THE PRODUCTION OF VESICULAR STOMATITIS VIRUS BY ANTIGEN- OR MITOGEN-STIMULATED LYMPHOCYTES AND CONTINUOUS LYMPHOBLASTOID LINES*

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In recent years a major thrust in immunology has been to clarify the morphological, surface, and functional properties of the complex populations comprising the lymphoid cell series. At the core of the problem is the mechanism of lymphocyte "activation," the process by which specific antigens or nonspecific agents stimulate resting lymphocytes to transform morphologically (1–5) and to exhibit a variety of measurable immunological functions (6, 7). Some bone marrow-derived lymphocytes (B lymphocytes)[1] may ultimately differentiate into cells of the plasmacyte series that produce immunoglobulin antibodies (8–10). Lymphocytes that are thymus derived or thymus dependent (T lymphocytes) are involved in cell-mediated immune reactions, such as graft and tumor rejection and resistance to certain intracellular infections (7, 11, 12). Although T lymphocytes may produce a number of factors that affect cells in their environment (13), they do not produce antibody (10) and it has been difficult to identify and enumerate sensitized T lymphocytes in a given population.

In contrast to many other body cells, resting lymphocytes are generally unable to support replication of a variety of RNA and DNA viruses (see references 13–15). When lymphocytes are activated, either by plant mitogens or by specific antigens, they rapidly acquire the ability to replicate viruses. By using an infectious center or virus plaque assay (VPA), in which lymphocytes are stimulated by specific antigens,

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[1] *Abbreviations used in this paper:* B lymphocytes, bone marrow-derived lymphocytes; Con A, concanavalin A; DME, Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum; EM, electron microscopy; LPS, *E. coli* lipopolysaccharide; MOI, multiplicity of infection; PFU, plaque-forming units; PHA, phytohemagglutinin, PPD, purified protein derivative; T lymphocytes, thymus-derived or thymus-dependent lymphocytes; VPA, virus plaque assay; VSV, vesicular stomatitis virus.

infected with a virus, and plated in agar above an indicator monolayer for the virus, it has been possible to estimate the number of antigen-sensitive cells in cell populations from delayed hypersensitive individuals. In exquisitely sensitive donors, the maximal number of virus plaque-forming cells is of the order of 0.5–2% (16).

The purpose of this study is to explore at the ultrastructural level the nature of the cells engaged in the production of vesicular stomatitis virus (VSV) in different lymphoid cell populations, particularly after stimulation with several different agents. Specifically, we have examined (*a*) lymph node cells from guinea pigs with delayed hypersensitivity activated by specific antigen, (*b*) murine spleen cells activated by selective B cell and T cell mitogens, and (*c*) cells of human and murine continuous lymphoblastoid or lymphoma lines.

Materials and Methods

Preparation of Sensitized Guinea Pig Lymph Node Cells.—Lymph node cells in suspension were prepared from guinea pigs sensitized with complete Freund's adjuvant 2–4 wk previously as described elsewhere (13). The cells were cultured for 3 or 4 days at 20×10^6 viable cells/1.5 ml in Leighton tubes in Eagle's minimal essential medium (MEM) supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), glutamine (2 mM), and 5% normal guinea pig serum. For specific activation 25 μg/ml tuberculin purified protein derivative (PPD) (Ministry of Fisheries, Food & Agriculture, Weybridge, Surrey, England) was added at zero time for a period of 4 days.

Preparation of Mouse Spleen Cells.—Spleens were removed aseptically from C57Bl/10 mice, rinsed in cold Hanks' balanced salt solution, and sliced into three pieces in MEM supplemented with 6% fetal calf serum, penicillin, streptomycin, and 2 mM glutamine in concentrations as listed above. The cells were gently dispersed from the tissue fragments with a loosely fitting Potter homogenizer, the capsule was allowed to settle, and the cell suspension was washed twice. Approximately 2×10^8 cells were obtained from a single spleen. The cells were cultured in Leighton tubes at a density of 15×10^6 cells in 1.5 ml of medium RPMI 1640 supplemented with 10% fetal calf serum, penicillin, streptomycin, glutamine, and 30 μg/ml of Mycostatin. The cultures were stimulated either with 2.5 μg/ml of concanavalin A (Con A) (Calbiochem, San Diego, Calif.), purified on Sephadex (17), or 20 μg/ml of *Escherichia coli* lipopolysaccharide (LPS-B) (Difco Laboratories, Detroit, Mich.). Control cultures were prepared without mitogens.

Maintenance of Continuous Cell Lines.—Human lymphoblastoid cell lines (Wil-2, 8866, and Raji) were maintained in stationary cultures in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (DME). Twice a week cells were counted and diluted with fresh medium to 0.4×10^6 cells/ml. Cell densities ranged from 0.4×10^6 to 2×10^6/ml. Mouse leukemic cell lines (L1210, L5178Y, and C57Bl/6 leukemia) at 0.5×10^6 cells/ml were grown in Fisher's medium containing 2.5% horse serum and 0.2% trypticase soy broth. The EL-4 mouse leukemic cell line grew best at 0.5×10^6–1×10^6 cells/ml in DME. Guinea pig leukemia L2C was passed in strain II guinea pigs and maintained in short-term culture in medium RPMI 1640 with 20% fetal calf serum. All media contained 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM glutamine.

VSV and Infection of Cells.—The stock of VSV used throughout these experiments was prepared in secondary chick embryo fibroblasts (18) and its titer on mouse L cells was 10^9 plaque-forming units (PFU)/ml. Guinea pig anti-VSV serum was prepared as previously described (18). Cells (2×10^6) were routinely infected at a multiplicity of infection (MOI) of 10 for 2 or 24 h at 37°C. At the end of this period cells were washed once with MEM and treated for 1 h

at 4°C with 0.02 ml of anti-VSV serum per sample, an amount of antiserum sufficient to neutralize completely all the input virus. Infected cells were then washed three times with MEM and used either for the VPA or electron microscopy (EM).

VPA.—Virus-producing cells were assayed as previously described (18). Briefly, 0.2 ml samples of infected cells at 10-fold dilutions (10^2–10^5 cells) were plated in duplicate on monolayers of L cells in 60 mm Falcon tissue culture dishes (Falcon Plastics, Oxnard, Calif.) and overlaid with warm 1% agar in MEM (Ionagar no. 2, Oxoid, Colab Laboratories, Inc., Chicago Heights, Ill.). The plates were incubated for 2 days at 37°C in 5% CO_2 in air, and plaques were counted after staining with neutral red (0.1 mg/ml in phosphate-buffered saline pH 7.2). The number of cells was determined at the time of plating by counting in a Coulter particle counter (Coulter Electronics, Inc., Hialeah, Fla.) a sample of infected cells diluted 1:10. Results were expressed as follows:

$$\% \text{ Infected cells} = \frac{\text{No. plaques/plate} \times 5 \times \text{dilution factor}}{\text{No. cells/ml}} \times 100.$$

EM.—Cells infected with VSV for 24 h were washed, chilled to 0°C, and the pellet was resuspended and fixed for 1 h at 0°C in a solution of 0.5% formaldehyde-2% glutaraldehyde (Ladd Corp., Wilmette, Ill.) in Millonig's buffer, pH 7.3, made freshly each time. After fixing cells were washed three times in Millonig's buffer, immersed for 1 h in cold 2% osmic acid (J. A. Samuel & Co., Inc., New York), washed twice in distilled water, and stained with 0.2% uranyl acetate (Fisher Scientific Co., Pittsburgh, Pa.) in water at room temperature overnight. The samples were then washed twice in distilled water and resuspended in 3-ml conical tubes in a 2% solution of agar in water, prewarmed to 50°C, and centrifuged to form a pellet. This procedure prevented any further loss of cells from samples. The tips of cooled, solidified agar cones containing the infected cells were loosened by injection of water between the agar and tube wall through a no. 18 needle, cut off, dehydrated, and embedded in Durcupan ACM-Fluka (ICN Corp., Chemical & Radioisotopes Div., Irvine, Calif.).

The number and percent of infected cells were calculated from an enumeration of intact cells with virus, of all intact cells, and of damaged cells. An intact cell was considered to be infected with virus if one or more identifiable VSV particles was budding from the surface membrane or was located not more than 650 Å (diameter of VSV) from the surface. At least three sections of each block were counted and the mean of infected cells, intact cells, and damaged cells was determined. Cell types with virus are noted in Results.

RESULTS

Replication of VSV in Lymphoid Cells from Hypersensitive Guinea Pigs.—Lymph node cells obtained from tuberculin-sensitive guinea pigs were cultured for 4 days in medium either with or without specific antigen, PPD. After infection they were plated for assay of infectious centers or fixed for EM. The results of 10 experiments of the VPA are presented in Table I *A*. There is a background of virus plaque-forming cells of approximately 1/1,000 cells (range 0.03–0.3%) in unstimulated cultures. In cultures stimulated by antigen the number of cells able to produce VSV by VPA rose to approximately 1.6% (range 0.8–6.0%). In these experiments ultrastructural examination of control unstimulated cultures revealed five virus-producing cells among 395 intact and damaged cells, an incidence of 1.3% of those seen, but none of these were lymphocytes. In PPD-stimulated cultures the incidence of lymphocytes with VSV was 1.9% of total cells. (See below for further comment on these results.)

TABLE I

Incidence of VSV Replication in Primary Lymphoid Cells

Cultured with	Total cells	Intact cells	Intact cells with virus	Intact cells with virus (% of total, EM)	Virus plaque-forming cells (% of total, VPA)
*A. Tuberculin-Sensitive Guinea Pig Lymph Node Cells**					
Medium	395	273	5‡	1.3	0.1 ± 0.02
PPD	1282	553	28§	2.2	1.6 ± 0.4
B. Normal Mouse Spleen Cells‖					
Medium	ND¶				0.09 ± 0.01
Con A	404	124	18	4.5	5.70 ± 0.06
LPS	422	137	17**	4.0	0.32 ± 0.06

* Cells were cultured 4 days with PPD. VSV was added at an MOI of 10 for an additional 20–24 h.

‡ Four of these five cells were macrophages; one cell, without visible nucleus, was either a macrophage or a blast cell.

§ 4 of these 28 cells were macrophages.

‖ Cells were cultured for 48 h with Con A or LPS. VSV was added at an MOI of 10 for an additional 20–24 h.

¶ ND, not determined.

** Two of these cells were macrophages.

Cells associated with virus were usually large lymphocytes which displayed some of the morphological features of transformed lymphocytes but were not typical mature blast cells (Figs. 1 and 2). Their cytoplasm, moderately abundant, contained numerous ribosomes and some polysomes, a few profiles of rough endoplasmic reticulum, inclusions, and several mitochondria, but no lysosomes. Nuclei were large and showed a relative decrease of heterochromatin and an increase of euchromatin. Virions associated with infected cells were few in number and localized to a short segment of cell membrane; occasionally only one was seen budding from the surface. Of the 28 cells seen to be producing virus in the PPD-stimulated culture, 4 appeared to be macrophages (Fig. 3). Of the five cells with virus in control populations, four were macrophages and one could not be classified since the section did not include the nucleus. This cell was large and was either a macrophage or a blast cell.

Replication of VSV in Mouse Spleen Cells Cultured with Con A or LPS.—In mouse spleen both T and B lymphocytes are present and may be selectively stimulated to undergo blast transformation by Con A (19) and by LPS (20). When mouse spleen cells were incubated with Con A for 48 h and then exposed to VSV for an additional 24 h in two experiments, approximately 4.5% of the total lymphocytes were seen in the electron microscope to be associated with VSV. In the VPA the percentage of VSV-producing lymphocytes found in 18 experiments averaged 5.7% (range 3–10%). When LPS was used to stimulate the spleen cultures in 10 experiments, the incidence of infected cells in the

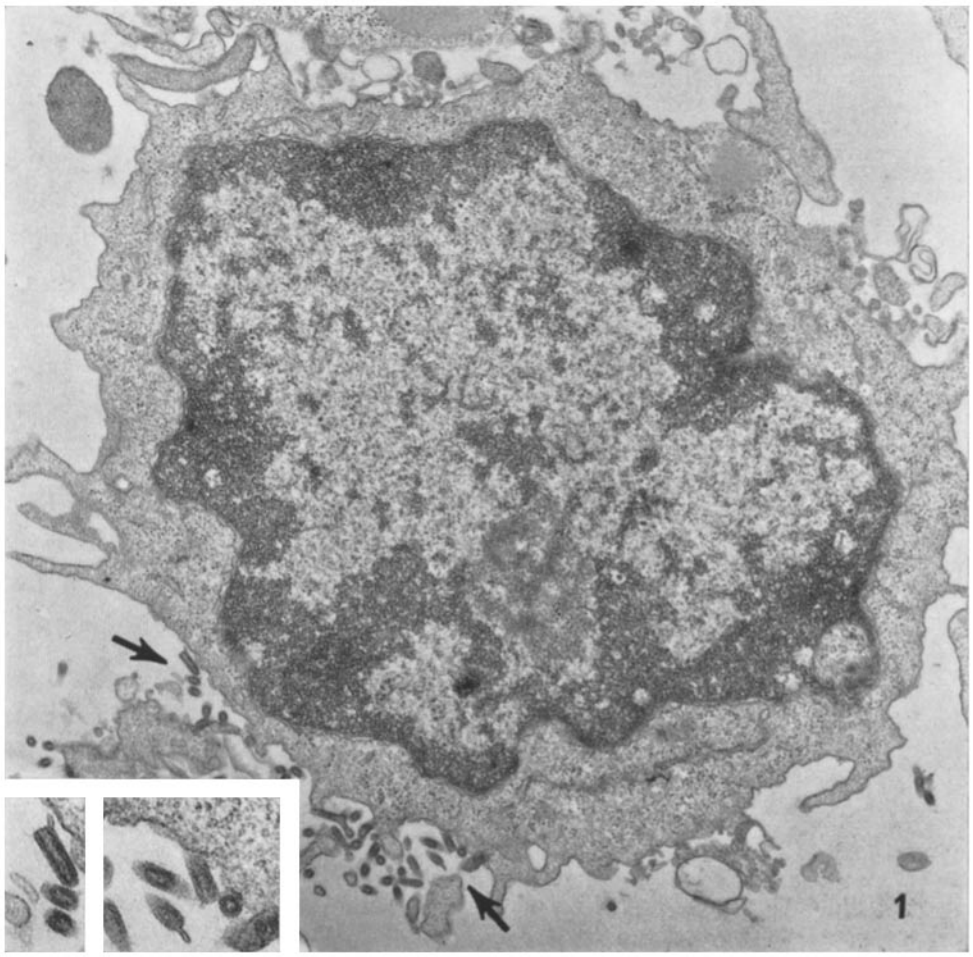


FIG. 1. Lymphocyte from sensitized guinea pig lymph node incubated for 3 days with PPD, and an additional day with VSV. Particles of VSV are clustered close to and budding from a narrow segment of the surfaces (inset). The nucleus is large and has peripheral heterochromatin and some euchromatin. The cytoplasm contains a small number of profiles of rough endoplasmic reticulum and numerous ribosomes. Arrows indicate areas shown in insets. × 20,000. *Insets:* Typical structure of VSV and budding are seen. × 47,000.

VPA was 0.32% (range 0.03–1.0%), while in two experiments the figure was 4% by EM. Mouse spleen cells cultured in medium without mitogens yielded an average of 0.09% virus plaques in 14 experiments and were consequently not scanned in the electron microscope.

The cells associated with VSV were atypical lymphocytes (Figs. 4 and 5) that displayed the morphology of transformed lymphocytes; i.e. they were large, with abundant cytoplasm and large irregularly shaped nuclei, and contained

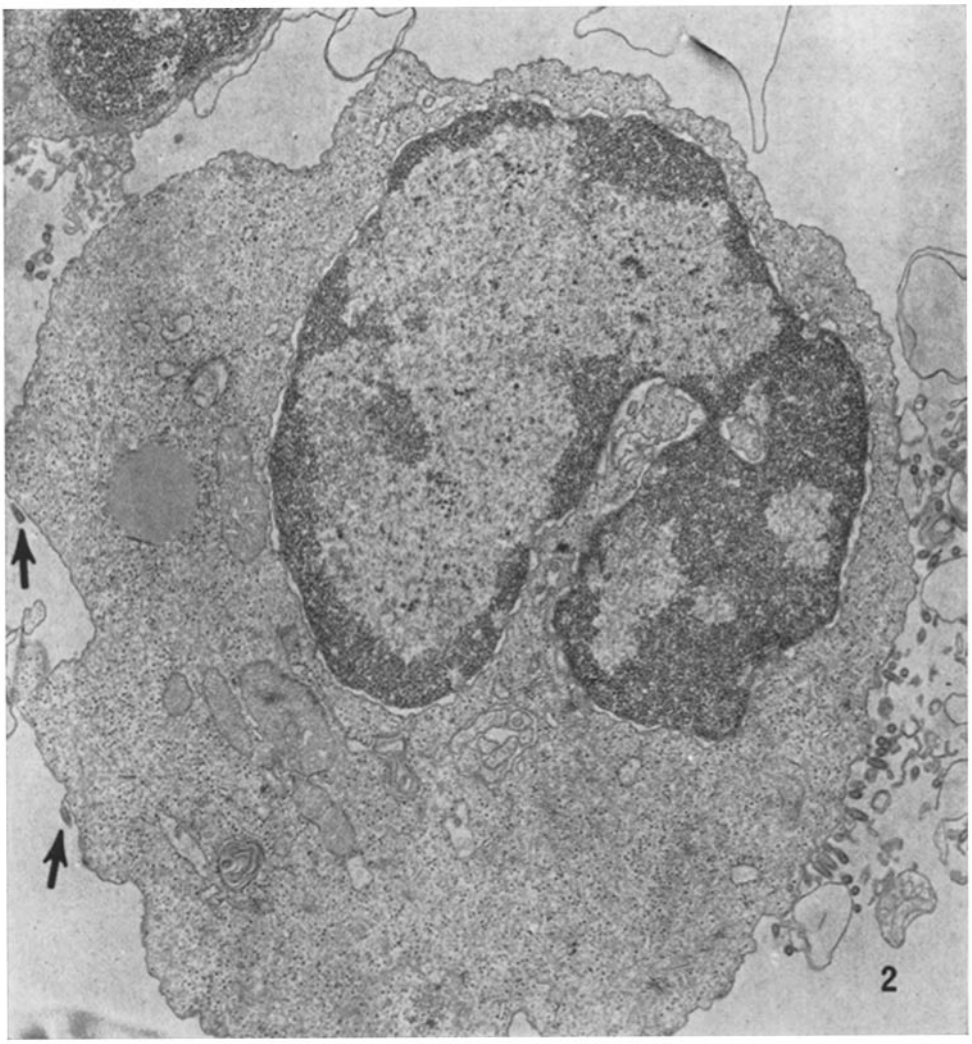


FIG. 2. Lymphocyte from sensitized guinea pig lymph node incubated for 3 days with PPD, and for an additional day with VSV. Particles of VSV are found adjacent to a segment of the surface and single particles (arrows) are seen on the opposite side. The cell is a large lymphocyte with an indented nucleus, abundant cytoplasm, numerous ribosomes, a few simple profiles of rough endoplasmic reticulum, two swirls of smooth membranes, and several mitochondria. × 14,000.

an increased amount of rough endoplasmic reticulum, an increase of mitochondria, several inclusions, and numerous ribosomes and polysomes. Spleen cells from LPS cultures could be distinguished from spleen cells of Con A cultures by an extensive and elaborate rough endoplasmic reticulum (compare

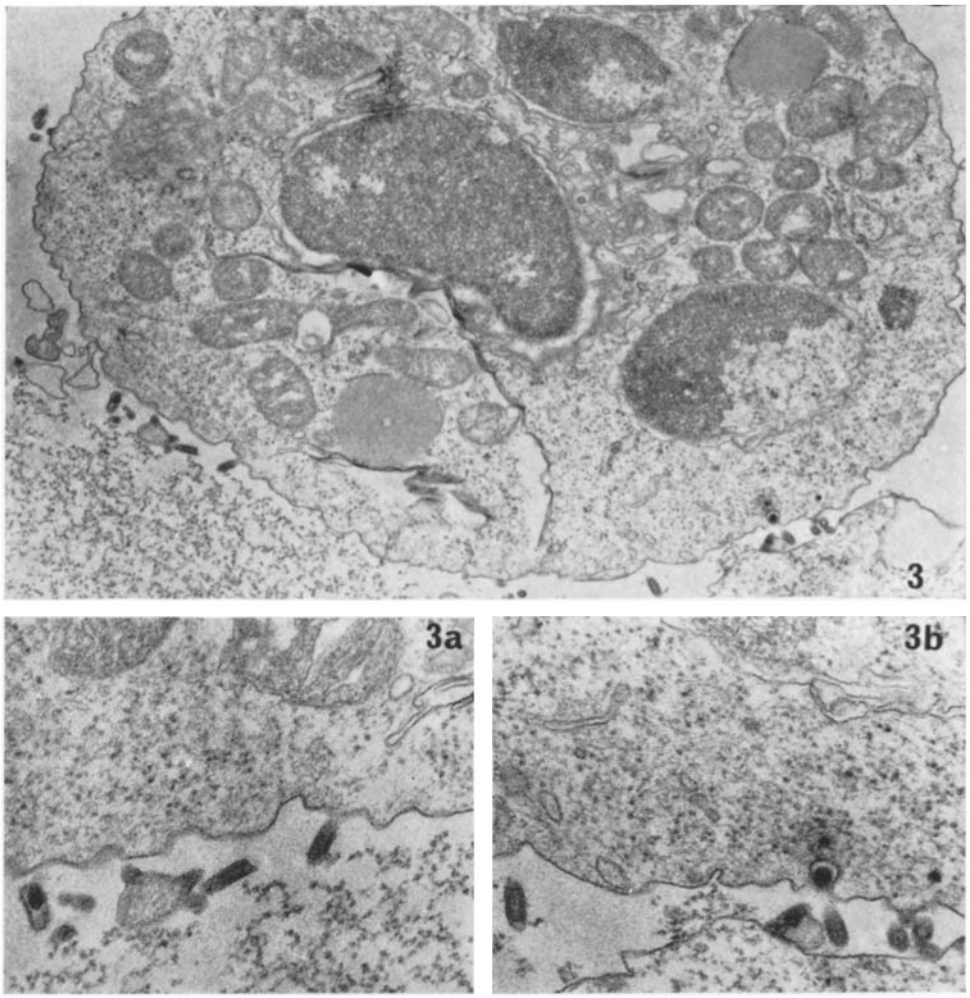


FIG. 3. Macrophage from sensitized guinea pig lymph node releasing and ingesting VSV. The nucleus appears as three lobes. Mitochondria are numerous. Two large granules are present but lysosomes are not seen in this section. × 17,000. (3 *a*, 3 *b*) Enlargements of segments of the macrophage surface showing typical structure of VSV, budding, and ingestion. × 37,500.

Figs. 4 and 5). Not all transformed cells were associated with virus. Of all LPS-treated cells found with surface virus, two were identified as macrophages.

Replication of VSV in Continuous Lymphoid Cell Lines.—Several long-term lymphocyte culture lines were examined for their capacity to replicate VSV and the data are shown in Table II. In these experiments, lymphocytes were infected with virus either for 2 or 24 h. Two murine leukemia lines, L1210 and

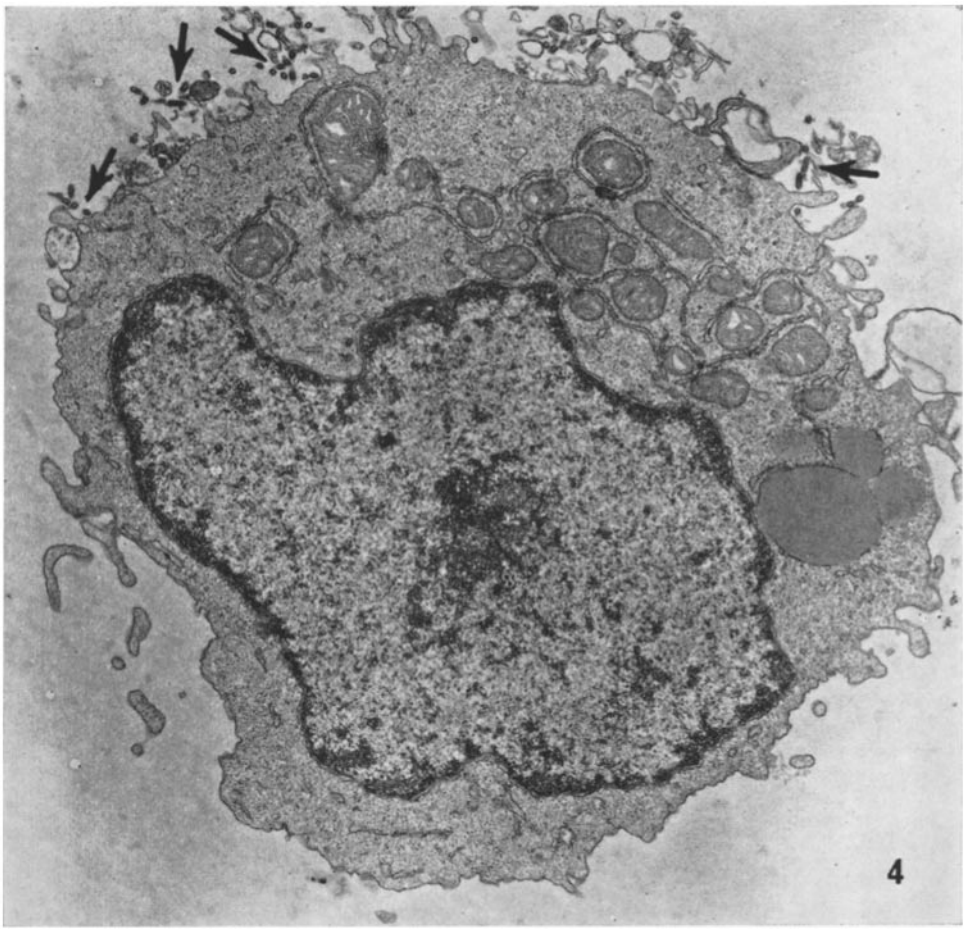


FIG. 4. Lymphocyte from mouse spleen stimulated with Con A for 48 h. A number of virus particles are scattered at periphery of cell (arrows). Cell shows features of a transformed lymphocyte: a large euchromatic nucleus, profiles of rough endoplasmic reticulum, many mitochondria, numerous ribosomes. × 12,500.

C57Bl/6, which do not carry the θ surface marker for T lymphocytes nor the complement receptor characteristic of B lymphocytes (10), were found by VPA to produce low levels of VSV, both at 2 and 24 h. Similarly, the L2C guinea pig leukemia, a known B cell leukemia (10), yielded a low percent of virus plaque-forming cells. A murine plasmacytoma, MOPC-104, that secretes large amounts of IgM immunoglobulin was active in producing VSV. In these cells typical cylindrical virions of VSV were seen budding from the surface and A-type particles were present in the cytoplasm (Fig. 6).

In contrast to the results observed with L1210 and C57Bl/6, two murine

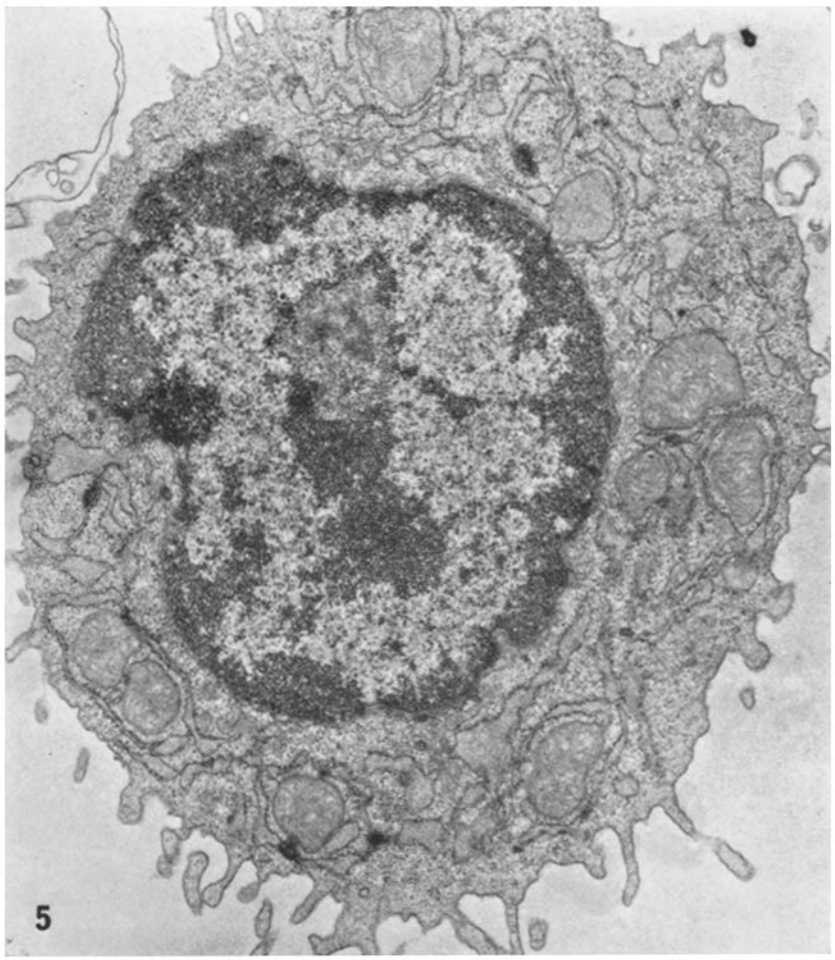


FIG. 5. Lymphocyte from mouse spleen stimulated by LPS for 48 h and exposed to VSV for an additional 24 h. No virus is seen in this micrograph. The cell displays an extensive and dilated rough endoplasmic reticulum, greater and more complex than that usually visible in mouse splenic lymphocytes. Many lymphocytes with such characteristics were seen after exposure to LPS, a few of which had virus on their surfaces. × 18,000.

lymphomas containing lymphocytes with θ surface antigens (L5178Y and EL-4) showed a 15-100-fold higher incidence of virus-producing cells when infected for 2 h. At 24 h the percent of virus-producing cells decreased to 1–3% of the 2 h level in the L5178Y line and to 4–10% of the 2 h level in the EL-4 line. From examination of these lines in the electron microscope at 24 h after infection, the number of intact cells was low and the percent of cells with virus was higher than that determined by VPA.

TABLE II

*Incidence of VSV Replication in Continuous Human and Murine Lymphoid Cell Lines**

Cell line	Origin	Total cells	Intact cells	Intact cells with virus	Intact cells with virus (% of total, EM)	Virus plaque-forming cells (% of total, VPA)	
						2 h infection	24 h infection
L1210	Mouse	ND‡				0.4–0.9	1.6–4.0
C57Bl/6	Mouse	ND				2.1–2.5	2.3
L2C(Ig+)	Guinea pig	ND				1.3	1.1–1.9
MOPC-104(Ig+)	Mouse	39	16	12	31	24	6.7
L5178Y(θ+)	Mouse	60	14	5	8	37–42	0.4–1.3
EL-4(θ+)	Mouse	34	3	1 or 2	3 or 6	39–50	1.7–4.8
Wil-2	Human	193	137	130	67§	8–15	70–80
8866	Human	180	124	44	24	1.5–2.7	5.5–14
Raji	Human	178	137	19	11	0.7–2.2	15–20

* For EM, cells were examined at 24 h after incubation with VSV at MOI of 10.

‡ ND, not examined by EM since the proportion of infectious centers in the VPA was very low.

§ In other studies with Wil-2 the percent of cells replicating virus ranged from 85 to 100%.

Three human lymphoblastoid lines supported replication of VSV but at different levels of efficiency (Table II). Wil-2 showed a high percent of infected cells, particularly at 24 h, reaching 80% by VPA and almost 70% by electron microscopic examination (Fig. 7). In the Raji and 8866 lines (the latter secretes IgG), virus plaque-forming cells at 24 h ranged from 5 to 20% by VPA and from 11 to 24% by morphologic examination (Fig. 8). In these lines, the percent of virus-producing cells was markedly lower at 2 h than at 24 h, a result that differed from that observed in some of the mouse lines. Virus particles on Raji and 8866 cells were less numerous than the numbers found associated with Wil-2, and often appeared over a few segments of the cell surface in clusters (Fig. 8). In all of the cell lines studied, both human and mouse, the number of virus particles per producer cell was greater than that seen in lymphoid cells from donor animals that were exposed to specific antigen, Con A or LPS.

DISCUSSION

The intent of this work was to examine morphologically the kinds of lymphoid cells, both in primary and in long-term cultured populations, that are capable of replicating VSV. VSV was chosen since it had been shown that human lymphocytes, stimulated by phytohemagglutinin (PHA) and infected simultaneously, produced maximum yields of VSV in 24 h (14); i.e., the ability to produce VSV reflects a relatively early step in the activation of lymphocytes. Secondly, the virus has a distinctive morphological appearance (21). Thirdly, it was the virus best studied in the VPA.

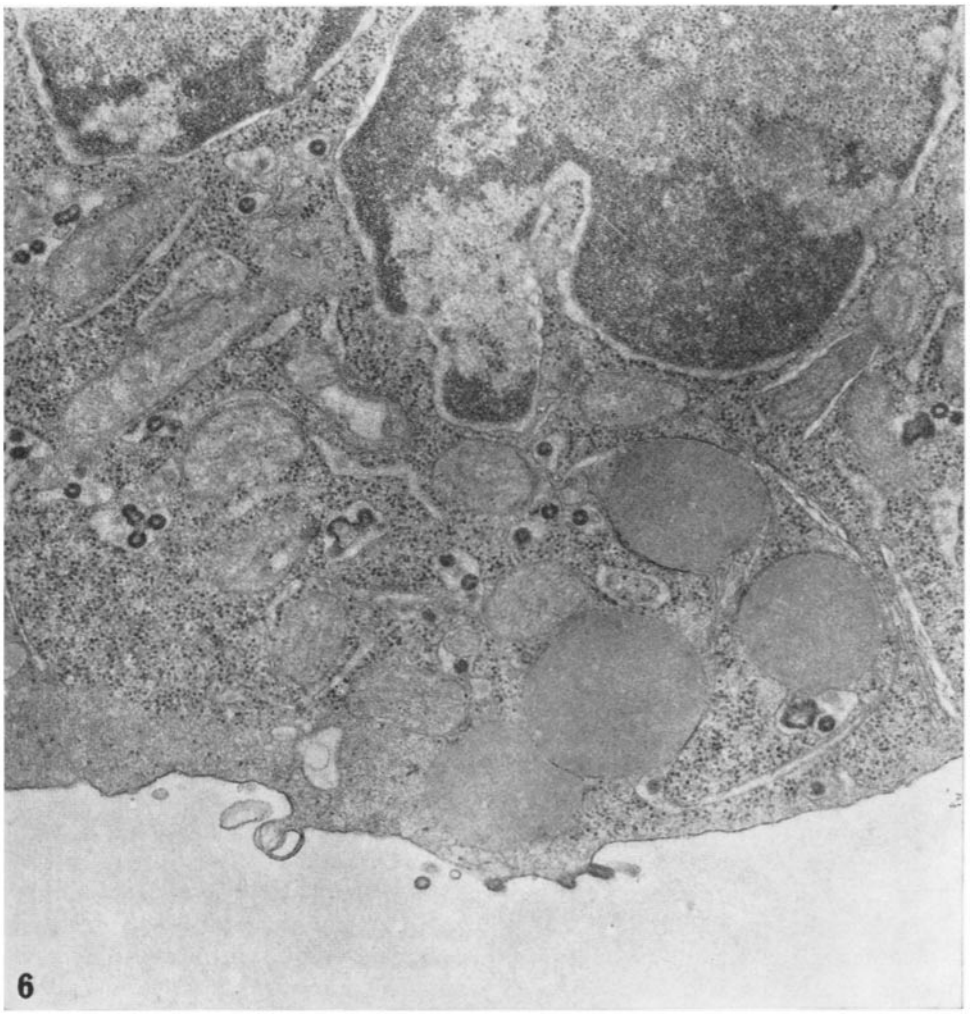
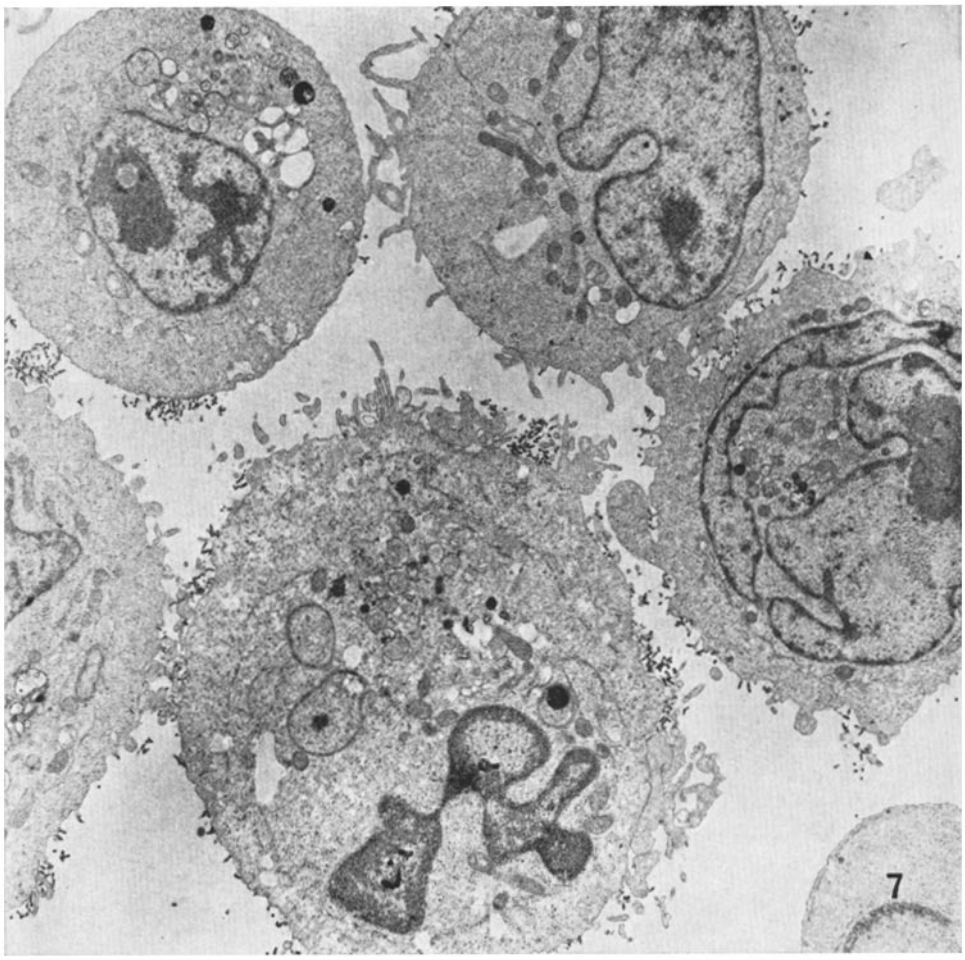


FIG. 6. Portion of a murine plasma cell tumor (MOPC-104) showing several particles of VSV budding from cell surface and A-type particles in cytoplasm. Suspension of myeloma cells was incubated with VSV, MOI = 10, for 24 h. × 14,000.

Replication of VSV, an enveloped RNA virus of the rhabdovirus group, is a complex process and consists of several stages, some of which are not entirely understood (22). Virus must first be adsorbed to the host cells, penetrate, and be uncoated. Then the genome must be transcribed to provide messenger RNA

FIG. 7. Several Wil-2 human lymphoblastoid cells showing the replication of VSV in large numbers around the periphery of the cells. 80–100% of cells in continuous culture were infected after exposure to MOI = 10 for 24 h. × 7,700. (7 *a*) Enlargement of portion of Fig. 7 to show viral detail and budding. × 34,500.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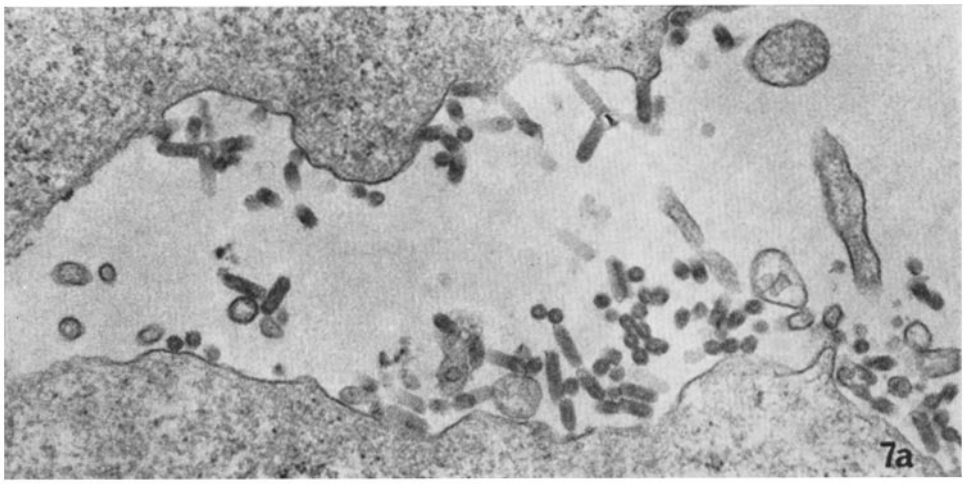
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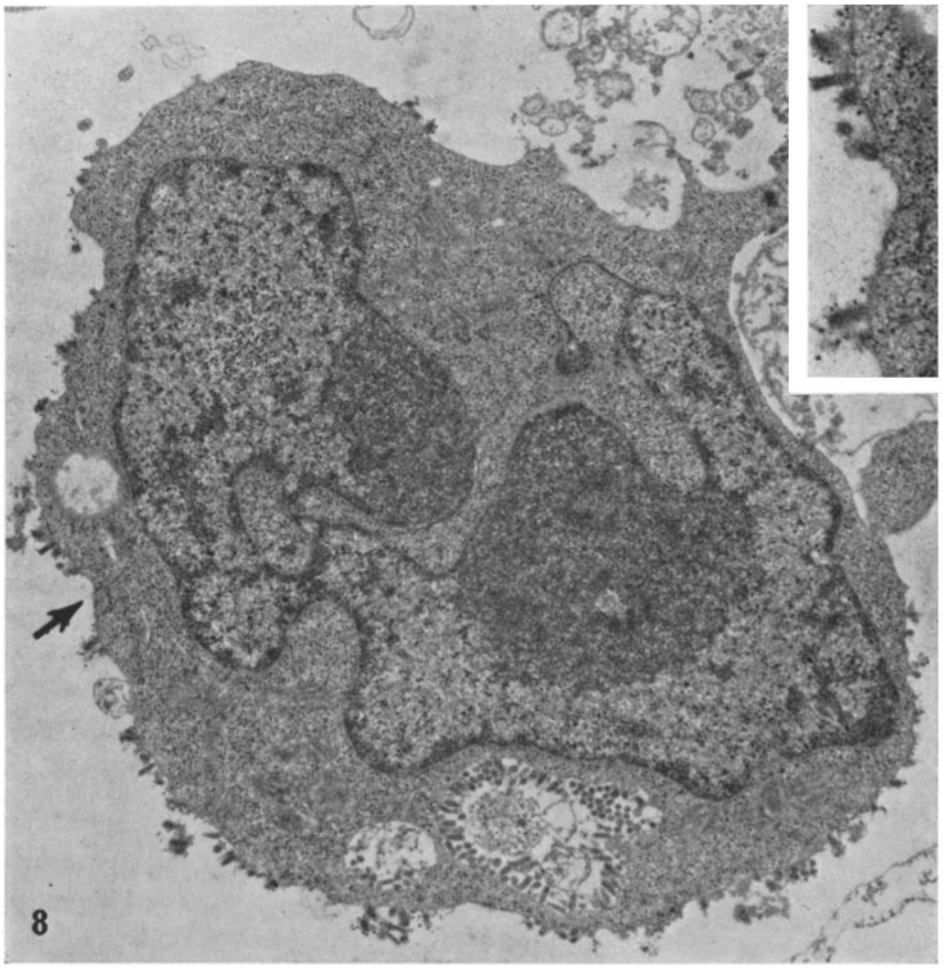

FIG. 8. 8866 human lymphoblastoid cell with VSV dispersed over a large part of the cell surface and several clusters of VSV enclosed in cytoplasmic vacuoles or section through cell projection. × 14,000. *Inset:* Enlargement of zone at arrow to show alteration and disintegration of VSV. × 40,000.

(mRNA) for viral protein synthesis, and then replicated. Finally the virus must be assembled before mature particles start budding at the host cell surface considerably. The host cell is ultimately lysed, but VSV buds at the membrane before lysis occurs. It is known that there is a rapid inhibition of host cell macromolecular synthesis after infection of cells that are highly susceptible to VSV (23–25). There is no information concerning the interaction of VSV with cells that do not replicate this virus efficiently. Activation of cells to produce VSV cannot at present be explained by what is known of the intra-

cellular events that occur in antigen or mitogen-stimulated cells. Nor is it feasible at this time to relate activation to one or more events associated with the different stages of the mitotic cycle. Nonetheless, replication of VSV seems to be an excellent means of enumerating a small number of sensitized lymphocytes that respond to antigen.

Among guinea pig lymph node cells from sensitized donors, 1.5–2.0% were capable of replicating VSV in vitro in the presence of antigen. Lymph node cells from the same pools cultured in the absence of antigen yielded a low background of infectious centers and a somewhat higher background level by morphologic examination. In the electron microscope four of five cells with VSV were macrophages and the fifth was not a lymphocyte. Among antigen-stimulated cells, 4 of 28 cells with VSV were macrophages and the remainder were large lymphocytes. Neither small lymphocytes nor blast cells were found to replicate the virus. These observations indicate that (*a*) the cells responsive to antigen, as detected by a marker virus, were lymphocytes; (*b*) cells other than lymphocytes were capable of replicating VSV even without antigenic stimulation; and (*c*) the correlation of results obtained by VPA and morphologic examination was very close.

When mouse spleen cells were stimulated by mitogens that act selectively on the T lymphocytes (Con A) or B lymphocytes (LPS), an increase in virus-producing cells was found. In the case of Con A this was of the order of 5.7% and in the case of LPS, 0.3% of the cells. The agreement between the VPA and EM counts was very good with Con A and less so with LPS. The cells producing VSV in the Con A-stimulated cultures, presumably T lymphocytes, showed the features of transformed lymphocytes, reminiscent of PHA-stimulated human lymphocytes (5). Cells with VSV in LPS-stimulated cultures, presumably B lymphocytes, showed an extensive rough endoplasmic reticulum resembling lymphocytes transformed by pokeweed mitogen (26, 27), and these were readily distinguished from lymphocytes transformed by Con A.

In an attempt to determine if there was a predilection of T or B lymphocytes for virus replication, several murine tumor lines were studied. Two murine lymphomas bearing the θ marker of T lymphocytes were capable of VSV replication. So was the MOPC-104, a plasma cell tumor line presumed to be derived from B lymphocytes. This finding raises the questions of whether VSV is produced equally well by activated B and T lymphocytes or whether tumor cells have an intrinsic quality, associated with neoplasia, that facilitates VSV replication. For example, granulocytes and mast cells are unable to produce virus (28), yet the mast cell tumor P 815 replicates VSV at approximately 80% efficiency.[2]

Determination of the percent of virus-producing cells in murine cell lines by VPA and EM examination yielded somewhat differing results. At 24 h

[2] Kano, S., and B. R. Bloom. Unpublished observations.

after VSV infection, the percent of cells with virus was higher by EM enumeration than the percent calculated by VPA. This may be explained on the basis that the lytic cycle in these cells was considerably shorter than 24 h. When infected cells were plated on monolayers 24 h after infection, many may have already been killed and could no longer form infectious centers during the ensuing 48 h. In addition, the numbers of intact and damaged cells of the murine lines remaining at 24 h after infection with VSV were small and did not provide a suitable basis for accurate calculation.

By contrast, the human lymphoblastoid lines showed at 24 h an increase of virus-containing cells over the 2 h levels. This may have been due to infection of new cells by VSV released from a small number, and to an enduring viability, at least for some hours, despite VSV infection. The ability to produce VSV among the human lymphoblastoid lines varied considerably. The basis for these differences is not clear. For example, in Raji, a nonsecretor of immunoglobulin (29) and in 8866, a secretor of immunoglobulin, virus production was poor, while in Wil-2, which only produces surface IgG, efficiency of VSV production was almost as great as in HeLa cells. Interestingly, at the beginning of this study the 8866 line produced VSV at an efficiency of only 3%, and 9 mo later its ability to replicate the virus had risen spontaneously to 30%. The possibility that Raji and 8866 cell lines produce interferon was examined by preincubating secondary human skin fibroblasts in supernatants from growing lymphoblastoid cells and challenging the monolayer with VSV. No protecting activity was found in the supernatants at dilutions of 1:8 or greater.

Technical Considerations: Comparison between EM and VPA.—For quantitative measurement by either assay, it must be possible to distinguish virus-producing cells from the remaining number of cells. It is difficult to obtain an accurate count of total cells after several days in culture, since the viability of the cultured cells varies drastically with cell source, type of stimulation, and duration of culture. Fewer cells survive 3–5 days in nonstimulated cultures of primary lymphoid cells than in cultures stimulated by antigen or mitogen. With VPA, one selects essentially for viable cells because during the procedures of infection, neutralization, eight centrifugations, etc., nonviable cells largely disintegrate. On the other hand, an unknown number of viable cells may be damaged in the handling. With respect to processing for EM study, there are losses of cells during fixation and dehydration, which are partially overcome by the agar-embedding technique. In the electron microscope the percentage of damaged cells that could be visualized ranged from 25 to 90%. The great advantage of the VPA is the ability to sample 10^4–10^6 cells on a single plate for virus plaque-forming cells. However, it is clear that cells other than lymphocytes can replicate VSV, and the great virtue of the electron microscope is that it enables one to distinguish cell types, and often stages of development within the lymphoid cell series. The major drawback

is that of screening enough sections and cells to detect low percentages of cells replicating viruses. In those instances in which sufficient numbers of cells were observed, the correlation between the EM analysis and VPA was excellent.

SUMMARY

A variety of lymphoid cell populations were examined in terms of their ability to replicate vesicular stomatitis virus (VSV), a lytic, RNA-containing virus maturing at the cell surface. The number of cells capable of producing VSV was estimated in terms of infectious centers by the virus plaque assay (VPA), and morphologically by electron microscopy (EM). The lymphoid cells examined in this study included: (*a*) lymph node cells from delayed hypersensitive guinea pigs stimulated by specific antigen, (*b*) mouse spleen cells activated by selective bone marrow-derived (B) cell and thymus derived (T) cell mitogens, and (*c*) cells of human and murine continuous lymphoblastoid or lymphoma lines.

In unstimulated cultures of guinea pig lymph node cells there is a background of approximately 1 in 1,000 cells which produces VSV; in purified protein derivative (PPD)-stimulated cultures the number of cells producing virus was 1.6% in the VPA and 1.9% by EM. These cells were large lymphocytes with some morphological features of transformed lymphocytes but were not typical blast cells. A few macrophages were associated with virus in both stimulated and control cultures. These observations indicate that (*a*) cells responsive to antigens, as detected by a marker virus, were lymphocytes; (*b*) cells other than lymphocytes (macrophages) were capable of replicating VSV even without antigenic stimulation; and (*c*) the correlation of results obtained by VPA and morphologic examination was usually quite good.

Of the total number of mouse spleen cells stimulated with concanavalin (Con A), a T cell mitogen, 4.5 (EM)–5.7% (VPA) were associated with VSV. These were characteristic transformed lymphocytes, similar to phytohemagglutinin (PHA)-stimulated human lymphocytes. In contrast *Escherichia coli* lipopolysaccharide (LPS)-treated mouse spleen cultures contained lower numbers of virus plaque-forming cells. The majority of such cells associated with virus displayed extensive rough endoplasmic reticulum.

Two cultured murine lymphomas containing lymphocytes with the θ surface marker (L5178Y and EL-4) showed a 15–100-fold higher incidence of virus-producing cells than leukemias (L1210 and C57Bl/6) which did not carry this marker. Similarly, the L2C guinea pig leukemia, a known B cell leukemia, yielded a low percent of virus plaque-forming cells ($<2\%$). However, MOPC-104, a plasma cell tumor presumed to be of B cell origin, was found to be an efficient virus producer. There was a wide variation in the efficiency of VSV replication among human lymphoblastoid lines. One line, Wil-2, produced 80% infectious centers after 24 h of exposure to VSV, and all cells were associated with virus at the EM level. The relationship between the

virus-producing cells and different lymphocyte subpopulations as well as the efficiency of the two assays for studying virus-producing lymphocytes is discussed.

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