Induction of Interferon in Lymphoblastoid Cell Lines[1] (34628)

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In the past few years, lymphoblastoid cell cultures have been established from individuals with a variety of lymphoproliferative and myeloproliferative disorders (1–7) and from apparently normal individuals (8). Particular interest in these cultures has been stimulated by the discovery of a new member of the herpes virus group, the Epstein-Barr virus (EBV), first in lymphoblastoid cell lines initiated from patients with Burkitt's lymphoma (9) and subsequently, in other disorders (7, 10, 11) and from some normal individuals (8). The demonstration of the EBV in a high proportion of the Burkitt cell lines and cell lines derived from patients with infectious mononucleosis (10) has strengthened seroepidemiologic data (11, 12) implicating the EBV in both of these conditions. Interferon production has also been described in cell lines derived from patients with Burkitt's lymphoma (6, 13), infectious mononucleosis (14), leukemia (15), and the Chediak-Higashi syndrome (16). The present study was undertaken to determine (1) if a relationship exists between interferon production by a lymphoblastoid cell line and the disease state in the patient from which it was established; and (2) if the presence of the EBV in a cell line could be correlated with interferon production by a cell line. It will be shown that interferon production by lymphoblastoid cells is not related to the presence of EB virus. Consequently, a broader study of the presence of other possible inducers of interferon in lymphoblastoid cell lines was undertaken and reported here.

Materials and Methods. Cultures. Fresh leukocyte cultures were prepared from blood of normal individuals as previously described (5). Lymphoblastoid cell cultures were obtained from several investigators whose cooperation is gratefully acknowledged. These cell lines had been established from normal individuals, from patients with leukocytosis of unknown etiology who were otherwise healthy and from patients with infectious mononucleosis, various leukemias, lymphosarcoma, and Burkitt's lymphoma. The cell lines were obtained from Dr. G. Moore, Roswell Park Memorial Institute, Buffalo, N. Y. and Drs. D. P. Stites, P. Glade, J. Fahey, R. Manaker, and A. Rabson of NIH. The cell line designation, reference to isolation, and disease states in the patient from which the culture was established are listed in Table I. Lymphoblastoid cell lines (LBCL) were maintained as suspension cultures by adding an equal volume of fresh growth medium (GM) consisting of RPMI 1640 medium with 20% heat-inactivated fetal calf serum (FCS), penicillin, streptomycin, and glutamine at 3- to 6-day intervals to the cultures.

Assay of antiviral activity. Tissue culture fluids obtained 72 hr after adjusting the cultures to equal cell densities with fresh GM were rendered cell-free by freezing and thawing once followed by centrifugation at 2000 rpm for 10 min. The cell-free fluids were examined for antiviral activity by modification of a vesicular stomatitis virus (VSV) yield reduction assay (17). The present assay employed Sindbis virus and AH1 cells, a fibroblast line derived from vaginal tissue (NIH media unit), because of its increased sensitivity in the detection of human interferons (18). Culture fluids shown to have antiviral activity in this assay were subjected to further testing to determine if they met criteria for interferons, *viz.*, activity

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TABLE I. Interferon Production and EBV Immunofluorescence of Lymphoblastoid Cells.

Cell line designation, reference to isolation		Disease state	Interferon titer ($\log_{10}$ yield reducing units/ml)	EBV (% fluorescent cells/1000 cells)
RPMI 82880			91	0.0
RPMI 84069	(8)	Normal individuals	250	0.0
RPMI 81954			8	0.0
RPMI 2177			270	0.1
GCS 1			18	0.15
GCS 2	D. P. Stites (unpub.)	Leucocytosis of unknown etiology	19	1.0
GCS 5			44	2.5
GCS 6			21	4.6
PGLC 334			21	1.6
PGLC 33J	(4)	Infectious mononucleosis	36	0
PGLC 42D			25	2.4
PGLC 42F			30	0
PGLC 44B			166	3.5
PT	J. A. Kasel (unpub.)		0	0
LKID	(1)	Acute lymphocytic leukemia	0	0
RPMI 4265	(4)	Chronic myelogenous leukemia	48	0
RPMI 8866	(4)	Acute myelogenous leukemia	8	1.6
IM1	(38)	Lymphosarcoma	8	4.4
Raji	(21)	Burkitt's lymphoma	0	0.0
AL1	(6)		10	0.8
P3J	R. Pulvertaft (unpub.)		0	5.0

against unrelated viruses, species specificity, nonsedimentability, sensitivity to trypsin, and loss of activity by pretreatment of the AH1 cells with actinomycin D.

Assay of EBV immunofluorescence. Serum from a patient with leukocytosis of unknown etiology (obtained from Dr. D. Stites) had an anti-EBV titer of 1:640 by indirect immunofluorescence testing with the P3J cell line. The IgG portion of this serum was isolated by chromatography on DEAE–cellulose and conjugated to fluorescein isothiocyanate (FITC) by the method of Wood *et al.* (19). The FITC-conjugated serum was exhaustively dialyzed to remove unbound dye and employed at a 1:6 dilution in direct immunofluorescence tests with the cell lines. Details of the test procedure and specificity tests for the fluorescent antiserum to the EB virus have been published (20). Cells from each lymphoblastoid culture were examined for EB virus immunofluorescent antigens after growth for 4–7 days in arginine-free medium and after aging the cultures for 9 days. Both culture conditions have been shown to increase the number of cells containing EB viral antigens (21, 22).

Tests for microbial organisma and endotoxin. Cell-free fluids from lymphoblastoid lines were examined for known viral, bacterial, and fungal organisms by published methods (23). Monitoring for mycoplasma was done through the courtesy of Dr. R. Purcell (LID, NIAID, NIH), and pyrogen assays were kindly done by Dr. S. M. Wolff.

Labeling and extraction of cellular RNA. Lymphoblastoid cells (10^8) were labeled in 100 ml of GM containing 5 μCi/ml of uridine-5-^{3}H (Amersham-Searle, specific activity 5 μCi/mmole) for 18 hr at 37°. Cellular RNA was extracted with sodium-dodecyl-sulfate (0.4% SDS), cold and hot phenol (24) and had specific activities of 1–4 $\times$ 10^5 cpm/μg of RNA. Presumptive double-stranded (ds) RNA was isolated after treatment of labeled cellular RNA with deoxyribonuclease and ribonuclease (in 0.2 *M* NaCl) on 6% agarose columns according to the method of

Colby and Duesberg (25). Minimal modifications in this method included collection of smaller effluent fractions (2.5 ml), counting of 300 μl aliquots in liquifluor-Biosolve (Beckman), and marking of the void volume of the column with ^{14}C-labeled bacteriophage DNA (obtained from K. Berns, NIH). Material appearing in the void volume was shown to be sensitive to alkaline hydrolysis (0.3 *N* KOH overnight at 37°).

Enzymes. Deoxyribonuclease and ribonucleases (pancreatic and T_1) were obtained from Worthington Biochemicals and employed at concentrations of 30 μg/ml and 100 units/ml (T_1). Enzymatic activity was monitored by counting the acid-insoluble radioactivity remaining after digestion of the cellular RNA.

Results. Interferon production. An inhibitor which met criteria for interferon was present in cell-free fluids from 17 of 21 lymphoblastoid cultures. As shown in Table I, interferon was detected in cell lines established from normal individuals, and from patients with leukocytosis of unknown etiology, infectious mononucleosis, leukemia, and lymphoma. Cell lines in which interferon was not detected ("nonproducer"-NP cultures) occurred in the Burkitt's lymphoma, leukemia, and infecctious mononucleosis groups. In contrast to the lymphoblastoid cultures, inhititor activity was not detectable in cell-free fluids obtained from fresh leukocyte cultures from normal donors or donors with infectious mononucleosis. Culture conditions and cell density in these experiments were comparable to those in the lymphoblastoid cell lines.

Interferon production and EBV immunofluorescence. The presence of the EBV immunofluorescent antigen in cells from aged and arginine-deficient lymphoblastoid cultures was determined. A comparison of interferon production by cell lines and their content of EBV detected by immunofluorescence is shown in Table I. It is evident that there are many cell lines that produce interferon but that do not contain detectable EBV at the time of interferon production; and, conversely, at least one culture contains EBV (P3J) but does not produce interferon. In addition, there was no relationship of interferon titers to the percentage of cells positive for EBV by immunofluorescence.

The above experiments indicated that the EB virus was not the inducer of interferon in lymphoblastoid cultures. Other experiments described below were then undertaken to systematically study other possible inducers of interferon in relation to the LBCL.

Microbial organisms. Endotoxin. Despite extensive testing, known viral, bacterial, fungal, or mycoplasma organisms were not isolated from cell-free harvests of the lymphoblastoid lines. Medium and cell-free harvests did not contain endotoxin as evidenced by negative pyrogen tests.

The possibility was considered that the lymphoblastoid lines might harbor a latent virus with replication limited to lymphocyte and/or lymphoblast cells as described for EBV (26). Accordingly, cell-free fluids and lysates of interferon-producing cultures were incubated with fresh leukocyte cultures and the interferon-negative LKID and PT lymphoblastoid lines, as follows. Cell-free lysates obtained from 10^8 cells of interferon producer cultures were adsorbed at 37° for 90 min to cell packs of 10^8 cells of NP cultures and fresh leukocyte cultures. At the end of 90 min, the cell packs were diluted to 3×10^6 cells/ml with GM and incubated at 37°. Assays of these cultures for antiviral activity over the next 2 weeks were persistently negative. These experiments suggested that interferon production was not induced by a transmissible agent in the lymphoblastoid lines under the described conditions.

Ribonucleic acids. Since heterologous ribonucleic acid (RNA) (27) and synthetic ribopolymers (28) induce interferon, experiments were designed to investigate the relationship of RNA to interferon production in the lymphoblastoid lines. These experiments were predicated on the following scheme. If RNA were the inducer of interferon in the lymphoblastoid culture, it would presumably come from dead cells in the culture. Exposure of RNA released from dead cells to the extracellular environment could result in alteration in the RNA to a non-native form that would be capable of stimulating viable cells to produce interferon analogous to exposure to heterologous RNA's. In this scheme, the hypothetical interferon inducer would be during its extracellular period, susceptible to destruction by ribonucleases. Thus, to try to answer the question of the role of RNA in interferon production by the lymphoblastoid lines, the experimental strategy was to assess changes in interferon production during ribonuclease treatment of the culture. The experimental procedure was as follows: RPMI 84069, a lymphoblastoid culture that consistently produced large amounts of interferon, was divided into two equal portions, centrifuged at 800 rpm for 10 min, washed once with fresh medium, and resuspended in 10 ml of 1640 medium with 10% FCS and glutamine. One portion served as the control. To the other T_1 and pancreatic ribonucleases were added.

In one set of experiments, cell-free fluids were assayed for inhibitor activity after 24 and 72 hr of enzyme treatment. In another experiment, the control and enzyme-treated cultures were centrifuged after 24 hr of ribonuclease exposure and, after washing one time with fresh medium, were resuspended in fresh GM. At intervals over the next 24 hr, cell-free fluids were assayed for antiviral activity to determine the rate of restoration of interferon production. Cell viability was not affected by enzyme treatment (determined by trypan blue dye exclusion) and ribonucleases were as active in the presence of cells and 10% FCS as in medium alone. The combination of pancreatic and T_1 ribonucleases degraded 97% of cellular RNA to acid-soluble nucleotides in 60 min at 37°.

Ribonuclease treatment failed to alter interferon production by lymphoblastoid line 84069. The ribonucleases did not decrease interferon production during the 72 hr of exposure of the cultures to enzymes. Ribonuclease treatment also did not affect the kinetics of restoration of interferon to fresh GM as shown in Fig. 1. In the latter experiment, it would be anticipated that if ribonuclease treatment removed the inducer of interferon,

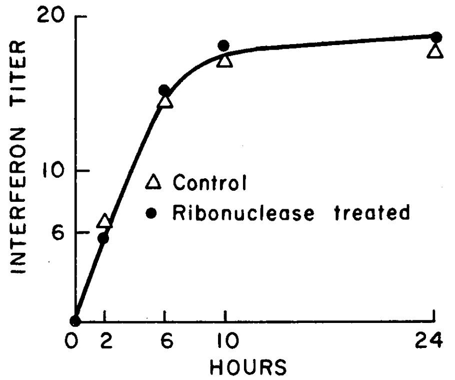

FIG. 1. Interferon production after exposure of cell line 84069 to ribonucleases. Cell line 84069 was exposed to pancreatic and T1 ribonuclease for 24 hr. The culture was then washed and resuspended in fresh GM. At intervals, aliquots of the culture were taken and assayed for interferon production.

then some time would be required after the enzymes were removed before induction of interferon would be detected compared to the control with constant exposure to inducer. No such lag in interferon production occurred in the enzyme-treated cultures.

Recent findings (25, 29) have disclosed ds RNA in animal cells in small amounts, 0.1 to 1% of the total cell RNA. Since such material is ribonuclease-resistant, it would not be excluded as the interferon inducer in lymphoblastoid cell lines by the ribonuclease experiments just described. A different experimental approach was required, therefore, to examine the role of ds RNA in interferon production. It seemed reasonable that if, in fact, ds RNA induced interferon in the lymphoblastoid cell lines, that it might be present in increased amounts in a producer compared to a nonproducer culture, and furthermore, that ds RNA isolated from a producer culture might induce interferon in the nonproducer culture. Accordingly, a producer culture (84069) and a nonproducer culture (Raji) were adjusted to the same cell density (10^6 cells/ml, 100-ml culture), labeled with ^{3}H-5-uridine, the RNA extracted, and the ribonuclease-resistant material appearing in the exclusion volume of a 6% agrose column (Fig. 2) collected. As shown in Table II, the interferon nonproducer culture contained a larger amount of ds RNA as determined by

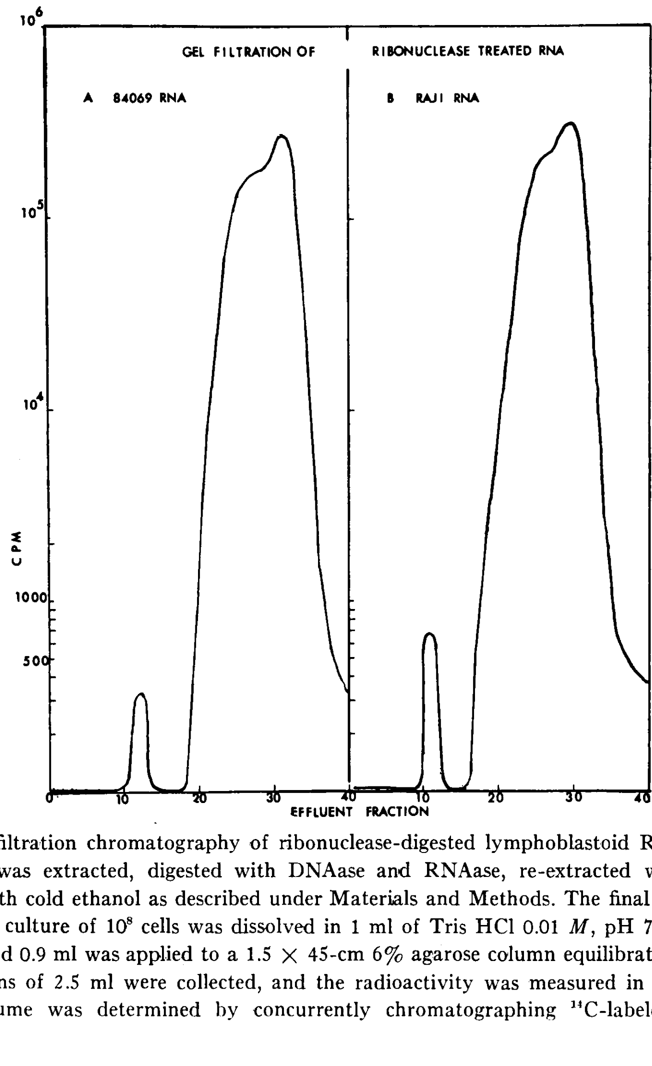

FIG. 2. Gel filtration chromatography of ribonuclease-digested lymphoblastoid RNA. ^{3}H-Uridine-labeled RNA was extracted, digested with DNAase and RNAase, re-extracted with phenol, and precipitated with cold ethanol as described under Materials and Methods. The final yield of RNA as treated from a culture of 10^8 cells was dissolved in 1 ml of Tris HCl 0.01 *M*, pH 7.4, 0.1 *M* LiCl, 2 m*M* EDTA, and 0.9 ml was applied to a 1.5 × 45-cm 6% agarose column equilibrated with the same buffer. Fractions of 2.5 ml were collected, and the radioactivity was measured in a 300 μl aliquot. The void volume was determined by concurrently chromatographing ^{14}C-labeled bacteriophage DNA.

percentage of recovery of the radioactivity in the RNAase-digested RNA applied to the column. In addition, the ds RNA was devoid of biological activity, *i.e.*, ds RNA isolated from either Raji or 84069 did not induce interferon in Raji cells, although Raji could be induced to synthesize greater than 20 units of interferon by exposure to 20 μg/ml of (In:Cn) polycytidylic-inosinic acid.

Deoxyribonucleotides have been demonstrated to stimulate antibody-forming cells, and it has been suggested that interferon might also be stimulated in this manner (30). To examine this possibility, the NP culture LKID was divided into control and test cultures. The latter was exposed to a digest of calf thymus DNA (Sigma) obtained by incubation of 1000 μg of DNA with deoxyribonuclease in Tris 0.002 *M*, pH 7.6, 0.005 *M* in $MgCl_2$ for 24 hr. After 24-hr exposure of LKID to 10–300 μg/ml of the DNA digest, cell-free fluids were assayed for antiviral activity. In addition to the untreated control, LKID stimulated with In:Cn was also assayed. In contrast to the positive In:Cn control, the DNA digest did not stimulate interferon production.

Relationship of interferon to the lymphoblastoid state. Four cultures were studied sequentially from the time that fresh leukocyte

TABLE II. Relation of Double-Stranded RNA to Interferon Production by Lymphoblastoid Cell Lines.

Cell line	IF production	% ds RNA[a]	Interferon induction[b]
84069	+	0.05%	—
Raji	—	0.1%	—

[a] Total radioactivity applied to agarose column (Fig. 2) was 8.8×10^6 dpm for 84069 and 9.1×10^6 dpm for Raji, of which >99.9% was recovered in the effluent fractions. The percentage of ds RNA was taken as the radioactivity recovered in the void volume fractions/total radioactivity applied.

[b] Raji cells (10^7) were exposed to the peak effluent fractions containing the 84069 and Raji RNA-ase-resistant RNA in GM containing 10 μg/ml of DEAE–Dextran (29). At the end of 3 hr, the cells were centrifuged, washed, and resuspended in 10 ml of fresh GM. After 24 hr of additional incubation, the tissue culture fluids were assayed for interferon.

cultures were initiated through the time that the cultures were considered continuous. A sudden increase in acid production, clumping of cells, and increase in cell number were taken as criteria for continuous cultures. Cell-free fluids obtained at weekly intervals from the time of initiation of each culture were assayed for inhibitor activity. In each case antiviral activity was first detected at the same time as signs of long-term proliferation became evident.

Discussion. In the present study, 17 of 21 human lymphoblastoid cell lines were found to elaborate an antiviral substance with the properties of interferon. Interferon production occurred in cell lines derived from patients with leukocytosis of unknown etiology, infectious mononucleosis, leukemia, lymphosarcoma, and Burkitt's lymphoma. Thus, interferon production by a lymphoblastoid culture does not correlate with any particular lymphoproliferative disorder and in fact, was found in the cell lines from four normal individuals. Similar conclusions were reached by Northrop and Deinhardt (31), in studies of spontaneous interferon production by human bone marrow tissue *in vitro* from normal individuals and patients with a variety of diseases.

A major interest in spontaneous interferon production by lymphoblastoid lines has been in its possible use as an indicator of latent viral infection, especially by EBV (13). The present investigation demonstrates that interferon production is not related to infection of lymphoblastoid cultures by EBV.

The failure to correlate interferon production with EBV infection prompted a broader investigation of possible inducers of interferon in the lymphoblastoid lines. Despite extensive testing in animals and tissue culture, known viral bacterial, fungal, or mycoplasma organisms were not isolated. Attempts to passage an agent, assumed to have infectivity restricted to lymphocyte or lymphoblast cells (similar to EBV), to interferon negative leukocyte cultures or lymphoblastoid lines were also unsuccessful. Endotoxin-stimulated interferon production, suggested as the inducer of low levels of spontaneous interferon elaboration in rabbit macrophage culture (32), was excluded by negative pyrogen tests.

Autostimulation of lymphoblastoid lines by altered homologous cell RNA was excluded by the failure of ribonucleases to alter interferon production. Stimulation of the cell lines by ds RNA would not be excluded by these experiments because of its ribonuclease resistance. The greater recovery of ds RNA under identical conditions from a nonproducer culture and the failure of ds RNA to induce interferon argues, however, against this possibility. It seems unlikely, therefore, that single- or double-stranded RNA released from dying cells is the inducer of interferon in lymphoblastoid lines. Heterologous deoxyribonucleotides at concentrations that produce marked stimulation of antibody-forming cells, failed to induce interferon in fresh leukocyte cultures of lymphoblastoid cultures. This finding and the failure of synthetic deoxyribonucleotides to induce interferon (38), make it unlikely that deoxyribonucleotides act as the stimulus to interferon production in the lymphoblastoid cultures.

The remaining known stimuli of interferon in leukocyte cultures include phytohemagglutinin (PHA), pokeweed mitogen (PWM), streptolysin, sensitized lymphocytes with their appropriate antigen, and antilymphocyte serum (34–37). All of these inducers share the common property of transforming resting lymphocytes to a lymphoblastoid cell and may induce interferon synthesis as one manifestation of derepression of gene expression at either the transcriptional or translational level. It is quite possible that a similar mechanism of interferon production is operative in the lymphoblastoid lines, i.e., in the dedifferentiation process that occurs as the lymphocyte assumes a lymphoblastoid character, interferon is produced as part of the increase in gene expression. The data presented are consistent with this interpretation in that transformation of leukocytes to lymphoblastoid cells was necessary for the expression of interferon production. The failure to detect interferon in some of the lymphoblastoid lines may be due to a population of leukocytes in which the interferon gene is destined not to be expressed analogous to occasional leukocyte cultures that undergo blast transformation without associated interferon production. Alternatively, "NP" cultures may produce small amounts of interferon that fall below the sensitivity of the assay system.

Summary. Lymphoblastoid cell lines derived from normal individuals and from patients with a variety of myelo- and lymphoproliferative disease states were examined for spontaneous interferon production. Interferon was synthesized by 17 of 21 lines investigated. No correlation was found with the disease state, and cultures from normal individuals produced interferon. Interferon production by lymphoblastoid cell lines is not correlated with infection of the cultures by EBV. The other nonviral inducers of interferon were studied and excluded as being responsible for the spontaneous production. It is proposed that interferon production by these cell lines is the result of a derepression of gene expression associated with the lymphoblastoid state analogous to interferon synthesis induced by lymphocyte mitogens.

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1. Armstrong, D., Proc. Soc. Exp. Biol. Med. **122**, 475 (1966).

2. Epstein, M. A., Henle, G., Achong, B. G., and Barr, Y. M., J. Exp. Med. **121**, 761 (1965).

3 Iwakata, S, and Grace, J. T., N. Y. State J. Med. **64**, 2279 (1964).

4. Pope, J. H., Nature **216**, 310 (1967).

5. Glade, P. R., Kasel, J. A., Moses, H. L., Whang-Peng, J., Hoffman, P F., Kammermeyer, J K., and Chessin, L. N., Nature **217**, 564 (1968).

6. Rabson, A. S., O'Conor, G. T., Baron, S., Whang, J. J., and Legallais, F. Y., Int. J. Cancer **1**, 89 (1966).

7. Zeve, V. H., Lucas, L. S., and Manaker, R. A., J. Nat. Cancer Inst. **37**, 761 (1966).

8. Moore, G. E., Gerner, R. E., and Franklin, H. A., J. Amer, Med. Ass. **199**, 519 (1967).

9. Epstein, M. A., Achong, B. G., and Barr, Y. M., Lancet **1**, 702 (1964).

10. Diehl, V., Henle, G., Henle, W., and Kohn, G., J. Virol. **2**, 663 (1968).

11. Henle, G., Henle, W., and Diehl, V., Proc. Nat. Acad. Sci. U.S.A. **59**, 94 (1968).

12. Evans, A. S., Niederman, J. C., and McCollum, R. W., New Eng. J. Med. **279**, 1121 (1968).

13. Henle, G., and Henle, W., J. Bacteriol. **89**, 252 (1965).

14. Kasel, J. A., Haase, A. T., Glade, P. R., and Chessin, L. N., Proc. Soc. Exp. Biol. Med. **128**, 351 (1968).

15. Deinhardt, F., and Burnside, J., J. Nat. Cancer Inst. **39**, 681 (1967).

16. Blume, R. A., Glade, P. R., Gralnick, H. R., Chessin, L. N., Haase, A. T., and Wolff, S. M., Blood **33**, 821 (1969).

17. Baron, S., Buckler, C. E., Levy, H. B., and Friedman, R. M., Proc. Soc. Exp. Biol. Med. **125**, 1320 (1967)

18. Baron, S., Personal communication.

19. Wood, B. T., Thompson, S. H., and Goldstein, G., J. Immunol. **95**, 225 (1965).

20. Hinuma, Y., Konn, M., Yamaguchi, J., Wudarski, D. J., Blakeslee, J. R., Jr., and Grace, J. T., Jr., J. Virol. **1**, 1045 (1967).

21. Henle, W., and Henle, G., J. Virol. **2**, 182 (1968).

22. Hinuma, Y., Konn, M., Yamaguchi, J., and Grace, J. T., Jr., J. Virol. **1**, 1203 (1967.

23. Knight, V., Progr. Med. Virol. **6**, 1 (1964).

24. Carter, W. A., and Levy, H. B., Arch. Biochem.

Biophys. **120**, 563 (1967).

25. Colby, C., and Duesberg, P. H., Nature **222**, 940 (1969).

26. Horoszewics, J. S., Dunkel, V. C., and Grace, J. T., Jr., Fed. Proc. **27**, 263 (1968).

27. Rotem, Z., Cox, R. A., and Isaacs, A., Nature **197**, 564 (1963).

28. Field, A. K., Tytell, A. A., Lampson, G. P., and Hilleman, M. R., Proc. Nat. Acad. Sci. U.S.A. **58**, 1004 (1967).

29. Montagnier, M. L., and Lacassagne, M. A., C. R. Acad. Sci. **267**, 1417 (1968).

30. Braun, W., and Plescia, O., "Nucleic Acids in Immunology," Springer, New York (1968).

31. Northrop, R. L., and Deinhardt, F. J., J. Nat. Cancer Inst. **39**, 685 (1967).

32. Smith, T. J., and Wagner, R. R., J. Exp. Med. **125**, 559 (1967).

33. Vilcek, J., Ng, M. H., Friedman-Kien, A. E., and Drawciw, T., J. Virol. **2**, 648 (1968).

34. Edelman, R., and Wheelock, E. F., Fed. Proc. **27**, 1949 (1968).

35. Freidman, R. M., and Cooper, H. L., Proc. Soc. Exp. Biol. Med. **125**, 901 (1967).

36. Green, J. A., Cooperband, S. R., and Kibrick, S., Science **164**, 1415 (1969).

37. Wheelock, E. F., Science **149**, 310 (1965).

38. Fahey, J. L., Finegold, I., Rabson, A. S., and Manaker, R. A., Science **152**, 1259 (1966).

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