-fold greater from the cell-free extracts of PBMC from cutaneous T-cell lymphoma than that synthesized by normal PBMC cell-free extracts (data not shown). Alternatively, when the

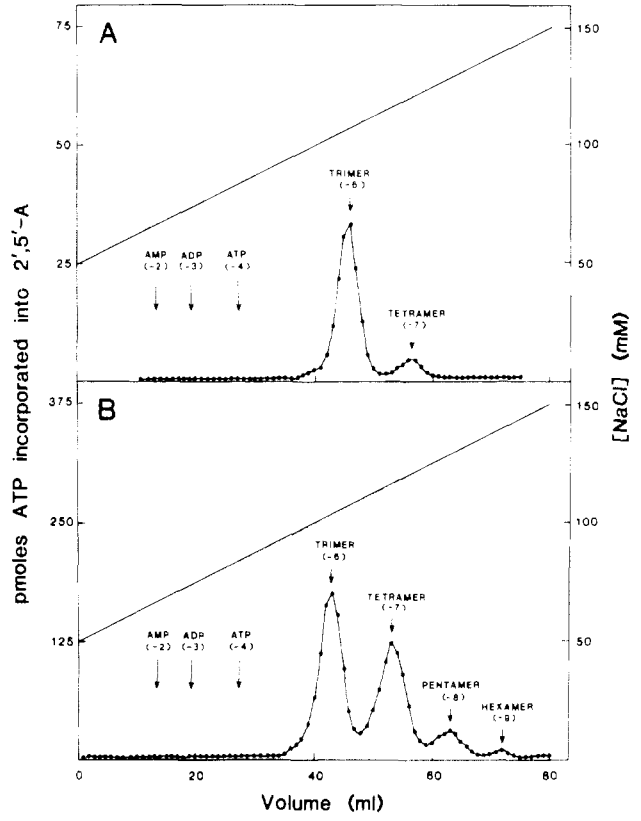

FIGURE 2: Separation of 2',5'-adenylates by DEAE-cellulose chromatography. The 2',5'-oligonucleotides displaced from the DEAE-cellulose columns by 350 mM KCl (fraction 3, Figure 1) were diluted to 50 mM KCl and applied to a second DEAE-cellulose column (0.5 × 17 cm) equilibrated with 50 mM NaCl–50 mM Tris-HCl (pH 8.0) in 7 M urea. Charge markers (AMP, ADP, ATP, and 2',5'-A_3) were added to the samples. Nucleotides and 2',5'-A_n were displaced with a 50–150 mM linear gradient of NaCl (40 mL/40 mL)–50 mM Tris-HCl (pH 8.0) in 7 M urea. The 2',5'-adenylates assigned to a particular radioactive peak and the corresponding charge are indicated directly above the peak fractions. Trimer peak tubes represent 184 500 (185 pmol) and 30 400 dpm (30 pmol) for cutaneous T-cell lymphoma and normal, respectively. (A) Normal no. 44; (B) cutaneous T-cell lymphoma no. 42. Micrographs of protein are the same as in Figure 1.

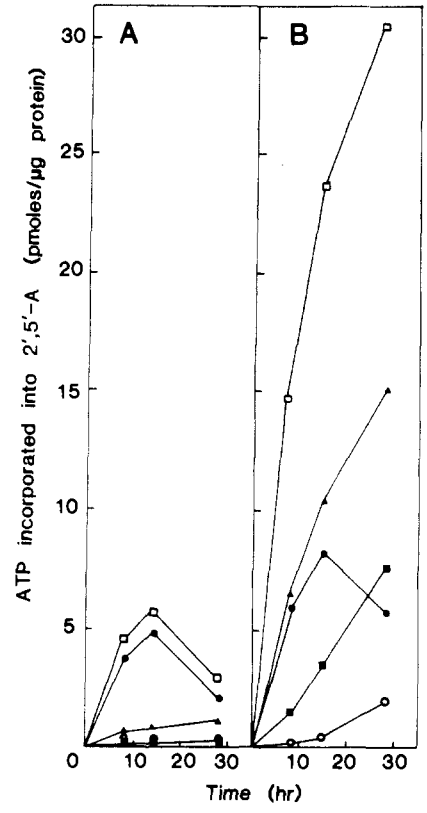

FIGURE 3: Synthesis of 2',5'-A_n by PBMC cell-free extracts from cutaneous T-cell lymphoma and normal. 2',5'-A_n synthetase activity was assayed as described under Materials and Methods except that at the indicated times 2-μL aliquots of reaction supernatants were removed and treated with BAP, followed by electrophoresis. (A) Normal; (B) cutaneous T-cell lymphoma; (●) core 2',5'-A_2; (▲) core 2',5'-A_3; (■) core 2',5'-A_4; (○) core 2',5'-A_5; (□) total 2',5'-adenylates.

bacterial alkaline phosphatase treated nucleotides were separated by high-voltage paper electrophoresis, there was more ^{32}P in the core 2',5'-A_n (n = 2–5) regions from the cutaneous T-cell lymphoma compared to that from 2',5'-A_n (n = 2–4) regions from the normals. There is a marked difference in both the total amount of 2',5'-A_n synthesized and the molecular size of the 2',5'-adenylates synthesized in PBMC cell-free extracts from cutaneous T-cell lymphoma compared with normals. When the 2',5'-adenylate dimer was included in the total 2',5'-adenylate synthesized, there was a 5.4- and 4.4-fold increase in the 2',5'-adenylates synthesized by PBMC cell-free extracts from cutaneous T-cell lymphoma compared to normal PBMC cell-free extracts. 2',5'-Adenylate dimers are usually not included in the 2',5'-adenylate total because the dimer does not inhibit protein synthesis.

Proof of Structure of 2',5'-Oligoadenylates following Biosynthesis from ATP by PBMC Cell-Free Extracts from Cutaneous T-Cell Lymphomas and Normals. To prove that the synthetase activity in PBMC cell-free extracts was due to the biosynthesis of 2',5'-A_n, an aliquot of [^{32}P]-2',5'-adenylate displaced from a DEAE-cellulose column (Figure 1, fraction 2) was treated with BAP, SVPD, and T2 RNase and chromatographed on a cellulose TLC developed in solvent A (Doetsch et al., 1981). The 2',5'-adenylates of the enzyme hydrolyses were detected by autoradiography. The R_f values of the enzyme digests were the same as those observed for authentic core 2',5'-A_n (n = 2–6) and 5'-AMP. BAP completely hydrolyzed ATP to adenosine and inorganic phosphate; T2 RNase hydrolyzed authentic core 3',5'-A_3 to adenosine and 3'-AMP but did not hydrolyze authentic core 2',5'-A_3; SVPD hydrolyzed authentic core 2',5'-A_3 to adenosine and 5'-AMP. BAP hydrolyzed the PBMC cell-free extract synthesized [^{32}P]-2',5'-A_n (n = 2–6) to core [^{32}P]-2',5'-A_n (n = 2–6) and inorganic phosphate. The radioactive product after SVPD hydrolysis of [^{32}P]-2',5'-A_n was [^{32}P]-5'-AMP. T2 RNase did not hydrolyze the [^{32}P]-2',5'-A_n, which established the 2',5'-phosphodiester bond.

Inhibition of Translation by 2',5'-A_n. The inhibition of protein synthesis by 2',5'-A_n synthesized by PBMC cell-free extracts from cutaneous T-cell lymphoma and normal samples was assayed by measuring the incorporation of [^{14}C]leucine into polypeptide with lysates from rabbit reticulocytes (Doetsch et al., 1981). While a 2500-fold dilution of the 2',5'-A_n isolated from the cutaneous T-cell lymphoma PBMC cell-free extracts inhibited protein synthesis by about 30%, a 2500-fold dilution of 2',5'-A_n from normal PBMC incubations did not inhibit protein synthesis (Figure 4). Therefore, the 2',5'-A_n synthesized and their inhibition of in vitro protein synthesis are markedly greater in the PBMC cell-free extracts from cutaneous T-cell lymphoma than from the normal.

2',5'-A_n Synthetase Activity in Cell-Free Extracts from Cutaneous T-Cell Lymphoma, Normals, and Psoriasis PBMC Samples. To verify the increase in 2',5'-A_n synthetase observed in Figures 1–3, a statistically significant number of samples were tested. 2',5'-A_n synthetase activities were compared in

Table I: Percent Distribution of 2′,5′-Adenylates Synthesized by PBMC Cell-Free Extracts

no. of samples	PBMC source	2′,5′-adenylates (sp act.)[a] dimer	trimer	tetramer	pentamer–hexamer	total
13	normal	1.45 (100)	0.55 (100)	0.080 (100)	negligible	2.1 ± 0.6
25	cutaneous T-cell lymphoma	4.62 (319)	2.13 (374)	0.29 (342)	0.142	7.2 ± 1.2 (<0.01)
10	psoriasis	2.81 (193)	1.32 (240)	0.22 (275)	0.5	4.4 ± 0.9 (>0.05)

[a] 2′,5′-A_n synthetase was assayed, and oligomers were separated by electrophoresis after BAP hydrolysis as described under Materials and Methods. Activity is expressed as picomoles of ATP incorporated per microgram of protein. The numbers given are the mean values for each group of individuals. Numbers in parentheses represent percent of normal. Total oligomers are expressed as mean values ± standard error. The numbers in parentheses represent the p values from a Dunnett's t test where each group is compared to the normal group.

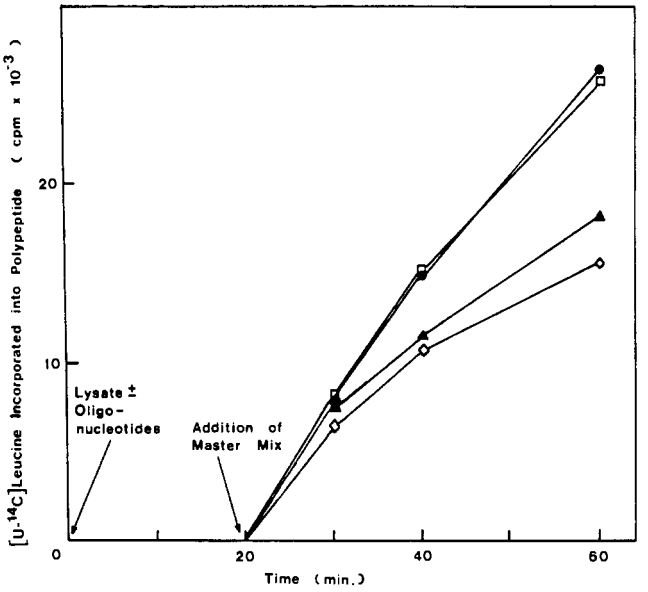

FIGURE 4: Inhibition of protein synthesis by 2′,5′-A_n synthesized by cell-free extracts of PBMC from cutaneous T-cell lymphoma and normal. 2′,5′-A_n displaced by 350 mM KCl buffer (fraction 3, Figure 1) from DEAE-cellulose columns of lymphoma and normal 2′,5′-A_n synthetase reaction supernatants were assayed for inhibition of protein synthesis in rabbit reticulocyte cell-free systems as described under Materials and Methods. (●) Control; (◇) 2′,5′-A_4, 100 nM; (□) normal no. 44, 1:2500 dilution; (▲) cutaneous T-cell lymphoma no. 42, 1:2500 dilution.

cell-free extracts of PBMC from 13 normal blood samples, 25 blood samples from cutaneous T-cell lymphoma, and 10 blood samples from psoriasis (Table I). The mean 2′,5′-A_n synthetase activity of the T-cell lymphomas was 3.5-fold higher than that of the normal samples. Although the mean activity in psoriasis samples was 2.1-fold higher than normal, a statistical analysis determined this difference to be insignificant. The psoriasis samples were included as a control skin disease group.

The 350% increase in the picomoles of 2′,5′-adenylates produced by the cell-free extracts of the lymphoma samples is reflected in the percent increases of 320%, 380%, and 340% of the dimer, trimer, and tetramer, respectively (compared to normals). Because negligible amounts of the pentamer–hexamer were synthesized in the normals, the 0.142 pmol of pentamer–hexamer 2′,5′-adenylates cannot be expressed as a percent-increased synthesis. In the 10 psoriasis samples used in these studies, the picomoles of the 2′,5′-oligoadenylates were similar to those of the normals.

Separation of PBMC Subpopulations. Because cutaneous T-cell lymphoma is a human cancer often characterized by neoplastic T-cells (Broder & Bunn, 1980) and because virus has been isolated from T-cells of patients with this disease (Poiesz et al., 1980, 1981), we subdivided the PBMC into T-cell-enriched and non-T-cell (T-cell-depleted) populations. The cell-free extracts from these populations were assayed for 2′,5′-A_n synthetase. The 2′,5′-A_n synthetase activity in extracts of normal T-cells and non-T-cells was lower than the 2′,5′-A_n synthetase activity in extracts of T-cells and non-T-cells from cutaneous T-cell lymphoma. The difference in 2′,5′-A_n synthetase activity between T-cells and non-T-cells was negligible for cell-free extracts of PBMC from four normals and seven lymphoma samples (data not shown). PBMC and separated subpopulations (T-cells and non-T-cells) from three lymphoma samples and three normal samples were treated with IFN-α for 24 h. Following incubation, cell lysates were prepared, and 2′,5′-A_n synthetase was assayed. Except for the T-cells of one lymphoma sample, all cells responded to IFN-α treatment with an increase in 2′,5′-A_n synthetase activity compared to untreated cells (data not shown). The increases in 2′,5′-A_n synthetase activity varied from 1.5- to 5-fold. The extent of the increase did not correlate with either the cell type or the presence of disease.

Discussion

Retroviruses have been shown to be the agents that cause lymphomas, leukemias, and sarcomas in animals (Klein, 1980. Because the demonstration of retrovirus particles is difficult in T-cell lymphomas and because there is good evidence that these lymphomas and leukemias are viral in origin, the aim of this study was to do a detailed biochemical analysis of cell-free extracts of PBMC from cutaneous T-cell lymphoma blood samples with respect to the induction of the 2′,5′-A_n synthetase that is known to be implicated in virus infections. Our data are especially significant in view of the recent reports of the isolation of type C virus particles from human cutaneous T-cell leukemia (Sēzary syndrome), cutaneous T-cell lymphoma (mycosis fungoides), adult T-cell leukemia, and a T-cell variant of hairy-cell leukemia (Poiesz et al., 1980, 1981; Miyoshi et al., 1981; Yoshida et al., 1982; Kalyanaraman et al., 1982). On the basis of the data reported here, we conclude that there is increased activity of 2′,5′-A_n synthetase demonstrated by increased biosynthesis of total 2′,5′-adenylates and increased biosynthesis of the higher molecular weight 2′,5′-adenylates. The increased 2′,5′-adenylates also inhibited protein synthesis by 30% following a 2500-fold dilution of the 2′,5′-adenylates synthesized by the PBMC cell free extracts from cutaneous T-cell lymphoma.

The consistent elevated activities of the 2′,5′-A_n synthetase in the cutaneous T-cell lymphomas might be an indication that these cells can not maintain normal cell functions. Evidence for this is the report of Drocourt et al. (1982) in which they demonstrated that cells in culture treated with 2′,5′-A_3 (5′-triphosphate trimer) for prolonged time periods maintained an inhibition of protein synthesis. More recently, Lee & Suhadolnik (1983) reported that prolonged exposure of L-cells and human fibroblasts to the naturally occurring 2′,5′-adenylate trimer and tetramer 5′-triphosphates resulted in cell death, whereas the structurally modified and metabolically more stable cordycepin analogues were not cytotoxic.

Although the 2′,5′-A_n synthetase is elevated in PMBC from cutaneous T-cell lymphomas, we observed that the 2′,5′-A_n synthetase activity in cell-free extracts and non-T-cells from four normal PBMC samples was essentially identical, in agreement with Kimchi (1981). The 2′,5′-A_n synthetase activities in cell-free extracts from T-cells and non-T-cells from seven lymphoma PBMC samples also did not appreciably differ. This study was done because the activity of 2′,5′-A_n synthetase has been shown to vary with growth and hormone status of cells as well as the result of interferon treatment (Stark et al., 1979; Kimchi et al., 1981; Baglioni, 1979).

Increased 2′,5′-A_n synthetase activity has been implicated in the antiviral and antiproliferative effects of interferon and has been demonstrated in numerous viral diseases (Schattner et al., 1981, 1982). The identification of a type C virus particle provides convincing evidence that certain T-cell malignancies may be caused by a viral agent. Monitoring 2′,5′-A_n synthetase as part of conventional diagnosis and therapy of viral and suspected viral diseases may be important in evaluation of the disease and patient response. Our results suggest that 2′,5′-A_n synthetase may be an important biochemical and physiological parameter of disease states.

Because there is the possibility that there are additional retroviruses or forms of slow-growing viruses that are responsible for other diseases (for example, the degeneration of the central nervous system in multiple sclerosis), we have expanded the study of 2′,5′-A_n synthetase to cell-free extracts of PBMC from patients with systemic lupus erythematosus and multiple sclerosis. We also observe elevated 2′,5′-A_n synthetase activity in these two diseases (unpublished results). It is essential that we continue to study the T-cell malignancies in terms of specific viral agents that may be responsible for these cancers as well as the associated enzyme activities, in view of the possibility of person-to-person transmission of these specific malignancies.

Added in Proof

A paper recently appeared by Friedman and co-workers (Preble et al., 1983) showing elevated 2–5A synthetase in SLE patients.

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