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Replication or Inactivation of Different Viruses by Human Lymphocyte Preparations

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Cultures of phytohemagglutinin-stimulated, unstimulated, and frozen and thawed mononuclear (as controls) cells from normal human donors were infected with different viruses. A variable pattern of virus infectivity was noted, and influenza and Coxsackie virus were rapidly inactivated even by stimulated lymphocytes. Direct inactivation by lymphocytes may be one form of host defense in infection by some viruses.

The ability of lymphocytes stimulated by phytohemagglutinin (PHA) or allogeneic cells in vitro to support the replication of some viruses is well recognized (9). Lymphocytes engaged in delayed hypersensitivity reactions in vivo possess similar properties (1). Nevertheless, lymphocytes have an important function in protecting the host against many forms of virus infection. We have recently found that several myxoviruses are inactivated after direct exposure to certain lymphocyte populations of human or murine origin (17), suggesting one mechanism by which these cells might contribute to host defense. Accordingly, we have measured the infectivity of several viruses in cultures of stimulated and control mononuclear cells obtained from normal individuals. The fate of these viruses in culture varied, and some appeared to be inactivated by exposure to lymphocytes.

MATERIALS AND METHODS

Virus stocks and assay. Viruses in current use in this Centre were propagated and assayed by the methods set out in Table 1. Plaque-forming units were assayed by a semi-micromethod (12) in which the indicator cells were cultured in wells of 1-ml capacity in disposable plates. After infection, the cells were overlaid with carboxymethylcellulose and, at the end of the incubation period, the plaques were stained through the overlay.

Antibody assays. Sera from the mononuclear cell donors were screened for antiviral antibody by the inhibition of plaque formation or virus cytopathic effect on the appropriate indicator cells. Antibody titers were expressed as the highest serum dilution that completely neutralized 100 tissue culture infective doses or reduced the plaque count by 50%.

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Lymphocyte cultures. Heparinized blood (100 ml) was obtained from each of four healthy male members of the laboratory staff aged 26 to 38 years, none of whom were suffering from any clinically apparent virus infection. The mononuclear cells were isolated by sedimenting the red cells with Plasmagel (Roger Bellon, Neuilly, Seine, France) and centrifuging the supernatant fluids on Ficoll-Triosil gradients (2). Cultures of 3.0×10^6 viable cells were established in conical-bottomed plastic tubes in 3.0 ml of medium consisting of RPMI 1640 medium (Flow Laboratories) with penicillin and streptomycin, 100 IU/ml each; 15 ml of 2 mM L glutamine per liter, 20 g of $NaHCO_3$ per liter, 20 ml of *N*-2-hydroxyethyl piperazine-*N'*-2'-ethane-sulfonic acid (HEPES), and 10% pooled serum from the four donors.

Cultures were either stimulated with PHA, (5 μg/ml; Burroughs Wellcome), which was added from the outset, or were left unstimulated. Control cultures consisted of equal numbers of mononuclear cells which had been subjected to six cycles of freezing and thawing. As a test for cell viability, selected cultures were labeled with [^{14}C]thymidine at 48 h, and the incorporation into DNA was measured by liquid scintillation counting. Because none of the viruses used in these studies significantly suppressed the normal response to PHA which was obtained, the data are not included in this report.

Lymphocyte stimulation by virus. Microcultures of mononuclear cells from all four donors were set up in microplates (Cooke Engineering, Microtitre R), and each well contained 0.2×10^6 cells in 0.2 ml of the medium already described. Triplicate cultures were challenged with 10 or 50 μliters of influenza, Coxsackie or herpes simplex virus suspensions which had been inactivated by ultraviolet irradiation for 30 min. The infectivity of each virus suspension before inactivation was the input infectivity indicated in Fig. 1. Some control cultures were challenged with equivalent amounts of the appropriate tissue suspension or fluid which had not been infected (Table 1), whereas others were left unstimulated. After 5 days of culture at 37 C in an atmosphere of 5% CO_2 in air, the cells

TABLE 1. *Viruses, media, and assay methods*[a]

Virus	Strain	Grown in	Assay method
Herpes simplex type 1	HFEM (P. Wildy)	CEF	PFU on CEF
Vesicular stomatitis	New Jersey	CEF	PFU on CEF
Yellow fever	17D (J. Porterfield)	Infant mouse brains	PS
Echovirus type 12	LEV 4	Vero cells	PFU on Vero cells
Vaccinia	CL (P. Wildy)	CEF	PFU on CEF
Adenovirus type 5	5	L 123 cells	$TCID_{50}$ on HeLa cells
Influenza	Clone 6 (PR8 × 939), recombinant of AO/PR8/34 and A2/England/939/69	Allantoic cavity of 10-day-old embryonated eggs	Hemagglutinating activity of allantoic fluid after incubation at 33 C for 2 days
Coxsackie virus B3	Nancy	Infant mouse brains	PFU on Vero cells
Measles	Philadelphia 26	V3 cells	$TCID_{50}$ on HeLa cells

[a] Abbreviations: CEF, chicken embryo fibroblast; PFU, plaque-forming units; PS, continuous pig kidney cells.

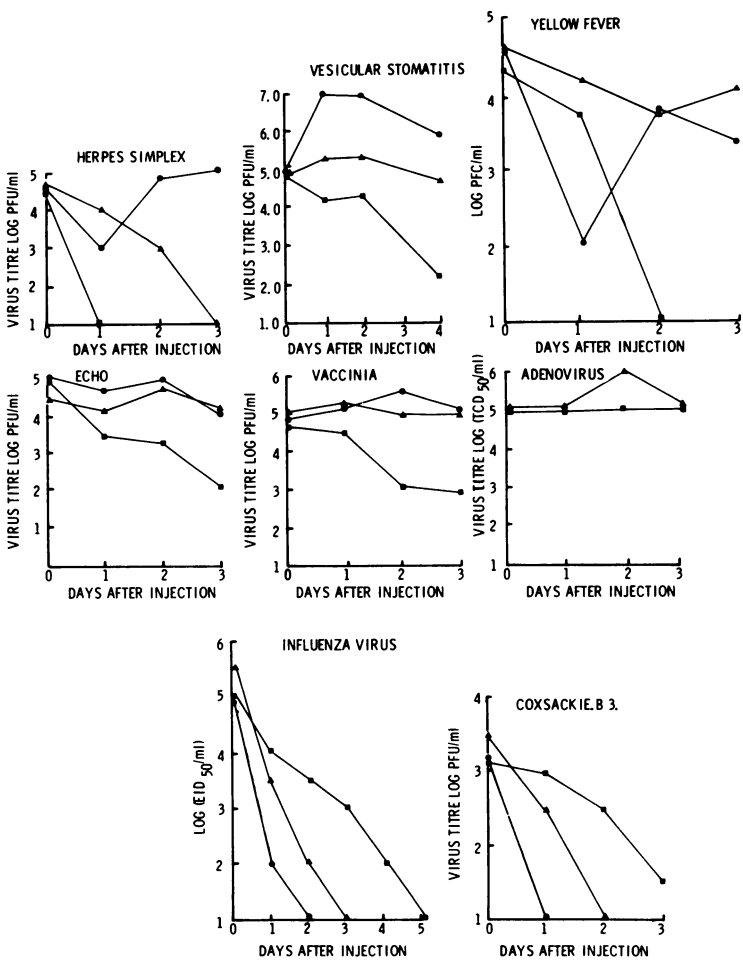

FIG. 1. *Replication or inactivation of different viruses by human lymphocytes.* ●, *PHA-stimulated lymphocytes;* ▲, *unstimulated lymphocytes;* ■, *frozen and thawed lymphocytes. Methods of assaying virus infectivity are given in Table 1.*

were labeled with 0.02 μCi of [^{14}C]thymidine per well. After an additional 24 h of culture, the amount of label incorporated was measured by standard techniques (17).

Infection of cultures. After 24 h, the cultured cells were centrifuged and resuspended in 1.0 ml of growth medium containing one of the viruses being studied. The viruses were added at a multiplicity which varied with individual viruses but which was always within the range of 0.1 to 1.0 infective units per cell. The cultures were incubated for 90 min at 37 C with continuous rotation, after which the cells were washed twice with medium and finally reincubated in 3 ml of medium. As a suitable time for infection, 24 h was selected on the basis of previous experiments with influenza virus, which was inactivated in lymphocyte cultures (17), and with herpes simplex virus, which replicated in (11) lymphocyte cultures at this stage. Cultures were assayed for virus infectivity at zero time and thereafter at 24-h intervals. Each culture was divided into three portions and stored at −70 C. The samples were frozen and thawed once before assay to disrupt the cells, but thereafter were discarded to avoid loss of infectivity from repeated refreezing.

RESULTS

Three patterns of interaction between virus and mononuclear cells were seen in the cultures (Fig. 1). First, herpes simplex virus, vesicular stomatitis virus, and yellow fever virus infectivity increased in PHA-stimulated cultures and even in unstimulated cultures, and persisted in higher titers than in control cultures containing frozen and thawed cells. Secondly, although they did not replicate, EHO virus and vaccinia virus infectivity titers remained stable in cultures of stimulated or unstimulated lymphocytes, whereas both declined in cultures of killed cells. Similar findings were noted in cultures of unstimulated cells infected with adenovirus. Finally, in the third pattern of results, influenza virus and Coxsackie virus titers declined more rapidly after exposure to either stimulated or nonstimulated mononuclear cells than did control, frozen and thawed cell preparations.

The sera of all four donors contained antibody titers of less than 0.5 to the viruses which were studied. Furthermore, none of the inactivated virus preparations induced significant lymphocyte transformation in cultures from any of the donors.

DISCUSSION

These experiments indicate that the results of infecting PHA-stimulated or nonstimulated lymphocytes in vitro vary with different viruses. Those viruses possess no obvious structural features which enable one to predict the outcome. The replication of herpes simplex (11), yellow fever (13), and vesicular stomatitis (5, 6, 15) viruses has been reported before as has the persistent infectivity of vaccinia virus (10, 14). On the basis of present evidence (1), it is the lymphocytes in deoxyribonucleic acid synthesis which support virus replication. Thus, herpes simplex virus replicated in 3-day-old cultures in which such cells predominated after stimulation with mitogens (8).

In contrast, the accelerated reduction of influenza and Coxsackie virus infectivity in cultures of viable lymphocytes compared with exposure to killed cells suggests that lymphocytes may directly inactivate certain viruses. Loss of infectivity of Sendai and influenza viruses in lymphocyte cultures was observed previously (17), when it was found that the reduction was due to interaction with a minority population of large and medium lymphocytes, possibly as a result of an incomplete cycle of replication within these cells; macrophages were not involved. Whether similar events occur in cultures infected with Coxsackie virus remains to be determined.

Mononuclear cells isolated from human blood by gelatine treatment followed by density sedimentation on Ficoll-Triosil gradients are heterogeneous mixtures of different lymphocyte populations and cells of the monocyte-macrophage series (4). It is possible that changes in virus infectivity in cultures of these cells represent the net result of concomitant replication and inactivation in different populations. Cooperative mechanisms between lymphocytes and macrophages may also be involved (7). These possibilities will need further evaluation by using cultures of homogeneous populations of lymphoid cells.

Experiments with yellow fever virus have shown that specific immunity may protect stimulated lymphocytes from infection by the immunizing virus (13). In our experiments, the lymphocyte donors had negligible titers of circulating antibody and, judged by the blast transformation test, undetectable delayed hypersensitivity to any of the viruses we employed. However, stimulation with purified viral components may prove more sensitive. It is unlikely, therefore, that the outcome of infecting their lymphocytes with viruses in vitro will be determined by specific immunity. Nevertheless some form of specific immunity cannot be entirely excluded. It is known that lymphocytes binding *Salmonella* flagellin can be detected in individuals who lack serum antibody to this organism (3).

Lymphocytes are known to possess receptors for Newcastle disease virus (16) and influenza (4), and receptors for other viruses can be

expected. It is likely that the structure of at least some of these receptors will prove to be similar to those for other antigens immunoglobulin in nature. Mitogens such as PHA might expand the numbers of lymphocytes bearing such receptors in cultures, and these could affect the distribution and survival of viruses added to the cultures.

Such reactivity could be relevant to the pathogenesis of virus infections. The spread of viruses which are inactivated by lymphocytes, even in the absence of specific sensitization, would be limited early in the course of infection. This factor might, for example, account for the failure to disseminate of many viruses affecting the respiratory tract.

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