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Interaction Between 6/94 Virus, a Parainfluenza Type 1 Strain, and Human Leukocytes

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6/94 virus, a parainfluenza type 1 virus recovered by lysolecithin fusion of multiple sclerosis brain cell cultures with CV-1 cells, replicated in monocyte macrophages and lymphocytes from normal human donors and from a patient with multiple sclerosis. In macrophage cultures, hemadsorption-positive cells and high levels of infectious virus became apparent within 24 to 48 h after infection, persisted for 6 days, and then began to decrease. Phytohemagglutinin-stimulated macrophages yielded similar titers of virus, but the levels were maintained for a longer period of time. Macrophage-produced virus appeared to be infectious for other macrophages in the same culture. Both unstimulated and phytohemagglutinin-stimulated lymphocytes also supported virus replication. Significantly higher titers were produced in the stimulated cultures, T cell-enriched populations producing more virus than unseparated populations whether stimulated or unstimulated. The presence or absence of antibodies to the virus in the donors did not appear to influence the levels of virus obtained in any of the leukocyte cultures. However, an increase in blastic forms after 6/94 virus infection was noted in lymphocytes from donors with antibodies as revealed morphologically and by increased incorporations of tritiated thymidine. Furthermore, 6/94 virus-infected lymphocytes, unlike Sendai virus-infected lymphocytes, were able to respond well to mitogenic stimulation by phytohemagglutinin.

Two preceding papers (22, 23) described studies on the interaction of mouse macrophages and lymphocytes with 6/94 virus, an agent recovered from brain cell-derived cultures from two patients with multiple sclerosis (MS) after lysolecithin-mediated fusion with CV-1 cells (20). The results of these studies indicated that the virus replicated in both macrophages and unstimulated lymphocytes.

Of interest was the finding that macrophages and lymphocytes derived from immunized mice that had high levels of specific humoral neutralizing antibodies were still variably susceptible to 6/94 infection. Zisman and Denman (26) reported that Sendai virus, an antigenic relative of the 6/94 agent (13), was inactivated by human and mouse lymphocytes independent of the presence of humoral antibodies. Differences in infectivity between 6/94 and Sendai viruses for mouse macrophages (4, 22) and lymphocytes (23, 26) made it of interest to investigate the interaction of 6/94 virus with human leukocytes. Accordingly, this study of the interaction of 6/94 virus with cultures of cells from healthy donors with and without antibodies against 6/94 virus and with cultures of cells from an MS patient was undertaken.

MATERIALS AND METHODS

Donors. Peripheral blood was obtained from six different subjects. Four of these donors were healthy members of the laboratory staff, aged 25 to 60 years, all of whom had circulating antibodies against 6/94 and Sendai viruses. One subject was a 24-year-old MS patient in the acute stage of the disease; he also possessed antibodies to 6/94 and Sendai viruses. Blood without antibodies against these viruses was obtained from a 5-year-old child who was bled for other purposes. Since infection with the HA_2 strain of parainfluenza type 1 virus induces antibodies that cross-react to a high degree with 6/94 and Sendai strains, it is difficult to find 6/94 and Sendai virus antibody-free donors among older age groups (F. S. Lief, unpublished data).

Separation of leukocytes. Separation of leukocytes from erythrocytes (RBC) was made either by buffy coat prepared in Plasmagel (R. Bellon, Neuilly, Seine, France), or by sedimentation in a Ficoll-Hypaque gradient (1). The leukocytes thus obtained were suspended at 5×10^6 to 10×10^6/ml in RPMI 1640 medium plus 10% fetal bovine serum and antibiotics as reported for mouse cells (22, 23). The cells were allowed to settle in plastic flasks (Falcon 3024) for 2 to

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3 h at 37°C. Some cell cultures were initially incubated in the absence of serum to promote the spreading of monocytes. The nonadherent cells were then transferred to other similar flasks. After 24 h of further incubation at 37°C, the floating cells in the second set of flasks were pooled and used as lymphocyte cultures. The adherent cells in both sets of flasks upon addition of fresh RPMI medium plus 10% fetal bovine serum and antibiotics served as macrophage cultures. Before infection, these cells were removed by trypsin-ethylenediaminetetraacetic acid treatment aided by a rubber policeman and after washing were resuspended in the medium as described above. Cells (4×10^5 to 6×10^5/ml were then seeded into petri dishes (Falcon 300I) for determinations of infectious virus production, into multi-dish Disposo-trays (Linbro FB-16-24-TC) for virus titrations, or onto round glass cover slips for determinations of hemadsorption (HAD) and tests for typical markers.

Separation of T and B lymphocytes. Populations enriched in T and B lymphocytes were obtained by the methods described for mouse T and B lymphocytes (23), i.e., by one cycle of adsorption on RBC monolayers.

Monocyte-macrophage characterization. Phagocytosis of sheep RBC or of India ink and tests for acid-phosphatase activity were employed to characterize adherent cells. Contamination of lymphocytes by monocytes was determined by the α-naphtol reaction. These tests have been previously described as applied to mouse leukocytes (22, 23).

T-lymphocyte characterization. Spontaneous rosette (E) formation, determined by the method of Jondal et al. (10), was used to identify T lymphocytes.

B-lymphocyte characterization. Immune rosette (EA) formation was determined by the method of Kedar et al. (12). The percentage of immunoglobulin G-bearing lymphocytes was also established by indirect immunofluorescence with anti-human immunoglobulin G produced in rabbits and fluorescein-conjugated anti-rabbit immunoglobulins (Hyland Laboratories, Costa Mesa, Calif).

Viruses and infections. 6/94 virus representing the fifth to sixth passage of the original isolate in the allantoic cavity of embryonated eggs was used in all the experiments. Infection, sampling and tests for hemagglutination (HA), for HAD, and for production of infectious virus were conducted as previously described (22, 23). In this study, infectivity titrations were carried out in embryonated eggs by using, unless otherwise stated, sonicated cell-medium mixtures. All eggs, as well as macrophage, lymphocyte, and other cell cultures, were incubated at 32°C after inoculation with 6/94 virus.

A laboratory-maintained Sendai virus (MN strain) was used in comparative studies under the same incubation conditions.

PHA stimulation of cells. Cells were incubated with phytohemagglutinin-P (PHA-P) (Difco Laboratories, Detroit, Mich.) at various dilutions in RPMI medium with 10% fetal bovine serum for 3 to 4 days before infection with 6/94 virus. In tests for blastogenesis inhibition, cells were incubated with PHA-P after infection with either 6/94 or Sendai virus. The effect of the mitogen on lymphocytes was determined either by counting blastic forms in smears stained with Giemsa, or by labeling the cells for 20 h with 0.05 mCi of [^{3}H]thymidine (specific activity, 18.4 Ci/mmol) (Radiochemical Centre, Amersham, England). For the latter test, the micromethod described by Hartzman et al. (8) was used in association with the method for harvesting cells on Whatman filter pads as reported by Zisman and Denman (26). Radioactivity uptake was determined in a liquid scintillation spectrometer (Tri-Carb; Packard Instrument Co., Inc., Rockville, Md.), and counts per minute were calculated per 10^6 cells.

Serological tests. The presence of neutralizing antibodies in the sera of leukocyte donors was assayed by the HAD-neutralization test according to previously described techniques (13), using 100 $TCID_{50}$ (50% tissue culture infective doses) of either 6/94 or Sendai virus as an antigen.

RESULTS

Table 1 shows the levels of neutralizing antibodies against both 6/94 and Sendai viruses in the blood of leukocyte donors. In each adult, the titer of antibody against 6/94 was similar to that of antibody against Sendai virus. The child lacked measurable antibodies to either virus.

Macrophages. Macrophages were obtained from three normal adults (designated NA-1, NA-2, and NA-4) and from one MS patient. The cultures were infected after 5 to 10 days of incubation in vitro, a time when the cells showed the best spreading and a typical morphology. The populations were 99% positive for phagocytosis of India ink or of sensitized sheep RBC and for acid-phosphatase activity.

Characteristics of infected macrophages. The responses of all the macrophage cultures to 6/94 virus infection were similar, regardless of the donors. Furthermore, cells derived from two or three repeated bleedings of the same adult donor also gave similar results. Most of the infected and uninfected cultures were observed for 3 to 4 days only. However, one set from a normal donor and two sets from the MS patient were kept for much longer periods of time, as

TABLE 1. *Titers of neutralizing antibodies in sera of leukocyte donors*

Subject[a]	Titer of antibody against:[b]	
	6/94 virus	Sendai virus
NA-1	16	16
NA-2	32	64
NA-3	40	40
NA-4	16	32
NC	0	0
MS	40	40

[a] NC, Normal child; MS, MS patient.

[b] Assayed by the HAD-neutralization test, using 100 $TCID_{50}$ of either virus.

described below. Initially, the infected cultures were indistinguishable from the uninfected controls either by morphology or by the presence of the typical macrophage markers. In the infected cultures, many HAD-positive cells became detectable after 24 h and their number increased over time. Interestingly, India ink phagocytosis and HAD were observed in the same cells. In the cultures maintained for more than 4 days, by 9 to 10 days most of the infected cells exhibited some cytopathic effect, showing rounding, although they remained spread with pale edges. This morphology was distinctly different from that of the control cells. Twenty days after infection, HAD was still detected in most of the cells. As the cultures grew older, they showed a decreased density, but the residual populations were still HAD positive. After 100 days, both infected and uninfected cultures showed marked degeneration, but in the infected cultures HAD-positive cells were still observed.

Sensitivity to infection of human macrophages compared with other cells. Egg-grown 6/94 virus gave the same HAD endpoint (usually $10^{-7.5}$) when titrated in human macrophages as when titrated in CV-1 cells. At 5 days after infection, the percentage of HAD-positive cells noted in both macrophage and CV-1 cell cultures decreased with the falling dilutions of virus. Only a few positive cells were detected in dilutions corresponding to the endpoint. However, when the same cultures were reincubated an additional 5 days, although the same endpoint of HAD was still detected, the CV-1 cells remained unchanged, whereas the macrophages showed a generalized cytopathic effect as described above. Nonetheless, in the macrophage cultures HAD became more extensive at the endpoint dilution, indicating that infectious virus must have been produced and transmitted to the remaining uninfected cells.

Production of infectious virus in unstimulated macrophages. When unstimulated macrophages were inoculated with 6/94 virus at a multiplicity of infection (MOI) of 2 to 4, they produced high levels of infectious virus within 24 to 48 h (Fig. 1). The titers did not increase significantly for the next 6 days, but soon thereafter began to decrease. This decrease was apparently related to the decrease in the number of cells and to the changes of medium. At 25 days, not more than 1 log of infectious virus was still detectable (data not shown). At the peak of infectious virus production, the HA titers of supernatants were between 1:4 and 1:8, and the $TCID_{50}$ was $10^{4.0}$ to $10^{4.5}$. Although the MS patient's cells produced about 0.5 log less virus at 6 days, and 2 logs less at 14 days, whether this finding can be considered significant is questionable.

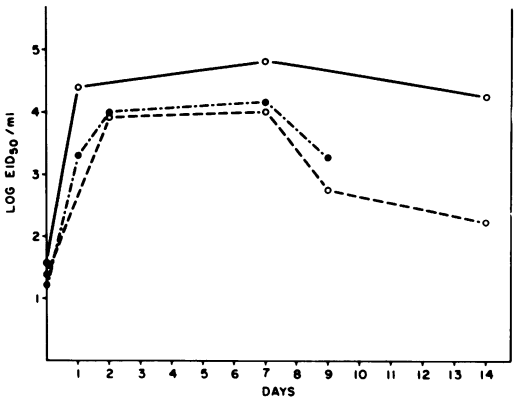

FIG. 1. *Production of infectious 6/94 virus in human macrophages. Symbols:* (O——O) *NA-1;* (●——●, O- - -O) *MS patient (cells obtained at different times).*

Production of infectious virus in PHA-stimulated macrophages. Dobrowolska and Kantoch (3) found that the increase in titers of poliovirus grown in mouse macrophages that were pretreated with PHA was related to macrophage replication. Therefore, to establish better conditions for viability and possibly to increase viral yield, cultures of human macrophages were exposed to 1% PHA for 3 days before infection. At that time, the treated cells showed an increase in size, some increase in number, and clumping. On inoculation of these stimulated cultures with 6/94 virus, it was found that after 7 days the amount of virus produced was the same as in unstimulated cells. However, in the stimulated cultures the viral titers were maintained at the same level for the next week, whereas the unstimulated cultures showed a 1-log decrease (Table 2). At 25 days after infection, in both PHA-treated and untreated cultures, the numbers of cells remaining dropped and, consequently, so did the titers of infectious virus produced.

Lymphocytes. The viability of lymphocytes used for infection and as controls after 24 h of stationary incubation at 37°C was always between 96 and 98%. The test for nonspecific esterases used for detection of contaminating monocytes was positive for less than 1% of the population.

Characteristics of infected lymphocytes. After the periods allowed for adsorption of virus, washing, and treatment with antiserum to neutralize residual virus, no HAD could be detected in inoculated cultures. At 24 h, however, HAD-positive cells appeared, indicating the development of new virus. In time, the percentage showing HAD increased slightly, but never reached

TABLE 2. *Production of infectious virus in human macrophages stimulated with PHA*

NA-1 macrophages	Titer after:[a]	
	7 days	15 days
Unstimulated	4.23	3.11
PHA stimulated	4.50	4.20

[a] Values indicate $\log_{10}$ EID_{50} per milliliter per 10^6 cells.

higher than 10% of the total number of cells present. HA became measurable after 3 days of incubation and showed a slight increase with time, but was never more than 1:4. The percentage of viable cells in infected cultures did not differ significantly from that in uninfected controls, but both decreased after 5 to 7 days at different rates in cultures from different subjects. Table 3 presents HA and HAD data obtained in lymphocytes from a normal adult and from the antibody-free child that were kept for 7 days. During this time there was little reduction in viability, i.e., from 96 to 86%. The notations of blastlike cells at 7 days after infection are also given. It can be seen that between days 3 and 7 the percentage of cells from the adult showing HAD and blast formation increased. Of interest was that both were considerably higher than in cell cultures from the child. Indeed, the percentage of blastlike cells seen in the child's cultures after 7 days was the same as that observed in uninfected control cultures from the same donors, i.e., 5%.

Production of infectious virus in unstimulated lymphocytes. Figure 2 shows the titers of 6/94 virus found at different times after infection of lymphocytes from two normal adults, from the child, and from two different bleedings of the MS patient. It can be seen that when cells from the child and an adult (NA-4) were inoculated with an MOI of 10, they produced almost identical titers of virus. The cells from the second adult (NA-2) and both cultures from the MS patient inoculated with an MOI of 2 also yielded infectious virus, but the titers were somewhat lower than those produced at the higher MOI. Cells that were obtained from the other two adults (NA-1 and NA-3, not shown) and also inoculated at an MOI of 2 produced similar levels (3.5 to 3.75 $\log_{10}$ EID_{50} [50% egg infective doses]). Maximal titers appeared quickly (1 to 3 days) and were sustained for 4 to 7 days.

Comparative titrations of lymphocyte-grown virus in eggs and CV-1 monolayers demonstrated that more virus was detectable by the former than by the latter system. However, slightly more virus infectious for CV-1 cells was produced after 7 days than after 1 day of incubation of lymphocytes (Table 4).

Production of infectious virus in enriched populations of T and B lymphocytes. To try to establish the type of lymphocyte principally involved in the replication of 6/94 virus, lymphocyte populations from two normal adults (NA-1 and NA-2), enriched in T or B cells, were infected with an MOI of 4 (Table 5). The titers of virus obtained from unseparated and enriched populations were about the same for both donors; therefore the average titers are given in Table 6. It can be observed that at 24 h the titers of virus produced in the T- and B-enriched cultures appear not to be significantly different from those of unseparated cells. After 3 days of incubation, however, 1 log more of virus was produced by the T as compared to the B cells. It should also be noted that the titers produced approached those shown in Fig. 2 when unstimulated lymphocytes were infected at an MOI of 10.

Production of infectious virus in PHA-stimulated lymphocytes. It is generally acknowledged that mitogenic stimulation of lymphocytes makes them more susceptible to viral infection (24). Hence, unseparated lymphocytes from a normal adult (NA-2) as well as a T-enriched population from the same donor were stimulated with 0.5% PHA. After 3 days at 37°C, stimulated and unstimulated cultures were in-

TABLE 3. *HAD endpoints, HA titers, and characteristics of unstimulated lymphocyte cultures infected with 6/94 virus*

Lymphocyte donor	Viability (%)	HAD (% positive)	HA titer	Blastlike cells (% present)
NA-4[a]				
Day 3	96	3.0	1	ND[b]
Day 5	87	4.5	2	ND
Day 7	86	7.7	2	20
NC[c]				
Day 3	95	2.2	0	ND
Day 5	ND	2.0	1	ND
Day 7	87	1.5	1	5

[a] Neutralizing antibody titer against 6/94 virus = 16.

[b] ND, Not determined.

[c] NC, Normal child. Neutralizing antibody titer against 6/94 virus = 0.

TABLE 4. *Production of 6/94 in unstimulated human lymphocytes titrated in eggs and CV-1 cells*

Assay system	Titer after incubation for:[a]	
	1 day	7 days
Eggs	4.4	4.2
CV-1 cells	1.5	2.7

[a] $\log_{10}$ EID_{50} or $TCID_{50}$ per milliliter per 10^6 cells.

TABLE 5. *Composition of human peripheral lymphocyte cultures depleted of monocytes, before and after separation of T and B cells*

Lymphocytes	% B cells			% T cells		
	Obtained		Expected[a]	Obtained		Expected[a]
	NA-1	NA-2		NA-1	NA-2	
Unseparated	24	32	22-25	76	62	75
T enriched	ND	4	4	96.5	94	95
B enriched	ND	61	46-79	28	37	ND

[a] Values determined by various methods (1, 6, 13). ND, Not determined.

TABLE 6. *Production of 6/94 virus by enriched T and B lymphocyte populations*

Lymphocytes	Titer of virus produced after:[a]	
	1 day	3 days
Unseparated	5.1	5.7
T enriched	5.0	6.2
B enriched	4.9	5.3

[a] Values indicate $\log_{10}$ EID_{50} per milliliter per 10^6 cells.

fected with 6/94 virus at an MOI of 4. The amount of infectious virus yields at different incubation times is shown in Fig. 3. It is apparent that between 1.5 and 2 logs more virus was produced in PHA-treated cells than in untreated cultures. Unfortunately, the unseparated and T-enriched populations could not be sampled on the same day. However, the figure suggests that on day 3, for example, the T-enriched populations produced slightly more virus than the unseparated populations. In addition, HAD and blast formation were much more extensive in PHA-stimulated lymphocytes than in unstimulated lymphocytes comprising either population (Table 7).

Effect of 6/94 infection on blastogenesis induced by PHA. The inability of PHA to induce blastogenesis in lymphocytes infected in vitro or in vivo by viral agents has been reviewed by Wheelock et al. (24). Since Sendai virus, which is antigenically related to 6/94 virus, has been found to be one of the strongest of such viral inhibitors (25), it appeared important to test 6/94 virus with respect to this activity. Accordingly, lymphocytes from a normal adult (NA-2) were divided into three groups: (i) uninfected control; (ii) infected with 6/94 virus at an MOI of 10; and (iii) infected with Sendai virus at the same MOI. The cells of each group were then treated with 0.05% PHA either immediately (experiment 1), or 24 h after infection (experiment 2). The cultures were incubated at 32°C for 3 days, and after an additional overnight incubation in the presence of [^{3}H]thymidine, were processed for radioactivity determinations.

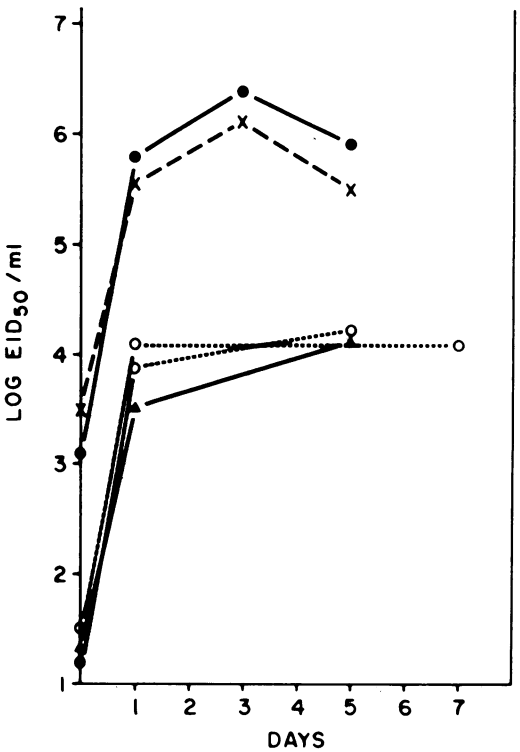

FIG. 2. *Production of infectious 6/94 virus in unstimulated human lymphocytes. Symbols:* (●——●) *NA-4 (MOI = 10);* (×---×) *normal child (MOI = 10);* (○----○) *MS patient (cells obtained at different times; MOI = 2);* (▲——▲) *NA-2 (MOI = 2).*

After PHA treatment, the levels of incorporation rose significantly in the control of uninfected cultures and increased with time (Table 8). In both experiments, the absolute values reached in control cultures were the same, although due to the higher basal value found in the unstimulated cultures of the second experiment, the stimulation ratios appeared to be different.

It became clear that Sendai virus depressed the incorporation of the labeled precursor in the unstimulated cultures of both experiments. PHA-treated Sendai-infected cultures showed

about the same degree of incorporation as found in unstimulated, uninfected controls. These values were 91 to 97% lower than those detected in PHA-treated control cultures.

However, 6/94 did not depress incorporation of the labeled precursor in the unstimulated cultures of experiment 1, and only slightly depressed that in experiment 2. PHA treatment of 6/94 virus-infected cultures revealed a higher degree of incorporation than the unstimulated uninfected cells. However, these values were only one-third those obtained in PHA-stimulated control cultures.

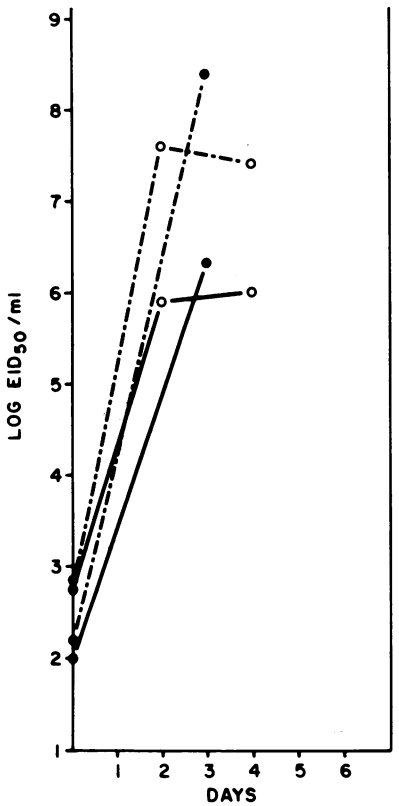

FIG. 3. *Production of infectious virus in PHA-stimulated human lymphocytes inoculated with 6/94 virus at an MOI of 4. Symbols:* (O——O) *unstimulated unseparated lymphocytes;* (●——●) *unstimulated T lymphocytes;* (O-----O) *PHA-treated unseparated lymphocytes;* (●-----●) *PHA-treated T lymphocytes.*

DISCUSSION

It is apparent from our results that 6/94 virus is able to replicate in monocyte-macrophages and lymphocytes from both normal human donors and a patient with MS. It is of interest that the five adult donors had circulating neutralizing antibodies to the virus. The degree of replication in their cells was the same as found in the antibody-free child. The identity of the immunizing agent responsible for the 6/94 virus-reactive antibodies detected is not known. However, past exposure to other parainfluenza agents, especially type 1 (HA_2 strain), could elicit such cross-reacting antibodies. Of interest also is that mice immunized intranasally with 6/94 virus and possessing much higher humoral antibody titers, which were known to be induced by 6/94 virus,

TABLE 7. *Comparison between unstimulated and PHA-treated lymphocytes after infection with 6/94 virus*

Lymphocytes	HAD (% positive)	HA titer	Blasts (% present)
Unstimulated			
Day 2	3	0	
Day 4	7.3	2–4	10
PHA treated			
Day 2	60	2	
Day 4	73	4–8	90

TABLE 8. *Response to blastogenic stimulation by PHA of human lymphocytes infected with either 6/94 or Sendai viruses*

Expt no.[a]	Group	Lymphocyte response				Stimulation ratio	
		Unstimulated		PHA treated			
		cpm/10^6 cells	%	cpm/10^6 cells	%	U[b]	I[c]
1	Controls	1.2	100	33.95	100	28	—
	6/94 infected	1.14	95	36	10	10	
	Sendai infected	0.00	0	0.97	2.8	0.7	ND
2	Controls	2.95	100	33.87	100	11	—
	6/94 infected	1.26	43	10.12	30	3.4	8
	Sendai infected	0.06	2	3.7	9	1.0	52

[a] Experiment 1: stimulation immediately after infection; experiment 2: stimulation 24 h after infection.
[b] Ratio of PHA-stimulated to unstimulated, uninfected cells.
[c] Ratio of PHA-stimulated to unstimulated infected cells. ND, Not determined.

yielded peritoneal macrophages and splenic lymphocytes that varied in susceptibility to infection (22, 23). The ability of viruses to grow in cultures of macrophages and leukocytes from donors possessing specific antibodies, at least under certain conditions, has been described, but usually the production of new virus was scanty (6, 18, 19, 21). Isolation of other viruses from humans and animals possessing humoral antibodies has been reported (5, 7, 24) indicating that the persistence of intracellular virus is not necessarily incompatible with the presence of humoral immunity.

In human leukocyte cultures, 6/94 virus appeared very quickly and reached maximal titers faster than it did in macrophages or lymphocytes from either normal or immunized mice. However, direct comparisons are not possible because of the different origins of the cultures (blood for humans; peritoneum-spleen for mice) and because different kinetics of virus production have been observed in different types of mouse cells (22).

As with mouse macrophages (22), 6/94 virus infection of human macrophages did not destroy their phagocytic activity. However, along with the accelerated virus production, the human macrophage cultures showed a much earlier cytopathic effect (9 to 10 days, versus 30 to 40 days for mouse cultures). As the infected human cultures grew older, they revealed a decreased density, and after 100 days both the infected and control cultures became markedly degenerated. In the mouse cultures, on the other hand, the macrophages were gradually replaced by fibroblast-like cells that continued to exhibit a high degree of HAD and to secrete virus into the supernatant fluids, which indicates that infection was transmitted to the new cell population. Indeed, persistently infected cultures were thus established in macrophage cultures from three strains of mice (23).

The fact that when egg-grown virus was titrated in human macrophages only a few cells were initially HAD positive but with time most of the cells became positive suggested that the newly produced virus was eventually able to spread to uninfected cells in the same cultures. A similar increase with time of HAD-positive cells at the endpoint dilution did not occur when egg-grown virus was titrated in CV-1 cells. Thus, it is conceivable that although 6/94 virus can cause degeneration of macrophages, as seen in vitro, these cells might play a distinct role in the pathogenesis of infections caused by the virus by transmitting the infection macrophage to macrophage or to other cells in the body.

The ability of 6/94 virus to replicate and reach high titers in PHA-stimulated lymphocytes is expected behavior, since blastic stimulation is known to be essential for, and sometimes to enhance, virus replication in human leukocytes (14, 16, 24). The production of relatively high titers of 6/94 virus in unstimulated lymphocytes, however, is rather unexpected. Zisman and Denman (26) demonstrated that Sendai virus, which is antigenically related to 6/94 virus, was inactivated in both mouse and human lymphocyte cultures; a finding duplicated in our experiments with Sendai virus in mouse lymphocytes (23). However, studies by Denman et al. (2) on the replication and inactivation of different viruses in human lymphocytes stressed the impossibility of predicting the outcome of viral infection on the basis of the structural features of the virus. The production of significant titers of infectious 6/94 virus in lymphocyte cultures indicates that, indeed, closely related viruses may behave very differently.

The titers of infectious virus produced seemed to be dependent on the initial input. Furthermore, under similar conditions no differences were observed in levels obtained in cultures from normal donors with or without antibodies, or from the MS patient.

An increase in blastic forms after 6/94 virus infection was noted in lymphocytes from adults with antibodies as revealed morphologically and demonstrated by the higher levels of tritiated thymidine incorporated into these cultures than in normal cells. However, this increase in blastic forms apparently did not modify the levels of infectious virus produced, since the titers were similar to those produced by the cultures derived from the child which barely showed an increased blastogenesis. More than likely, therefore, the blastogenesis observed in the adult cultures resulted from the stimulation of immunological memory from a previous contact with a related antigen, and not the acquisition of a permanent cellular immunity.

A particularly striking difference between 6/94 and Sendai virus is the low inhibition of the PHA blastogenic response exhibited by lymphocytes infected by the former virus in comparison with the strong inhibition observed in Sendai virus-infected cells. The ability to respond to mitogenic stimulation, as demonstrated in the 6/94-infected lymphocytes, and the enhancement of virus growth in stimulated lymphocytes would support the hypothesis that these cells, as well as macrophages, may also be involved in maintaining and carrying the infectious agent to distant parts of the body, although in this instance the role of 6/94 virus in producing disease is unknown. In addition, they could be the selective host cell for a variant of Sendai virus that

normally is inactivated by lymphocytes.

As in mice, human T lymphocytes seem to support 6/94 virus growth better than B lymphocytes. However, experiments employing pure populations of each, rather than populations enriched in one type over the other, remain to be done.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. **Bobrove, A. M., S. Strober, L. A. Herzenberg, and J. D. DepPamphilis.** 1972. Identification and quantitation of thymus-derived lymphocytes in human peripheral blood. J. Immunol. **112**:520–527.
2. **Denman, A. M., B. Rager-Zisman, T. C. Merigan, and D. A. Tyrrell.** 1974. Replication or inactivation of different viruses by human lymphocyte preparations. Infect. Immun. **9**:373–376.
3. **Dobrowolska, H., and M. Kantoch.** 1968. Badania Nadreaktywnoscia makrofagow na zakazenie enterowirusami. Med. Dosw. Mikrobiol. **20**:201–206.
4. **Eustatia, J. M., E. Maase, P. Van Helden, and J. Van Der Veen.** 1972. Viral replication in mouse macrophages. Arch. Gesamte Virusforsch. **39**:376–380.
5. **Frank, A. L., J. A. Bissell, D. S. Rowe, N. R. Dunnick, R. E. Mayner, H. E. Hopps, P. D. Parkmann, and H. M. Meyer, Jr.** 1973. Rhesus leukocyte-associated herpes virus. J. Infect. Dis. **128**:618–637.
6. **Gresser, I., and C. Chang.** 1964. Multiplication of poliovirus type I in preparation of human leukocytes and its inhibition by interferon. J. Immunol. **92**:889–894.
7. **Gresser, I., and D. J. Lang.** 1966. Relationship between virus and leukocytes. Prog. Med. Virol. **8**:62–130.
8. **Hartzman, R. J., M. Segall, M. L. Bach, and F. H. Bach.** 1974. Histocompatibility matching. VI. Miniaturization of the mixed leukocyte culture test; a preliminary report. Transplantation **11**:268–273.
9. **Haspel, M. V., P. R. Knight, R. G. Duff, and F. Rapp.** 1973. Activation of a latent measles virus infection in hamster cells. J. Virol. **12**:690–695.
10. **Jondal, M., G. Holm, and H. Wigzell.** 1972. Surface markers on human T and B lymphocytes. J. Exp. Med. **136**:207–215.
11. **Kantoch, M., A. Warwick, and F. B. Bang.** 1963. The cellular nature of genetic susceptibility to a virus. J. Exp. Med. **117**:781–792.
12. **Kedar, E., M. Ortiz de Landazuri, and B. Bonavida.** 1974. Cellular immunoadsorbents: a simplified technique for separation of lymphoid cell populations. J. Immunol. **112**:1231–1243.
13. **Lewandowski, L. J., F. S. Lief, M. A. Verini, M. M. Pienkowski, V. ter Meulen, and H. Koprowski.** 1974. Analysis of a viral agent isolated from multiple sclerosis brain tissue: characterization as a parainfluenza virus type 1. J. Virol. **13**:1037–1045.
14. **Miller, G., and J. F. Enders.** 1968. Vaccinia virus replication and cytopathic effect in cultures of phytohemagglutinin-treated human peripheral blood leukocytes. J. Virol. **2**:787–792.
15. **Nagata, I., Y. Kimura, Y. Ito, and T. Tanaka.** 1972. Temperature sensitive phenomenon of viral maturation observed in BHK persistently infected with HVJ. Virology **49**:453–461.
16. **Nahmias, A. J., S. Kibrick, and R. Rosan.** 1964. Viral leukocyte interrelationships. 1. Multiplication of a DNA virus-herpes simplex in human leukocyte cultures. J. Immunol. **93**:69–74.
17. **Preble, O. T., and J. S. Youngner.** 1973. Selection of temperature-sensitive mutants during persistent infection: role in maintenance of persistent Newcastle disease virus infections of L cells. J. Virol. **12**:481–491.
18. **Roberts, J. A.** 1964. Growth of virulent and attenuated ectromelia in cultured macrophages from normal and ectromelia immune mice. J. Immunol. **92**:837.
19. **Rockborn, G.** 1958. Further studies on viraemia and neutralizing antibodies in naturally acquired distemper in dogs. Arch. Gesamte Virusforsch. **8**:500–510.
20. **ter Meulen, V., H. Koprowski, Y. Iwasaki, Y. M. Käckell, and D. Müller.** 1972. Fusion of cultured multiple sclerosis brain cells in indicator cells: presence of nucleocapsids and virions and isolation of parainfluenza-type virus. Lancet **ii**:1–5.
21. **Ueda, S., and T. Nozima.** 1973. Delayed hypersensitivity in vaccine infected mice. II. Resistance of peritoneal macrophages against vaccinia infection. Acta Virol. **17**: 41–49.
22. **Verini, M. A., and F. S. Lief.** 1979. Interaction between 6/94 virus, a parainfluenza type 1 strain, and mouse macrophages. Infect. Immun. **24**:720–728.
23. **Verini, M. A., and F. S. Lief.** 1979. Interaction between 6/94 virus, a parainfluenza type I strain, and mouse lymphocytes. Infect. Immun. **24**:729–733.
24. **Wheelock, E. F., S. T. Toy, and R. L. Stjernholm.** 1971. The interaction of viruses with human lymphocytes, p. 787–801. *In* Proceedings of the First International Congress of Immunology. Academic Press Inc., New York.
25. **Willems, F. C., J. L. Melnick, and W. E. Rawls.** 1969. Viral inhibition of the phytohemagglutinin response of human lymphocytes and application to viral hepatitis. Proc. Soc. Exp. Biol. Med. **130**:652–660.
26. **Zisman, B., and A. M. Denman.** 1973. Inactivation of myxoviruses by lymphoid cells. J. Gen. Virol. **20**:211–233.