Volume 17 Number 3 1989 Nucleic Acids Research

Activation of interferon-regulated, dsRNA-dependent enzymes by human immunodeficiency virus-1 leader RNA

Dibyendu N.SenGupta and Robert H.Silverman*

Department of Pathology, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814-4799, USA

Received November 7, 1988; Revised and Accepted January 3, 1989

ABSTRACT

Human immunodeficiency virus-1 (HIV-1) leader RNA, which contains double-stranded regions due to inverted repeats, was shown to activate the dsRNA-dependent enzymes associated with the interferon system. HIV-1 leader RNA produced in vitro using SP6 RNA polymerase was characterized using probes for antisense and sense-strand RNA. The RNA preparation was free from significant levels of antisense RNA. HIV-1 leader RNA was shown to activate dsRNA-dependent protein kinase in a cell-free system from interferon-treated HeLa cells. Affinity resins, consisting of HIV-1 leader RNA covalently attached to cellulose, immobilized and activated dsRNA-dependent protein kinase and 2-5A-synthetase. HIV-1 leader RNA, therefore, may be a contributing factor in the mechanism by which interferon inhibits HIV replication.

INTRODUCTION

The 5' or leader region of HIV-1 mRNA contains two adjacent stem-loop structures with 17 and 23 imperfectly matched base-pairs (1). The leader RNA is present on all of the HIV-1 mRNA species (2) and is likely to be a critical element in the regulation of translation during infection. It is, therefore, important to identify and characterize host proteins which interact with the leader RNA. Here we have identified two such host proteins, dsRNA-dependent protein kinase and 2-5A-synthetase, which not only bind HIV-1 leader RNA but are enzymatically activated in the process. Significantly, these proteins have been implicated in the antiviral mechanisms of interferons (3, 4). In the former pathway, dsRNA induces the autophosphorylation of an interferon-regulated protein kinase. The phosphorylated kinase is capable of phosphorylating exogenous substrates (5). In particular, the kinase will phosphorylate the alpha subunit of protein synthesis initiation factor, eIF-2, thus inhibiting the normal recycling mechanism for eIF-2 (reviewed in ref. 6). These events lead to an inhibition of viral and cellular protein synthesis. The 2-5A-system also inhibits protein synthesis but through a separate mechanism (3, 4). Three types of enzymatic activities are involved: (i) 2-5A-synthetases which are interferon-inducible, dsRNA-dependent enzymes producing 2-5A [$p_x(A2'p)_nA$; x = 2 or 3, n $\geq$ 2] and pyrophosphate from ATP; (ii) 2',5'-phosphodiesterase which degrades 2-

5A from the 2',3'-termini; and (iii) 2-5A-dependent RNase which is activated by 2-5A to cleave single-stranded RNA on the 3'-side of UpNp sequences. The 2-5A-pathway, therefore, inhibits virus replication by bringing about the degradation of viral and cellular RNA. Results presented suggest that the dsRNA-dependent mechanisms have the potential to limit HIV-1 replication.

MATERIALS AND METHODS

Preparation of HIV-1 leader RNA.

The plasmid, pSP6HIV+1 (1) containing the HIV-1 leader sequence (nucleotides +1 to +232) downstream of an SP6 promoter was linearized after HIV-1 nucleotide +232 using XhoI. The linear DNA was purified first by centrifugation in cesium chloride gradients containing ethidium bromide (7) and then through three successive 1% low-melting agarose gels (Sea Plaque, FMC) (7) to remove trace levels of uncut DNA. RNA was synthesized using the linear pSP6HIV+1 DNA and SP6 RNA polymerase (Promega) as described by Melton et al. (8). The plasmid DNA was then digested with RQ1 DNase (Promega) and the RNA was isolated by phenol-chloroform extraction and ethanol precipitation (7). The RNA preparation was purified by centrifugation in a 5-20% sucrose gradient of 5.0 ml containing 10 mM HEPES, pH 7.5, 0.1 M NaCl, and 1 mM EDTA at 40,000g for 12h at 15^{0}C. About 1 ml of the top portion of the gradient containing the leader RNA was removed and layered on 10% sucrose in the same buffer and centrifuged at 106,000g for 24h at 15^{0}C. About 400 ul from the bottom of tube containing the leader RNA was collected and the RNA was twice precipitated in ethanol, washed with 70% ethanol, and dissolved in 10 mM HEPES, pH 7.5, 0.1 M NaCl, and 1 mM EDTA.

Northern Blots.

Electrophoresis of the RNA (about 0.7 ug RNA per lane) was on denaturing, glyoxal/1.8% agarose gels according to McMasters and Carmichael (9) with modifications described previously (10). To prepare probes for sense and antisense RNA, the plasmid pSP6HIV+1 was cleaved with NheI and XhoI. The double digest produced a 473 base-pair fragment containing the entire 232 base-pair HIV-1 sequence and an upstream region; this fragment was subcloned in M13mp18 and M13mp19 after the vectors were each digested with both SalI and XbaI. The resulting recombinant single-stranded M13 DNAs contained complementary strands of HIV-1 leader DNA. Probes of ^{32}P-labeled recombinant M13 DNA (4 to 6 X 10^7 counts/min/ug of DNA) were prepared using M13 hybridization primer (New England Biolab, cat. no. 1202) according to Hu and Messing (11). Transfer of RNA to nitrocellulose, 0.45 um, or NYTRAN, 0.2 um, (both from Schleicher and Schuell), pre-hybridization, hybridization, and washing of the filter (4 changes in 2 x SSC/0.1% SDS for 15 min at 50^{0}C) was according to Williams and Mason (12).

Preparation of affinity resins.

Covalent linkage of poly(I):poly(C), poly(U) (Pharmacia), or HIV-1 leader RNA to cellulose was by a modification (10) of the method of Wells et al. (13). The amount of HIV-1 leader RNA linked to cellulose was determined by adding trace levels of ^{32}P-labeled HIV-1 leader RNA, prepared using [alpha-^{32}P]UTP, to the unlabeled RNA preparation and then monitoring the bound radioactivity.

Assay of dsRNA-dependent protein kinase in solution.

HeLa cells were cultured, treated with 100 units/ml of human alpha interferon (1.2 X 10^8 units/mg of protein, Interferon Sciences) for 16 h, harvested, and postmitochondrial supernatants (75 ug of protein per assay) were prepared as described previously (10). Assays were with 1.0 uCi of [gamma-^{32}P]ATP for 15 min at 30^{0}C as described (10) except there was 1.0 mM magnesium chloride and 1.0 mM manganese chloride (14) in the assays. Proteins were separated on 10% polyacrylamide/ SDS gels. Dried gels were used for autoradiography.

Assay of dsRNA-dependent protein kinase immobilized on affinity resins.

The affinity resins were incubated with postmitochondrial supernatant of interferon-treated or control HeLa cells (80 ug of protein/ 20 ul) on ice for 1h, and then washed and incubated in the presence of 2 uCi of [gamma-^{32}P]ATP in a final volume of 40 ul for 30 min at 30^{0}C as described previously (10) except there was 1.0 mM of magnesium chloride and 1.0 mM manganese chloride (14). Each assay was adjusted to contain the same amount cellulose. Some assays were in presence of added purified eIF-2 (0.5 ug/assay, a generous gift of Dr. Brian Safer, N.I.H.).

Assay of 2-5A synthetase.

Assays for 2-5A-synthetase activity were performed as described by Silverman and Krause (10) except the adsorption of 2-5A-synthetase to the resins was at 0^{0}C for 1h and the incubations with 4 mM ATP for 2-5A synthesis were at 30^{0}C for 15 min in a final volume of 60 ul. 2-5A-synthetase activity was from cell extract of interferon-treated HeLa cells (120 ug of protein per assay). 2-5A-synthetase was adsorbed to various amounts of RNA attached to cellulose or to an equivalent volume of linker-cellulose. Levels of 2-5A synthesized were determined using a modification (10) of the radiobinding assay of Knight et al. (15).

RESULTS

Synthesis and characterization of HIV-1 leader RNA produced from plasmid pSP6HIV+1

HIV-1 leader RNA was produced from plasmid, pSP6HIV+1, which contains a sequence from the HIV-1 LTR located 3' to an SP6 promoter (1). RNA synthesized from this plasmid by SP6 RNA polymerase contained HIV-1 nucleotides +1 to +232; this RNA was purified (Materials and Methods) to remove components of the SP6 transcription system. It was critical to exclude the presence of antisense RNA because such aberrant transcripts could anneal to the sense strand and produce a dsRNA. DNA fragments containing the entire HIV-1 leader sequence from pSP6HIV+1 were subcloned into M13 vectors in order to produce probes for sense and antisense RNA. The northern blot of HIV-1 leader RNA using complementary strand probes is shown in Fig. 1. The absence of significant levels of antisense RNA (lane 1) was consistent with the fact that XhoI, the enzyme used to linearize the plasmid, does not produce 3' protruding ends. Hybridization to RNA blotted on Nytran, instead of nitrocellulose, did produce a band with antisense probe at the position of the HIV-1 leader RNA with less than 1% of the intensity of that produced with sense strand probe amongst a very high nonspecific background (data not shown). This was almost certainly due to complementary sequences in the inverted repeats of HIV-1 leader RNA and not to antisense RNA.

HIV-1 leader RNA activates dsRNA-dependent protein kinase in a cell-free system

The purified HIV-1 leader RNA and poly(I):poly(C) were compared for their ability to induce autophosphorylation of the dsRNA-dependent protein kinase in a cell-free systems (Fig. 2). Addition of poly(I):poly(C) (lanes 2 to 6) or HIV-1 leader RNA (lanes 8 to 12) to the cell-free system from interferon-treated HeLa cells resulted in

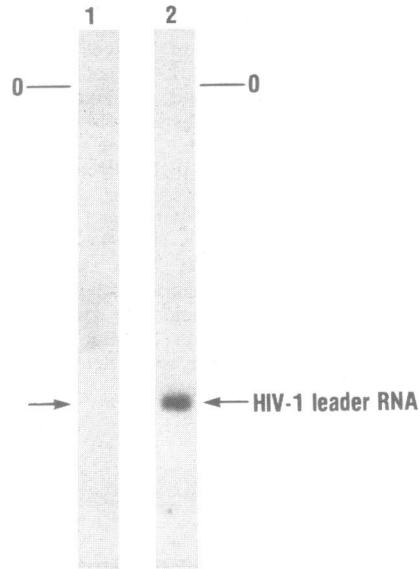

Fig. 1. Northern blot analysis of HIV-1 leader RNA using complementary strand probes. RNA on nitrocellulose was reacted with either probe for antisense RNA (lane 1, from HIV-1 DNA cloned in M13mp18) or with probe for sense RNA (lane 2, from HIV-1 DNA cloned in M13mp19). The position of HIV-1 leader RNA is indicated (arrow).

a dose-dependent activation of dsRNA-dependent kinase. The levels of phosphorylation were determined by excising the kinase bands from the dried gel and measuring the radioactivity (data not shown). The minimum concentrations of RNA resulting in activation of the kinase were 0.1 ug/ml for poly(I):poly(C) (lane 2) and 1.0 ug/ml for HIV-1 leader RNA (lane 9). Maximum activation of the kinase was obtained with 1.0 ug/ml of poly(I):poly(C) (lane 3) and 5.0 ug/ml of HIV-1 leader RNA (lane 12). The level of kinase activity in the control cell extract (lanes 13 and 14) with poly(I):poly(C) and HIV-1 leader RNA was 2.9-fold and 2.0-fold less, respectively, than was obtained using extract of interferon-treated cells. For reasons unknown, the basal level of the protein kinase in the control cells was unusually high.

HIV-1 leader RNA-cellulose is an activating affinity resin for dsRNA-dependent protein kinase

Affinity resins were synthesized to study the binding and activation of the dsRNA-dependent enzymes by HIV-1 leader RNA. Either HIV-1 leader RNA, poly(I):poly(C), or poly(U) were covalently attached at their 3' termini through a caproic acid-derivative linker to finely-divided cellulose (13). A mock cellulose preparation contained the linker without RNA (subsequently, "linker-cellulose"). The various

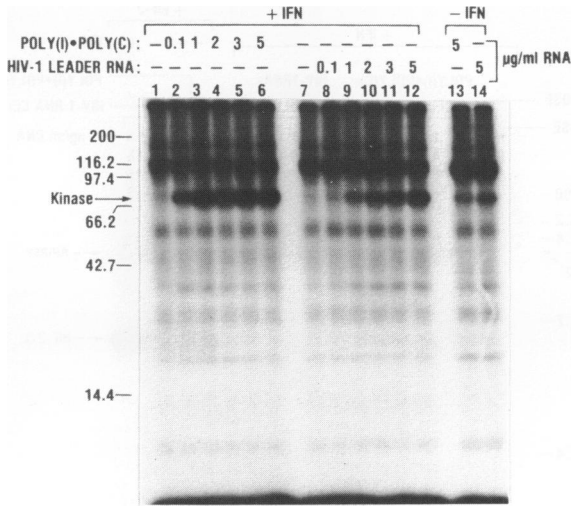

Fig. 2. Activation of dsRNA-dependent protein kinase by HIV-1 leader RNA or by poly(I):poly(C). Activation of dsRNA-dependent protein kinase was using the in solution assay (Materials and Methods). The cell extracts were from control or interferon-treated HeLa cells as indicated. The concentrations of HIV-1 leader RNA or poly(I):poly(C) and the positions of the molecular weight markers (in kdal) and of the protein kinase (arrow) are all indicated in the figure.

resins were preincubated with extracts of interferon-treated or control HeLa cells and then, after extensive washing, these were incubated in the presence of [gamma-^{32}P]ATP (Fig. 3). The autoradiogram of the gel revealed significant levels of the interferon-regulated kinase were immobilized and activated on resins containing either poly(I):poly(C) (Fig. 3, lanes 3 to 7) or HIV-1 leader RNA (lanes 8 to 12). In contrast, little or no kinase was detected using either linker-cellulose or poly(U)-cellulose (Fig. 3, lanes 1 and 2, respectively). Determination of the radioactivity in the excised kinase bands from the dried gel shown in Fig. 3 indicated that poly-(I):poly(C)-cellulose was about 8- to 10-times as active as HIV-1 leader RNA-cellulose. Induction of kinase by interferon was also demonstrated using the affinity resins (Fig. 3, lanes 13 to 16). When purified eIF-2 was added to these kinase reactions, both poly(I):poly(C) and HIV-1 leader RNA activated the kinase to phosphorylate the alpha subunit of eIF-2 (see lower arrow, Fig. 3).

2-5A synthetase was immobilized and activated on HIV-1 leader RNA-cellulose.

The ability of HIV-1 leader RNA to stimulate 2-5A-synthetase activity was similarly investigated. The affinity resins were first incubated with extracts of interferon-treated HeLa cells to allow binding of 2-5A-synthetase and then they were

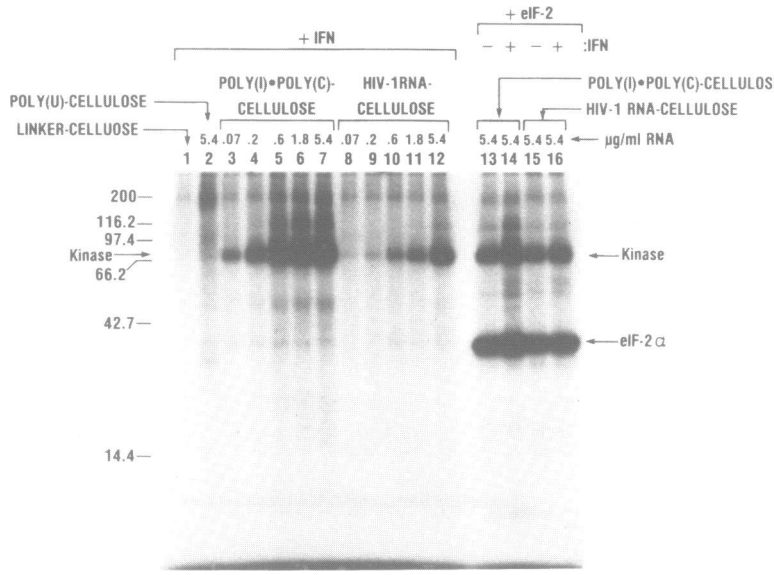

Fig. 3. Assay of dsRNA-dependent protein kinase immobilized on affinity resins. Activation of dsRNA-dependent protein kinase immobilized on various affinity resin was as described (Materials and Methods). The concentration of poly(U), HIV-1 leader RNA, or poly(I):poly(C) linked to cellulose is indicated as is the presence of added eIF-2. Cell extracts were from either control or interferon-treated HeLa cells as indicated. The positions of the molecular weight markers (in kdal) and of the kinase (upper arrow) and eIF-2 alpha (lower arrow) are shown.

washed to remove contaminating proteins. 2-5A synthesis was performed by incubating in the presence of ATP and magnesium. The 2-5A levels were determined using a highly specific radiobinding assay (15). Although HIV-1 leader RNA-cellulose bound and activated 2-5A-synthetase it did so at a low efficiency compared to poly-(I):poly(C) (Table 1). Nevertheless, there was an exponential increase in 2-5A synthesis with increasing levels of HIV-1 leader RNA (Table 1). As a result, the level of 2-5A produced using 50 ug per ml of HIV-1 leader RNA was 19% of that obtained using the same concentration of poly(I):poly(C). Furthermore, because 2-5A-dependent RNase is activated by subnanomolar levels of 2-5A even low levels of 2-5A synthetase activation could be significant. There were only trace levels of 2-5A synthesized in the incubations containing either linker-cellulose or poly(U)-cellulose (Table 1).

DISCUSSION

The current pandemic of AIDS has stimulated intense investigation into mechanisms for controlling the replication of HIV. The interferons are a family of broad

Table 1. Binding and induction of 2-5A-synthetase activity by HIV-1 leader RNA-cellulose and poly(I):poly(C)-cellulose.

RESIN	RNA bound (ug per ml)	2-5A synthesized (nM)
Linker-cellulose	0	0.41
Poly(U)-cellulose	50.0	0.36
Poly(I):poly(C)-cellulose:	0.07	76.0
	0.2	250
	0.6	750
	1.8	1,950
	5.0	3,353
	15.0	4,525
	50.0	8,225
HIV-1 leader RNA-cellulose:	0.07	2.0
	0.2	2.5
	0.6	5.4
	1.8	11.5
	5.0	24.9
	15.0	169
	50.0	1,578

spectrum antivirals (16) of potential use in the control of HIV infection. In several studies human interferons showed various levels of inhibition of HIV replication in infected cells in culture (17-21). Additional reports demonstrated synergistic inhibition of HIV replication by interferons used with azidothymidine (22), phosphonoformate (23), tumor necrosis factor-alpha (24), and granulocyte-macrophage colony-stimulating factor (25). Studies with patients indicated that interferon showed promise in the treatment of AIDS or AIDS-related conditions. Notably, interferons have been shown to have some activity against Kaposi's sarcoma associated with AIDS (26-29).

Here we have presented data indicating a possible mechanism for inhibition of HIV-1 replication in the interferon-treated cell. A computer analysis (RNAFOLD) of the structure of the first 200 bases of different isolates of HIV-1 and HIV-2 indicated extensive secondary structure and, therefore, a potential to activate dsRNA-dependent enzymes (data not shown). Activation of the dsRNA-dependent pathways could augment effects of interferon on the maturation and release of virus from infected cells (30). Because there are basal levels of the dsRNA-dependent protein kinase and 2-5A-synthetases in most if not all mammalian cells, HIV-1 leader RNA may also contribute to the virus-carrier state. For instance, suppression of HIV

replication could be achieved by a localized activation of the dsRNA-dependent antiviral pathways as proposed by Baglioni and coworkers (31, 32). Significantly, a recent report (33) indicated that translational inhibition mediated by HIV-1 leader RNA was relieved by mutations which disrupted its secondary structure. In that study, the inhibition was correlated with 5'-terminal cap accessibility. However, decreased stability of an mRNA species containing the leader structure (33) was at least consistent with activation of the 2-5A system.

There are additional indications that the dsRNA-dependent pathways could have importance in the control of replication of HIV. For instance, interferon-treated human T cells were reported to show high levels of 2-5A synthetase and 2-5A-dependent RNase (34). In addition, many patients with AIDS have an acid-labile form of interferon alpha in their serum (35) and elevated levels of 2-5A synthetase in their peripheral blood mononuclear cells (36, 37).

Viral or host factors with affinity for HIV leader RNA, perhaps the product of the tat gene (1, 38), could prevent the activation of the kinase and synthetase resulting in production of virus. In this respect there are precedents for viral-mediated repression of both the 2-5A system (39, 40) and the protein kinase pathway (5, 41-46). Therefore, it may be necessary to interfere with viral or host factors to obtain activation of the antiviral pathways in infected cells. Analysis of the 2-5A- and protein kinase systems in interferon-treated, HIV-1 infected cells (in progress) will reveal whether there is activation or repression of these pathways in intact cells.

ACKNOWLEDGMENTS

We thank Dr. D. Capon (Genentech) for the gift of plasmid pSP6HIV+1 and for valuable discussions with him and with Dr. C. W. Czarniecki (Genentech), Dr. I. M. Kerr (I.C.R.F., London), and Drs. K. Minton, P. Grimley, K.V. Holmes, and R.M. Friedman (U.S.U.H.S.). This investigation was supported by United States Public Health Service Grant number 1 RO1 CA 44059 awarded by the Department of Health and Human Services, National Cancer Institute and by the Uniformed Services University of the Health Sciences grant number CO74BK.

*To whom correspondence should be addressed

REFERENCES

1. Muessing, M.A., Smith, D.H., and Capon, D.J. (1987) Cell **48**, 691-701.
2. Muessing, M.A., Smith, D.H., Cabradilla, C.D., Benton, C.V., Lasky, L.A., and Capon, D.J. (1985) Nature **313**, 450-458.
3. Johnston, M.I. and Torrence, P.F. (1984) In Friedman, R.M. (ed) Finter, N.B. (gen. ed.), Interferons 3: Mechanism of Production and Action, Elsevier, New York, pp. 189-341.

4. Pestka, S., Langer, J.A., Zoon, K.C., and Samuel, C.E. (1987) Ann. Rev. Biochem. **56**, 727-777.
5. Katze, M. G., DeCorato, D., Safer, B., Galabru, J., and Hovanessian, A.G. (1987) EMBO J. **6**, 689-697.
6. Safer, B. (1983) Cell **33**, 7-8, 1983.
7. Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982) In, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory.
8. Melton, D.A., Krieg, P.A., Rebagliati, M.R., Maniatis, T., Zinn, K., and Green, M.R. (1984) Nucleic Acid Res. **12**, 7035-7056.
9. McMasters, G.K. and Carmichael, G.G. (1977) Proc. Natl. Acad. Sci. U.S.A. **74**, 4835-4838.
10. Silverman, R.H. and Krause, D. (1987) In Clemens, M.J., Morris, A.G., Gearing, A.J.H. (eds), Lymphokines and Interferons: A Practical Approach, IRL Press, Washington, D.C., pp. 149-193.
11. Hu, N.-t. and Messing, J. (1982) Gene **17**, 271-277.
12 Williams, J.G. and Mason, P.J. (1985) In Hames, B.D., Higgins, S.J. (eds), Nucleic Acid Hybridization: a practical approach, IRL Press, Washington, D.C., pp. 139-160.
13. Wells, J.A., Swyryd, E.A., and Stark, G.R. (1984) J. Biol. Chem. **259**, 1363-1370.
14. Galabru, J. and Hovanessian, A. (1987) J. Biol. Chem. **262**, 15538-15544.
15. Knight, M., Cayley, P.J., Silverman, R.H., Wreschner, D.H., Gilbert, C.S., Brown, R.E., and Kerr, I.M. (1980) Nature **288**, 189-192.
16. Stewart II, W.E. (1981) In, The Interferon System, 2nd edn., Springer-Verlag, New York.
17. Ho, D.D., Rota, T.R., Kaplan, J.C., Hartshorn, K.L., Andrews, C.A., Schooley, R.T., and Hirsch, M.S. (1985) Lancet **i**, 602-604.
18. Dolei, A., Fattorossi, A., D'Amelio, R., Aiuti, F., and Dianzani, F. (1986) J. Interferon Res. **6**, 543-549.
19. Yamamoto, J.K., Barre-Sinoussi, F., Bolton, V., Pedersen, N.C., and Gardner, M.B. (1986) J. Interferon Res. **6**, 143-152.
20. Nakashima, H., Yoshida, T., Harada, S., and Yamamoto, N. (1986) Int. J. Cancer **38**, 433-436.
21. Hartshorn, K.L., Neumeyer, D., Vogt, M.W., Schooley, R.T., and Hirsh, M.S. (1987) AIDS Research and Human Retroviruses **3**, 125-133.
22. Hartshorn, K.L., Vogt, M.W., Chou, T.-C., Blumberg, R.S., Byington, R., Schooley, R.T., and Hirsch, M.S. (1987) Antimicrob. Agents Chemother. **31**, 168-172.
23. Hartshorn, K.L., Sandstrom, E.G., Neumeyer, D. Paradis, T.J., Chou, T.-C., Schooley, R.T., and Hirsch, M.S. (1986) Antimicrob. Agents Chemother. **30**, 189-191.
24. Wong, G.H.W., Krowka, J., Stites, D.P., and Goeddel, D.V. (1987) J. Interferon Res. **7**, 773, abstr. II-45.
25. Hammer, S.M., Gillis, J.M., Groopman, J.E., and Rose, R.M. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 8734-8738.
26. Krown, S.E., Real, F.X., Cunningham-Rundles, S., Myskowski, P.L., Koziner, B., Fein, S., Mittelman, A., Oettgen, H.F., and Safai, B. (1983) New Engl. J. Med. **308**, 1071-1076.
27. Groopman, J.E., Gottlieb, M.S., Goodman, J., Mitsuyasu, R.T., Conant, M.A., Prince, H., Fahey, J.L., Derezin, M., Weinstein, W.M., Casavante, C., Rothman, J., Rudnick, S.A., and Volberding, P.A. (1984) Ann. Intern. Med. **100**, 671-676.
28. Gelman, E.P., Preble, O.T., Steis, R., Lane, H.C., Rook, A.H., Wesley, M., Jacob, J., Fauci, A., Masur, H., and Longo, D. (1985) Amer. J. Med. **78**, 737-741.
29. Contu, L., Cerimele, D., Del Giacco, G.S., and Pintus, A. (1987) Antibiol. Chemother. **38**, 121-131.
30. Friedman, R.M. and Pitha, P.M. (1984) In Friedman, R.M. (ed) Finter, N.B. (gen. ed.), Interferons 3: Mechanism of Production and Action, Elsevier, New York, pp. 319-341.

31. Nilsen, T.W. and Baglioni, C. (1979) Proc. Natl. Acad. Sci. USA **76**, 2600-2604.
32. De Benedetti, A. and Baglioni, C. (1984) Nature **311**, 79-81.
33. Parkin, N.T., Cohen, E.A., Darveau, A., Rosen, C., Haseltine, W., and Sonenberg, N. (1988) EMBO J. **7**, 2831-2837.
34. Schmidt, A., Crisp, B., Krause, D., Silverman, R.H., Herberman, R.B., and Ortaldo, J.R. (1987) Nat. Immunity Cell Growth Regul. **6**, 19-27.
35. DeSefano, E. Friedman, R.M., Friedman-Kien, A.E., Goedert, J.J., Henriksen, D., Preble, O.T., Sonnabend, J.A., Vilcek, J. (1982) J. Infect. Dis. **146**, 451-455.
36. Preble, O.T., Rook, A.H., Steis, R., Silverman, R.H., Krause, D., Quinnan, G.V., Masur, H., Jacob, J., Longo, D., and Gelmann, E.P. (1985) J. Infect. Dis. **152**, 457-465.
37. Read, S.E., Williams, B.R.G., Coates, R.A., Evans, W.K., Fanning, M.M., Garvey, M.B., and Shepard, F.A. (1985) J. Infect. Dis. **152**, 466-472.
38. Patarca, A. and Haseltine, W.A. (1987) AIDS Research and Human Retroviruses **3**, 1-2.
39. Cayley, P.J., Knight, M., and Kerr, I.M. (1982) Biochem. Biophys. Res. Commun. **104**, 376-382.
40. Silverman, R.H., Cayley, P.J., Knight, M., Gilbert, C.S., and Kerr, I.M. (1982) Eur. J. Biochem. **124**, 131-138.
41. O'Malley, R.P., Mariano, T.M., Siekierka, J., Mathews, M.B. (1986) Cell **44**, 391-400.
42. Kitajewski, J., Schneider, R.T., Safer, B., Munemitsu, S.M., Samuel, C.E., Thimmappaya, B., and Shenk, T. (1986) Cell **45**, 195-200.
43. Whitaker-Dowling, P. and Youngner, J.S. (1983) Virology **131**, 128-136.
44. Rice, A.P. and Kerr, I.M. (1984) J. Virol. **50**, 229-236.
45. Paez, E. and Esteban, M. (1984) Virology **134**, 12-28.
46. Hovanessian, A.G., Galabru, J., Meurs, E., Buffet-Janvresse,C., Svab, J., and Robert, N. (1987) Virology **159**, 126-136.