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Replication and Persistence of Measles Virus in Defined Subpopulations of Human Leukocytes[1]

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Replication of Edmonston strain measles virus was studied in several human lymphoblast lines, as well as in defined subpopulations of circulating human leukocytes. It was found that measles virus can productively infect T cells, B cells, and monocytes from human blood. These conclusions were derived from infectious center studies on segregated cell populations, as well as from ultrastructural analyses on cells labeled with specific markers. In contrast, mature polymorphonuclear cells failed to synthesize measles virus nucleocapsids even after infection at a relatively high multiplicity of infection. Measles virus replicated more efficiently in lymphocytes stimulated with mitogens than in unstimulated cells. However, both phytohemagglutinin and pokeweed mitogen had a negligible stimulatory effect on viral synthesis in purified populations of monocytes. In all instances the efficiency of measles virus replication by monocytes was appreciably less than that of mitogenically stimulated lymphocytes or of continuously cultured lymphoblasts. Under standard conditions of infection, all of the surveyed lymphoblast lines produced equivalent amounts of measles virus regardless of the major histocompatibility (HL-A) haplotype. Hence, no evidence was found that the HL-A3,7 haplotype conferred either an advantage or disadvantage with respect to measles virus synthesis in an immunologically neutral environment. A persistent infection with measles virus could be established in both T and B lymphoblasts. The release of infectious virus from such persistently infected cells was stable over a period of several weeks and was approximately 100-fold less than peak viral titers obtained in each respective line after acute infection.

The concept that measles virus infection in man can disrupt normal immune responsiveness has gained broad scientific support since the report of von Pirquet in 1908 (42). Numerous studies have validated von Pirquet's observation that delayed cutaneous hypersensitivity to tuberculin antigen(s) may be transiently lost or severely impaired during natural measles virus infection, and there is evidence that similar hyporesponsiveness can occur after vaccination with attenuated measles virus (for review, see reference 10). Recent in vitro studies collectively indicate that measles virus can impair the responsiveness of lymphoid cells to assorted antigens and mitogens (11, 12, 38, 39, 49), and it has been suggested that direct infection of immunocytes with measles virus might be responsible for the depressed reactivity of these cells to diverse stimuli (28, 39).

Several different lines of evidence support the proposition that measles virus can replicate in human leukocytes. Papp (35) demonstrated that measles virus could be passed to susceptible humans by washed buffy coat cells from patients with naturally acquired measles virus infection. Others showed that lymphoreticular organs were among the principal sites for measles virus replication during acute illness (23, 34, 44, 48). Peebles (unpublished observation), as well as Gresser and Chany (13), reported that circulating leukocytes from patients with measles virus infection could transfer infectivity to cultured cell monolayers; in addition, Berg and Rosenthal (2) demonstrated that peripheral blood leukocytes from normal human adults and from patients with lymphoblastic leukemia could support measles virus replication in vitro.

The purpose of the present study was to determine which subpopulations of circulating human leukocytes can support measles virus replication and to establish whether any of these subpopulations can be persistently infected

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with measles virus. It was hoped that answers to these questions might shed additional light on (i) immunologic disturbances known to occur during acute in vivo infections with measles virus and (ii) on the pathogenesis of protracted or latent forms of measles virus infections.

MATERIALS AND METHODS

Virus. Wild-type Edmonston strain measles virus was obtained from the American Type Culture Collection, Rockville, Md. After repeated passage in HeLa cells, fluids from infected cell cultures were clarified by centrifugation at 700 × *g* for 10 min and stored in 3-ml aliquots at −70 C until use. This virus pool contained 3×10^6 PFU/ml by assay on Vero cell monolayers.

Continuously cultured human lymphoblastoid cells. The following human lymphoblastoid cell lines were used in this study: Daudi, CCRF-HSB-2, LD-7A, MOLT-4, Raji, RPMI 6410, RPMI 8866, UM-56, UM-61, and WI-L2. For experimental purposes all of these lines were grown in RPMI 1640 medium supplemented with 1% glutamine, antibiotics, and 20% heat-inactivated (56 C, 30 min) fetal calf serum (standard medium). HL-A typing on several of these lines was kindly performed by M. A. Pellegrino, Department of Molecular Immunology, Scripps Clinic and Research Foundation. Lines were classed as "B" or "T" subtype according to published accounts (3, 7, 22, 31, 40) or by direct analysis in our laboratory.

Cultured peripheral blood leukocytes from normal human adults. Heparinized venous blood from healthy adults, having 1:16 to 1:64 measles hemagglutination inhibition titers, was mixed with 3% dextran-phosphate-buffered saline in a 2:1 ratio and allowed to settle at 37 C for 30 to 45 min. Cells contained in the supernatant were pelleted by centrifugation, suspended in standard growth medium, and then segregated into polymorphonuclear (PMN) and mononuclear enriched cell fractions by Ficoll-Hypaque gradient centrifugation (4) in clear plastic, wide-bore tubes.

Leukocytes pelleting with erythrocytes were almost exclusively PMN cells as judged by electron microscopy. Contaminating erythrocytes were removed by treating washed pellets with 0.83% ammonium chloride. PMN cells were placed in culture after three additional washes in standard medium.

Cells suspended at the interface of Ficoll-Hypaque gradients (lymphocytes and monocytes) were likewise washed three times with medium. In some instances, these cells were utilized for experimental purposes without further purification.

The following regimen was used to segregate the mononuclear fraction into populations enriched for T cells, B cells, and monocytes (macrophages). After repeated washing, mononuclear cells from the interface of Ficoll-Hypaque gradients were mixed with a 50-fold excess of sheep erythrocytes (E cells) that had been pretreated with 2-aminoethylisothiouronium hydrobromide (Sigma Chemical Co.) as described by Pellegrino et al. (36). Immediately after mixing, the suspension was cooled to 4 C and centrifuged at 600 × *g* for 10 min. The resulting pellet was incubated at 4 C for 6 h. At the end of this interval, approximately 70 to 75% of the leukocytes in most preparations were firmly and fully rosetted with E-2-aminoethylisothiouronium hydrobromide cells. (The procedure rosettes T cells but not B cells or macrophages.) The resuspended pellet was then refractionated by means of centrifugation on Ficoll-Hypaque gradients. Cells pelleting in these gradients were then subjected to ammonium chloride treatment with subsequent washing as described above. Cells collecting to gradient interfaces (B cells and macrophages) were likewise washed repeatedly.

The purity of the T-cell-enriched fraction (pellet) and the non-T-cell-enriched fraction (interface) was monitored by immunofluorescent staining for surface immunoglobulin. As judged by this assay, populations of 96% purity or greater were reproducibly obtained by the methods described.

To segregate B cells from monocytes, cells failing to form E-2-aminoethylisothiouronium hydrobromide rosettes were cultured under standard stationary conditions in T75 plastic flasks at a density of 2×10^7 to 3×10^7 cells per flask. After 12 to 18 h, cultures were washed repeatedly to remove nonadherent cells which were then pooled, counted, and recultured. Approximately 50% of interface cells remained residually attached to flasks. The purity of segregated populations was monitored by neutral red staining. Virtually 100% of adherent cells (macrophages) exhibited positive staining; less than 15% of nonadherent cells took up neutral red in each preparation.

Conditions of infecting and sampling cultured cells. Cells (10^7) of each continuous lymphoblast line were infected with stock measles virus at a multiplicity of infection (MOI) of 1.0. Viral adsorption was carried out at 37 C for 4 h in a closed tube on a continuous inverter. Cells were then washed three times and cultured in 40 ml of standard medium in T75 plastic flasks. After incubation at 37 C in 5% CO_2, 3-ml samples were withdrawn from each culture immediately, 24, 48, 72, and 96 h after plating. Samples were clarified by centrifugation at 700 × *g* for 10 min, and individual supernatant fluids were frozen at −70 C in duplicate for subsequent assay.

PMN cells (2×10^7) were infected in an analogous manner at MOIs of either 1.0 or 5.0. At 24, 48, and 72 h after viral adsorption, samples of approximately 5×10^6 cells were pelleted and fixed in 2% glutaraldehyde–phosphate-buffered saline for subsequent ultrastructural analysis.

Mononuclear cells (8×10^7) were cultured for 4 days in either standard medium, in medium supplemented with 50 μg of phytohemagglutinin (PHA-P; Difco) per ml, or medium supplemented with 50 μg of pokeweed mitogen (PWM; Gibco) per ml. PHA acts as a selective stimulant of human T cells (18); at the dosage employed, PWM would be expected to stimulate B cells and T cells (29). Viral adsorption was performed as above by using 4×10^7 viable cells at an MOI of 1.0. After infection, all preparations were cultured in standard medium without mitogens and were sampled as described for infected lymphoblastoid lines.

Preparations of macrophages (approximately 5×10^6 cells per flask) were likewise grown in the presence or absence of mitogens for 4 days, infected in situ with measles virus at an MOI of 1.0, and sampled serially over a period of 96 h.

Populations of peripheral blood leukocytes enriched for T cells (2×10^7), B cells (2×10^7), and macrophages (2×10^7) were grown independently in medium supplemented with 50 μg of PWM per ml. After a 4-day incubation, cells were infected with measles virus at an MOI of 1.0. Cells were harvested 48 h after viral adsorption and were assayed for infectious centers. This experiment was performed twice with blood cells drawn from the same donor on separate occasions.

Standard plaque assay and infectious center assay. Confluent monolayers of Vero cells grown in multi-well (35 by 10 mm) Linbro trays were inoculated in duplicate with 0.2-ml aliquots of each serially diluted sample. Plates remained for 2 h at 37 C on a rocker platform in a humidified incubator. Each compartment was then overlaid with 3 ml of basal Eagle minimal essential medium-1% agarose containing glutamine, antibiotics, and a 10% final concentration of fetal calf serum. Plates were incubated at 37 C for 7 days and were then overlaid with an additional 3 ml of basal Eagle minimal essential medium-agarose solution containing 1:12,000 neutral red. Plaques were read on day 9 after plating samples.

Infectious center assays were performed in the same test system using an identical overlay protocol. Populations of T cells, B cells, and macrophages, which had been prestimulated with PWM and then infected with measles virus, were washed 3 to 5 times with standard medium. Only macrophages that remained adherent were used in this experiment, and these were suspended by brief treatment with a 0.05% trypsin-EDTA solution (Gibco). The viability of each population was checked with trypan blue. Suspensions were adjusted to approximately 10^5 viable cells/ml. Using 50-μl Hamilton syringes affixed with autodispensors, either 1 or 2 μl was then sterilely discharged into each of three Linbo tray wells from which medium had been aspirated. Trays were rocked side to side for several minutes and then incubated at 37 C for 2 h without further shaking. To quantitate titers of non-cell-associated virus, each cell suspension was centrifuged at $700 \times g$ for 10 min, and supernatant fluids were plated in a manner identical to that outlined above. These plates were rocked continuously throughout the period of viral adsorption.

In the infectious center assays, plaques were counted on day 9 after plating samples. The number of plaques was averaged for each replicate series and the number of infectious centers for each series was then determined by subtracting the number of plaques formed by clarified samples from the number of plaques produced by each cell suspension. The incidence of infectious center formation for each subpopulation was obtained by dividing the infectious center number by the number of viable cells plated (e.g., 1 μl of 13×10^4 viable cells/ml would contain approximately 130 viable cells; 2 μl, 260 viable cells).

Persistent infection of lymphoblastoid lines. Lines MOLT-4 and RPMI 8866 were acutely infected with stock measles virus at an MOI of 0.2. After viral adsorption, cultures were fed with complete RPMI 1640 medium supplemented with 20% heat-inactivated fetal calf serum. Cultures were refed with fresh medium every other day during the first 2 weeks of infection and twice weekly thereafter. Thirty days after infection, cells from each culture were examined (i) for their ultrastructural appearance, (ii) for their growth rates relative to uninfected cells, and (iii) for their ability to release infectious virus. In the last instance, 10^7 viable cells of each line were suspended in medium and sampled as described above for acutely infected lymphoblasts.

Ultrastructural studies. For routine analysis, cells were pelleted, fixed, and processed according to procedures outlined elsewhere (26).

To assess directly whether T cells, B cells, and monocytes from adult human blood could replicate measles virus nucleocapsids, the following test systems were employed. For T cells, Ficoll-Hypaque purified mononuclear cells were prestimulated with PHA for 3 days and were then infected with measles virus at an MOI > 1.0, and on day 3 of infection, cells were washed repeatedly in complete medium and were then reacted with washed E cells according to the method of Jondal et al. (19) for forming E rosettes. For B cells and monocytes, mixed populations of freshly derived mononuclear cells were prestimulated with PWM for 4 days and then infected with measles virus at an MOI > 1.0. On day 3 of infection, nonadherent cells were removed and reacted with EA cells that had been sensitized with C5 deficient mouse serum. The reaction mixture was incubated under conditions known to maximize formation of EAC3d rosettes (40). Glass-adherent cells harvested by trypsin-EDTA (Gibco) treatment were incubated with colloidal carbon for 45 min at 37 C to provide an ultrastructural marker of phagocytosis.

Cells treated by each of the above methods were pelleted and fixed in 2% glutaraldehyde–phosphate-buffered saline. Pilot studies indicated that HeLa cells infected with measles virus would not form E or EAC3 rosettes and that concentrated preparations of measles virus would not cause hemagglutination of E cells.

RESULTS

(i) Continuously cultured human lymphoblastoid cells—acute infection. Sequential samplings of each line of continuously cultured lymphoblasts acutely infected with measles virus (MOI = 1.0) indicated that infectious virus was released in similar amounts over the time periods studied and that peak titers of infectious virus were produced at 72 or 96 h after infection. As seen in Table 1, all the cell lines, whether of T or B subtype, proved capable of replicating measles virus, and peak viral titers

TABLE 1. *Release of infectious measles virus from acutely infected lymphoblast cell lines*[a]

Cell line	Cell type	HL-A type	Viral titer (PFU/ml)				
			0	24 h	48 h	72 h	96 h
Daudi	B	NI[b]	2.5×10^2	7.5×10^2	4.0×10^4	2.8×10^5	5.0×10^5
LD-7A	B	2,5,12	1.5×10^3	1.3×10^3	6.0×10^4	6.0×10^5	5.0×10^5
Raji	B	3	0.0×10^1	2.8×10^2	1.8×10^5	2.0×10^6	4.0×10^6
RPMI-6410	B	2,7,12	2.3×10^2	ND[c]	ND	1.0×10^6	1.4×10^6
RPMI-8866	B	2,3,7,12	ND	8.0×10^3	1.6×10^5	1.2×10^6	5.8×10^6
UM-56	B	3,5,7	1.3×10^2	ND	ND	2.8×10^5	6.0×10^5
UM-61	B	1,2,5	4.0×10^2	ND	ND	2.2×10^5	1.8×10^6
WI-L2	B	1,2,5	ND	1.3×10^4	1.1×10^6	1.5×10^6	6.5×10^6
CCRF-HSB-2	T	1,2,5	0.0×10^1	4.0×10^1	1.6×10^3	4.0×10^6	6.0×10^6
MOLT-4	T	NI[b]	2.0×10^1	1.6×10^2	4.0×10^3	1.2×10^5	1.6×10^5

[a] In each instance, 10^7 cells were infected with stock measles virus at an MOI = 1.0. After washing, infected cells were incubated at 37 C in 75-cm² plastic flasks containing 40 ml of RPMI 1640 medium supplemented with 20% heat-inactivated fetal calf serum, glutamine, and antibiotics. Samples of culture fluid were removed various times after infection. These fluids were clarified by centrifugation and supernatant fluid was frozen at −70 C for subsequent plaque assay on Vero cell monolayers. Peak titers were obtained in all systems at 72 or 96 h after infection.

[b] HL-A type not identifiable (NI) using a standard panel of typing sera.

[c] ND, Not determined.

fell within a range, i.e., 1.6×10^5 to 6.5×10^6 PFU/ml. Furthermore, equivalent amounts of measles virus were made in B cell lines as in T cell lines. The efficiency of viral replication in lymphoblasts carying HL-A determinants 3 and 7 (e.g., RPMI-8866, UM-56) did not significantly differ from that in lines lacking determinants 3 and 7 (e.g., UM-61, WI-L2). Lymphoblast line LD-7A that putatively carries the LD-7 mixed lymphocyte culture determinant showed no distinguishing features with regard to measles virus replication.

(ii) Continuously cultured human lymphoblastoid cells—persistent infection. Persistent infection of several lymphoblast lines was readily established by frequent refeeding of cells after acute infection with measles virus. One B cell line, RPMI 8866, and one T cell line, MOLT-4, were studied in detail after becoming persistently infected. In each instance, virtually 100% of cells expressed measles virus antigens on their plasma membranes as detected by immunofluorescence. The majority of persistently infected cells were lysed upon the addition of antibodies to measles virus and complement. Virtually all persistently infected lymphoblasts contained nucleocapsid structures within their cytoplasm (Fig. 1A and B). From an ultrastructural standpoint, the morphology of nucleocapsids in the cytoplasm of persistently infected cells was indistinguishable from acutely infected cells.

Viral replication was assayed in lines which were persistently infected for over 8 weeks. In all instances these persistently infected lines produced constant amounts of infectious virus over a 72-h sampling period (Table 2); however, in each case, daily titers were 1.5 to 2.5 logs less than the peak titer obtained in each respective cell line during acute infection (Table 1). For example, 10^7 persistently infected 8866 cells produced titers in range of 10^4 to 3×10^4 PFU/ml each day over a 3-day period, whereas a comparable number of acutely infected cells from the same line produced a peak titer of 5.8×10^6 PFU/ml under identical culture conditions. Studies on the growth of persistently infected lymphoblasts indicated that their growth rate was equivalent to that of uninfected cells during the first 3 days after refeeding. This was the period during which viral samples had been collected. By day 4 after refeeding, the growth of persistently infected lymphoblasts began to lag behind uninfected lymphoblasts. By day 5, the disparity in growth rates was pronounced.

Virus recovered from persistently infected lymphoblast lines caused acute cytopathic degeneration when seeded onto HeLa cell monolayers; plaques on Vero cells were comparable to those induced by wild-type virus with respect to both size and clarity.

(iii) Peripheral blood leukocytes from normal human adults—acute infection. Mixed populations of circulating mononuclear cells (i.e., lymphoid cells and monocytes) obtained from healthy human adults supported measles virus replication to a limited extent even in the absence of exogenous mitogenic stimuli (Fig. 2). Prestimulation of such cells with PWM or PHA increased the efficiency of measles virus replica-

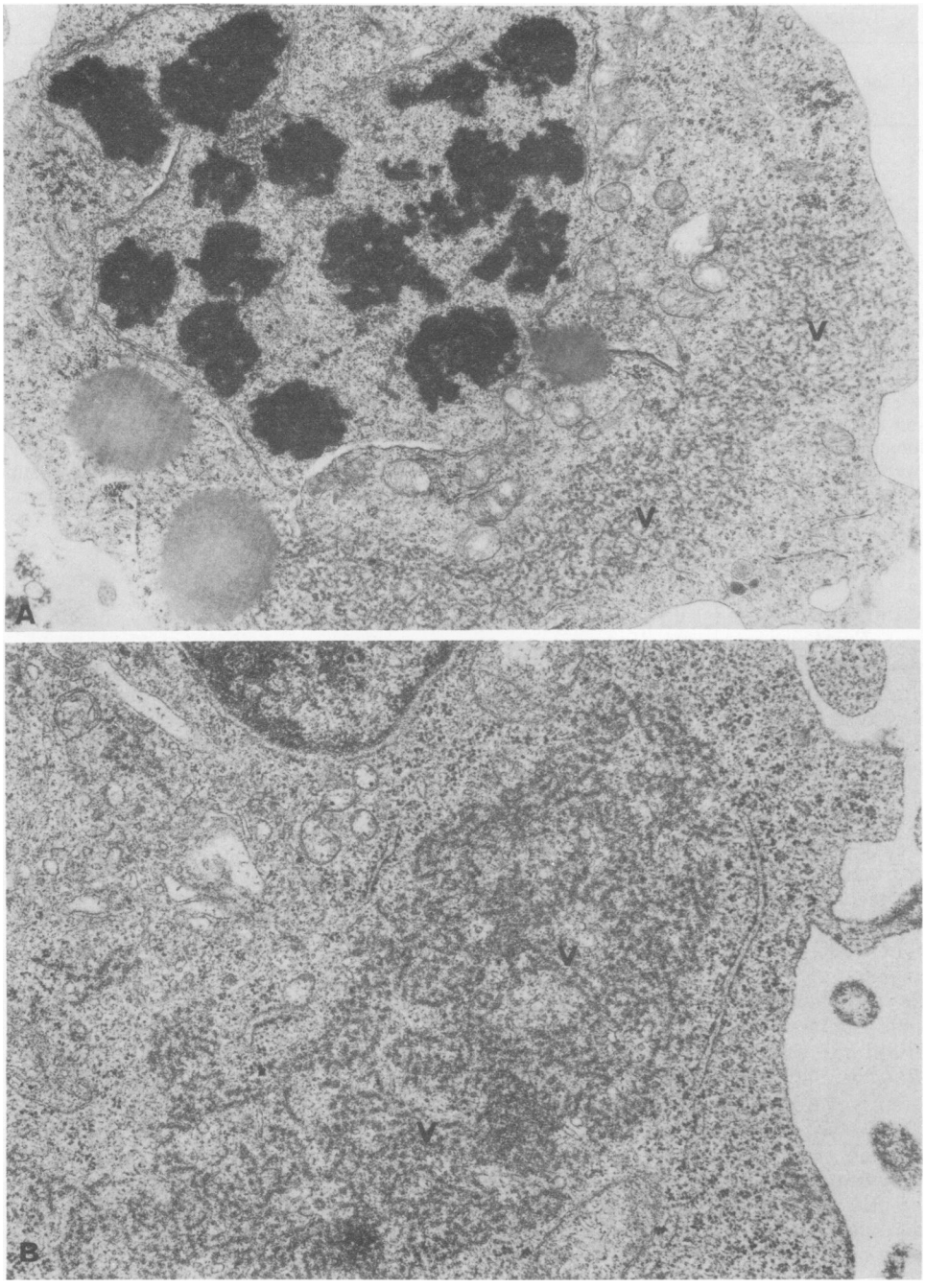

FIG. 1. *Electron photomicrographs of T and B lymphoblastoid cells persistently infected with Edmonston strain of measles virus. (A) MOLT-4 cell in mitosis with aggregates of viral nucleocapsids (V) in the cytoplasm. Magnification, ×12,000. (B) RPMI-8866 cell showing intracytoplasmic aggregates of viral nucleocapsids (V). Magnification, ×22,000. MOLT-4 are characterized as T cells and RPMI-8866 as B cells by various immunologic markers. All the cells in each population contained measles virus antigens as judged by direct immunofluorescence assay.*

TABLE 2. *Release of infectious measles virus from persistently infected lymphoblast lines*[a]

Cell line[b]	Viral titers (PFU/ml) obtained at designated intervals after replating cultures		
	24 h	48 h	72 h
RPMI-8866 (P/M)	1.0×10^4	3.0×10^4	2.5×10^4
MOLT-4 (P/M)	4.8×10^3	5.0×10^3	5.0×10^3

[a] 10^7 persistently infected cells were incubated and sampled under conditions identical to those described in Table 1 for acutely infected lymphoblasts. Lymphoblasts used in this experiment were studied 8 weeks after acute infection with measles virus.

[b] RPMI-8866 is a B cell line; MOLT-4 is a T cell line. The notation (P/M) indicates persistent infection with measles virus.

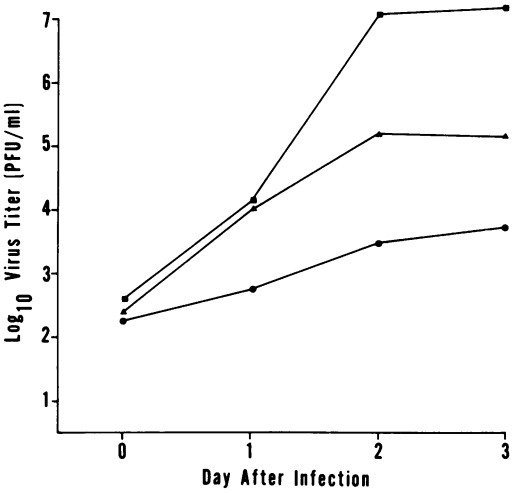

FIG. 2. *Replication of infectious measles virus by mononuclear cells from human blood. Cells were cultured without mitogen (●) or with either PHA (■) or PWM (▲) for a period of 4 days before being infected with measles virus at an MOI of 1.0. Cells were washed repeatedly after viral absorption, and clarified culture fluids were assessed sequentially for infectious measles virus by plaque assay on Vero cell monolayers.*

tion. The increase was more pronounced with PHA than with PWM; in the latter circumstance, virus titers peaked at an approximate level of 10^5 PFU/ml, whereas 10^7 PFU/ml were obtained after PHA prestimulation. These cultures contained approximately four times as many cells as employed for our studies on continuous lymphoblast lines. Thus, when an adjustment is made for this difference, the number of PFU replicated per PHA-prestimulated mononucleated cell is comparable to the maximum replicative index obtained for continuously cultured lymphoblasts. In specific terms, approximately 10 to 20 PFU were replicated per cell per 24 h in both instances.

PMN cells showed no evidence of nucleocapsid replication throughout a 72-h sampling period. This result was obtained even at an MOI of 5 and despite the fact that the majority of PMNs continued to maintain a viable ultrastructural appearance throughout the first 24 to 36 h in culture. At 72 h, nearly all PMNs appeared degenerate.

Ultrastructural investigations indicated that monocytes (glass-adhering cells ingesting carbon particles) (Fig. 3) as well as lymphoid cells have the capacity to replicate measles virus nucleocapsids. Selective rosetting techniques additionally indicated that cells forming E rosettes (i.e., T cells) and nonadherent cells forming EAC3 rosettes (i.e., B cells) could each replicate viral nucleocapsids (Fig. 4 and 5).

To determine whether infectious measles virus was being synthesized in each of these cell types, the incidence of infectious center formation in relatively pure populations of cells was studied. The results obtained are detailed in Table 3. In two sets of experiments, 7 to 8% of T cells, 6 to 8% of B cells, and 8 to 10% of macrophages formed infectious centers. These results indicate that each of the above subpopulations can produce infectious measles virus.

Replication of measles virus by monocytes (macrophages) was further evaluated by studying the effect of mitogenic prestimulation on such replication (Table 4). Cells employed in this segment of the study had a purity of 98% or greater as judged by neutral red uptake. Unstimulated monocytes produced titers of approximately 2×10^3 and 4×10^3 PFU/ml at 72 and 96 h, respectively. Slightly higher titers were recorded at both intervals for PWM- and PHA-prestimulated cells, but in each case the difference was less than 10-fold. Using the highest viral titers obtained, it is calculated that macrophages produce infectious measles virus at an efficiency of less than 1 PFU/cell per day.

DISCUSSION

The present study demonstrates that measles virus can replicate in circulating T cells, B cells, and monocytes (macrophages) of man. These conclusions were derived from ultrastructural studies and infectious center assays which were mutually confirmatory. In contrast, mature PMN cells were apparently incapable of synthesizing measles virus. Whether PMNs might be capable of such synthesis at an earlier point in their maturation has not been evaluated here, but a report by Gresser and Lang (14)

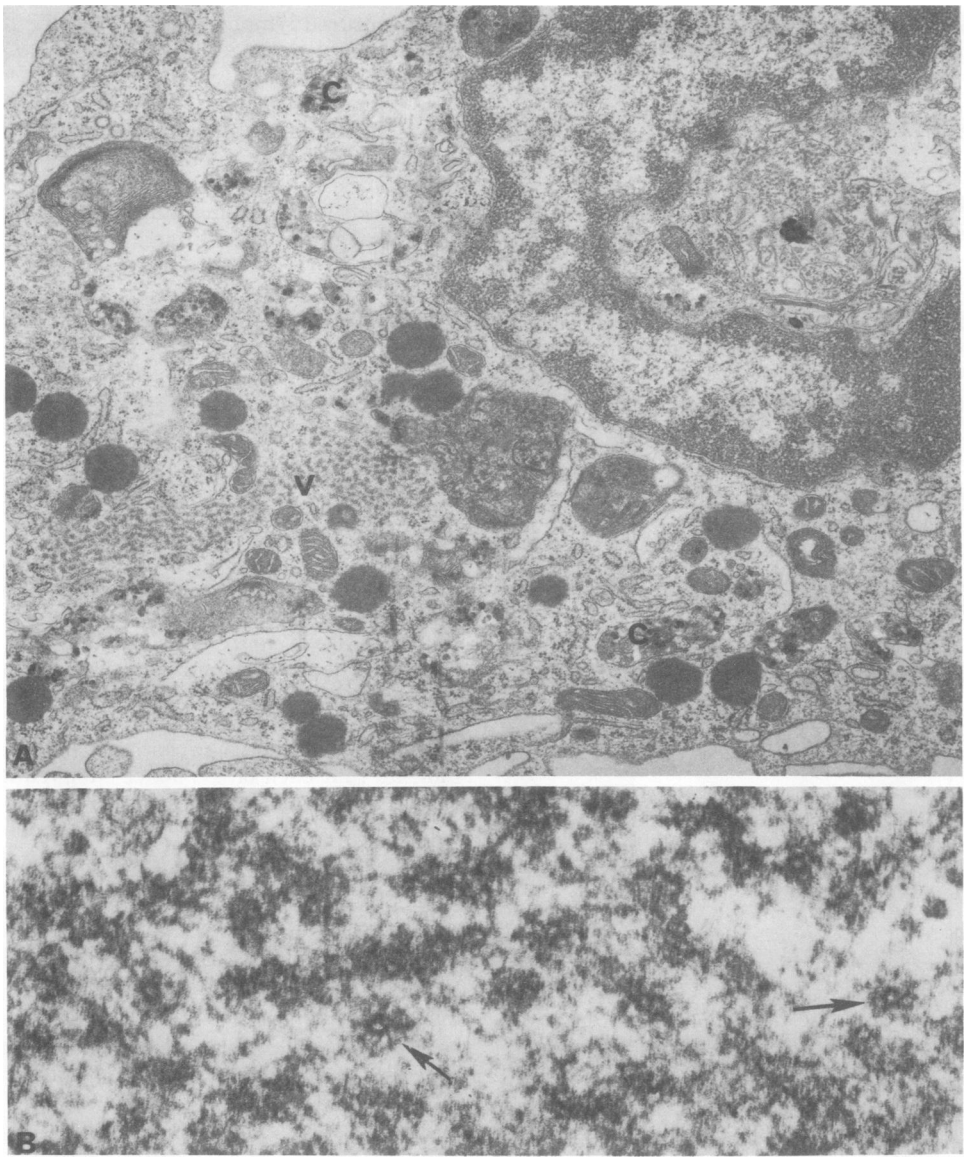

FIG. 3. *Electron photomicrographs of a macrophage obtained from the peripheral blood of a normal human adult and infected with Edmonston strain of measles virus. (A) Intracytoplasmic viral nucleocapsids (V) and carbon particles (C) are seen. Magnification, ×18,000. (B) Higher magnification of the intracytoplasmic viral nucleocapsids (arrows). Magnification, ×190,000.*

suggests that this may be the case.

In view of the diversity of leukocyte subtypes that are infectible with measles virus, it is not surprising that transient defects in immunologic defenses should occur during systemic measles virus infection. The precise means whereby measles virus infection of immunocytes leads to disturbed immunocyte function in vivo is unclear. Among the possibilities are (i) viral-induced cytolysis, (ii) immune lysis of infected leukocytes by antiviral antibody and complement (21), (iii) disturbed trafficking of infected leukocytes due to nonlytic, viral-related modifications in cell surface properties (47), (iv) inhibition of DNA synthesis (30, 39), and (v) suppression of nonvital, specialized cell functions (33a). Although there is as yet no evidence of B cell or macrophage dysfunction associated with measles virus infection, others have presented theoretical (5) and experimental (28) ar-

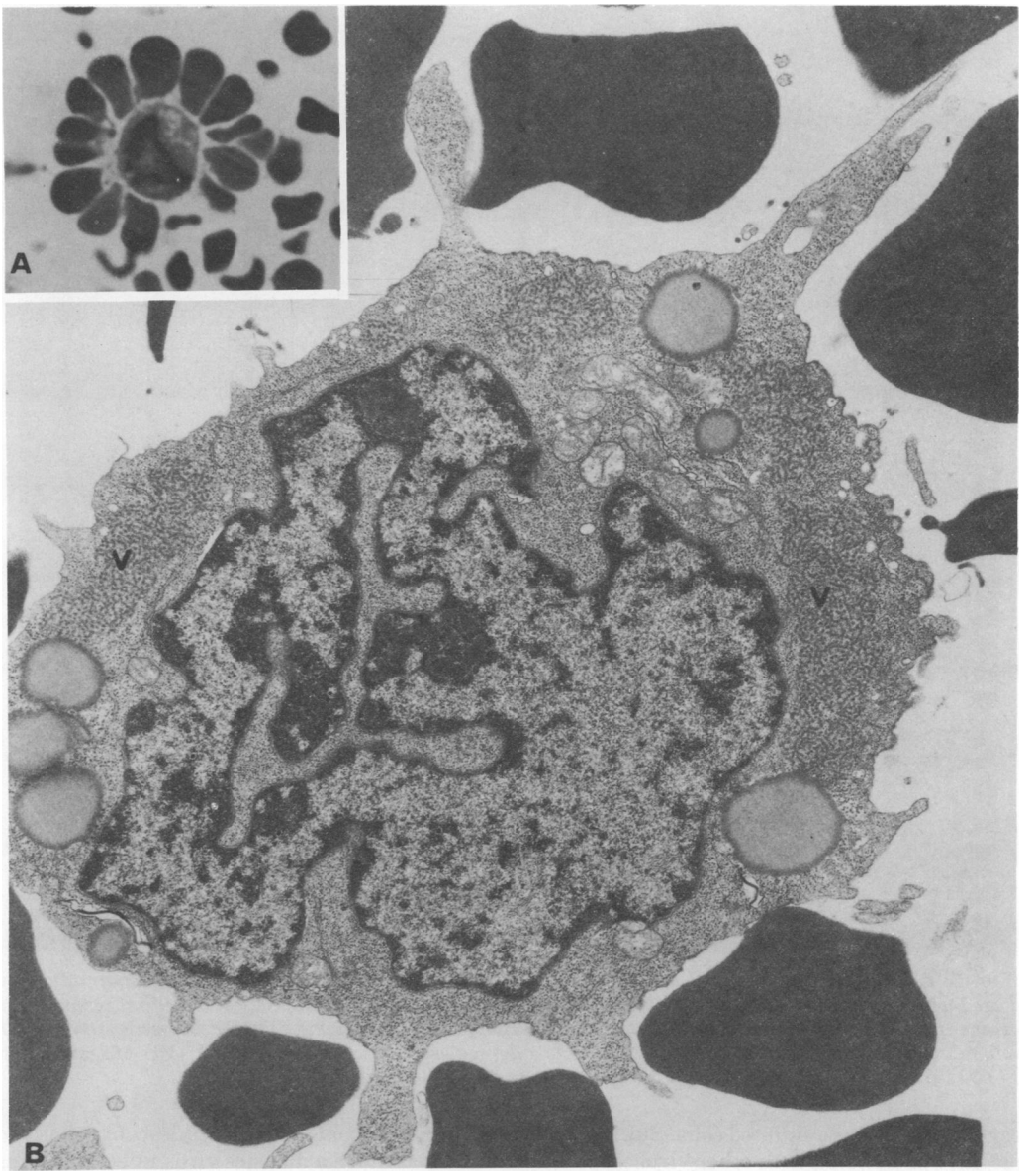

FIG. 4. *Photomicrographs of a T cell obtained from the peripheral blood of a normal human adult and infected with Edmonston strain of measles virus. The T cell was rosetted with E cells. (A) Light micrograph of the T cell rosette. (B) Electron photomicrograph showing intracytoplasmic aggregates of viral nucleocapsids (V). Magnification, ×14,000. (See text for techniques used to ensure purity of T cell population.)*

guments suggesting that measles virus has a selective effect on T cells.

The susceptibility of diverse types of leukocytes to measles virus infection stands in contradistinction to other viral systems in which infectibility is apparently limited to selected leukocyte subclasses (22, 33, 37). Such selective infectibility is likely related in part to a differential distribution of surface viral receptors among the leukocyte subclasses (20, 46). Human T cells, B cells, and monocytes would all be expected to contain surface receptors for measles virus; this has been demonstrated for T cells (41).

We observed that measles virus replicates more efficiently in mitogenically stimulated leu-

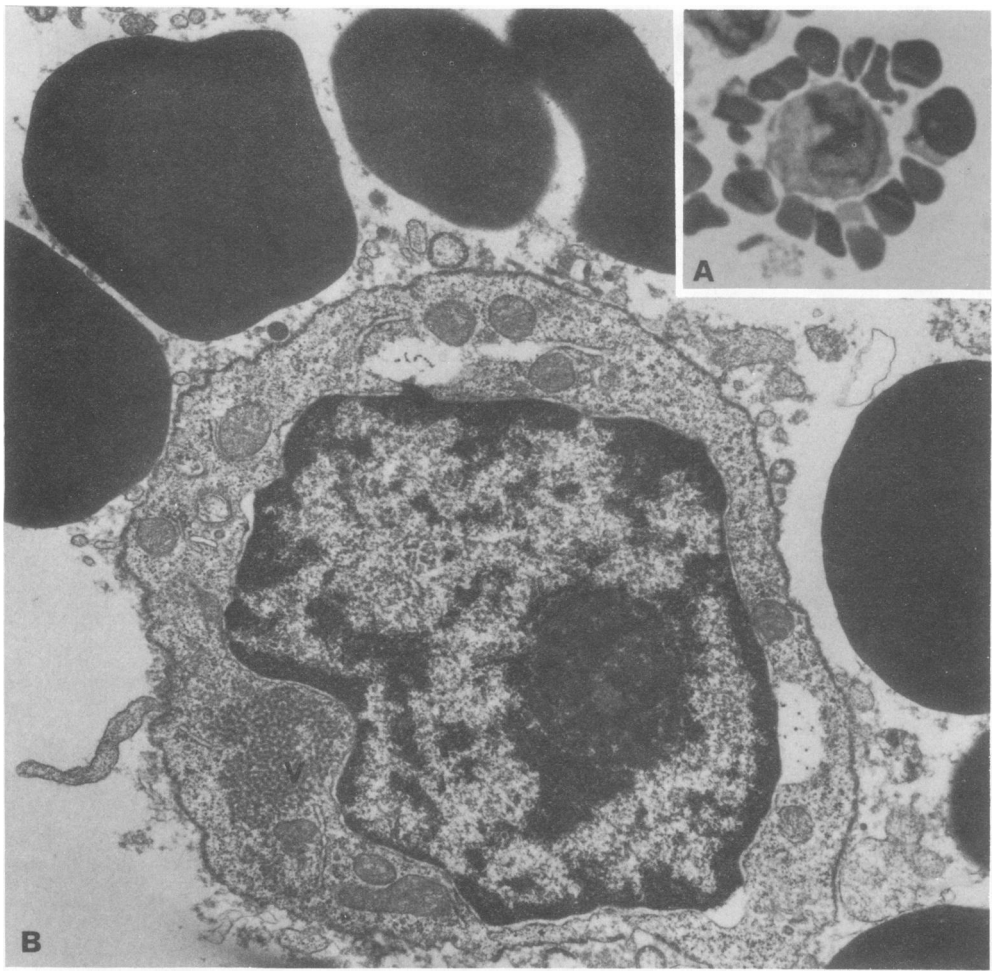

FIG. 5. *Photomicrographs of a B cell obtained from the peripheral blood of a normal human adult and infected with Edmonston strain of measles virus. Glass-adherent cells (macrophages) were first removed and then the B cell was rosetted by use of EAC cells. (A) Light photomicrograph of the B cell rosette. (B) Electron photomicrograph demonstrating intracytoplasmic aggregates of viral nucleocapsids (V). Magnification, ×14,000. (See text for techniques used to ensure purity of B cell population.)*

kocytes than in unstimulated cells. Similar findings have been noted with other viruses, particularly herpes simplex virus (24), poliovirus (45), and vesicular stomatitis virus (8). Why stimulated leukocytes should have an increased capacity to reproduce virus, particularly an RNA virus such as measles, remains an enigma. However, our observations suggest that germinative areas within the lymphoreticular system could serve as major centers of measles virus replication during infection. This prediction is supported by experimental findings (23, 34, 44, 48). Further, replication of measles virus should also be amplified in leukocytes turned on by specific antigenic stimuli. Conceivably, such a mechanism might permit preferential infection of tuberculin-sensitive immunocytes in patients with quiescent tuberculosis who acquire a measles virus infection. Thus, flareups and dissemination of tuberculosis in patients subjected to measles virus infection (10) might be related to a narrow, rather than a broad, impairment of cell-mediated immunity.

We have shown that circulating human monocytes can replicate measles virus. Replication by circulating human monocytes has been demonstrated with certainty for only two other viruses: vesicular stomatitis virus (9) and 17D yellow fever virus (43). Poliovirus can replicate in human peritoneal macrophages, but whether circulating monocytes have a similar capability has not been examined (45). Replication of measles virus was found to proceed with less efficiency in monocytes than in mitogeni-

TABLE 3. *Replication of infectious measles virus by defined subpopulations of circulating human leukocytes*[a]

Cell type	Incidence of infectious centers[b] (%)	
	Expt 1	Expt 2
T cells	8	7
B cells	6	8
Monocytes/macrophages	10	8

[a] T cells were isolated as E-2-aminoethylisothiouronium hydrobromide (AET) rosettes. Cells that failed to form E-AET rosettes were divided into adherent (i.e., monocyte/macrophage) and nonadherent (i.e., B cells) subgroups by culturing mixed cell populations at low density in flat-bottomed, plastic culture flasks. Each subpopulation was prestimulated with PWM (50 μg/ml) for 4 days and then infected with measles virus at an MOI > 1.0. Cells were harvested for assay 48 h after infection. As judged by neutral red and surface immunoglobulin staining, T cell and mononuclear cell preparations exhibited a purity of 96% or greater; B cell preparations exhibited a purity of 85% or greater.

[b] See text for a description of the infectious center assay employed. The experiment was repeated on two separate occasions on cells drawn from the same donor. The results are expressed as a percentage of viable cells plated. Viability was determined by trypan blue dye exclusion. Under conditions used no infectivity was observed in cultures of polymorphonuclear leukocytes where almost all HeLa cells acutely infected with measles virus produced infectious virus.

TABLE 4. *Release of infectious measles virus from human monocytes*[a]

Pretreatment	Viral titer (PFU/ml)		
	48 h	72 h	96 h
No mitogen	1.5×10^3	1.8×10^3	4.3×10^3
PHA (50 μg/ml)	1.0×10^4	1.3×10^4	4.0×10^4
PWM (50 μg/ml)	2.8×10^3	3.5×10^3	9.5×10^3

[a] Cells employed in this experiment failed to form E-2-aminoethylisothiouronium hydrobromide rosettes but did attach to plastic culture flasks after a relatively brief period of incubation. Neutral red staining indicated that 98 to 99% of the cells employed had phagocytic potential. Adherent cells (approximately 5×10^6 cells/flask) were cultured in the presence or absence of PHA-P or PWM for 4 days and then infected with measles virus at an MOI of 1.0. Cultures were then incubated and sampled in a manner identical to that described for acutely infected lymphoblasts (see footnote, Table 1).

cally stimulated lymphocytes or in continuously cultured lymphoblasts. Slight amplification of measles virus synthesis by monocytes was observed in cultures pre-exposed to PHA and PWM; however, we cannot exclude the possibility that these relatively small differences may have been related to mitogenic effects on lymphoid cells contaminating the test cultures. PHA and PWM are not known to have a mitogenic influence on human macrophages (25).

Epidemiologic studies indicate that measles virus antibody titers are increased in persons carrying HL-A3,7 determinants (1, 16). The present study indicates that several lymphoid cell lines carrying HL-A3,7 markers do not show a differential capacity to replicate measles virus when compared with lymphoid cell lines lacking these determinants. Our findings favor the possibility that the unusually elevated antibody response to measles virus seen in HL-A3,7 individuals may be related to aberrant host immune responsiveness rather than to an enhanced viral replicative capacity. Such aberrant responsiveness could be instrumental in changing a normally acute and uncomplicated viral disease into one with a protracted course with serious residual. However, we can not exclude the alternative possibility of enhanced virus replication and/or virus-induced cytotoxicity in cells of the central nervous system bearing the HL-A3,7 haplotype. Both of these possibilities have particular relevance to multiple sclerosis and paralytic poliomyelitis since both diseases have associations to the HL-A3,7 genotype (17, 32).

It is widely appreciated that persistent states of infection can be readily established in vitro with many paramyxoviruses, including measles virus (6). To date, however, there is no specific data available regarding the capacity of leukocytes to become persistently infected with measles virus or other paramyxoviruses. This issue has acquired considerable biologic importance in view of recent demonstrations that lymphoreticular organs may serve as a primary reservoir for the scrapie agent (27) and that measles virus can be rescued from lymphoreticular tissues in patients with subacute sclerosing panencephalitis (15). Our demonstration that lymphoid cells of both T and B subtype can be persistently infected with measles virus lends credence to the hypothesis that long-lived leukocytes could be the seat of latency and a vehicle whereby viruses enter the central nervous system in some "slow" or recurrent degenerative diseases affecting that organ.

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ADDENDUM

Recent independent studies using human peripheral blood lymphocytes and measles virus have also indicated a 6 to 10% incidence of infectious centers in B and T lymphocytes (J. L. Sullivan, D. W. Barry, P. Albrecht, and S. J. Lucas, J. Exp. Med., in press).

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