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Replication of Poliovirus and Measles Virus in Cultures of Human Lymphoblastoid and of Burkitt Lymphoma Cell Lines

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With 4 Figures

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Summary

Poliovirus type 1 replicated in 4 different human lymphoblastoid cell lines (LBL) transformed *in vitro* by EBV virus or isolated from cases of mononucleosis. Maximal virus titers were reached 2—4 days after inoculation. There was a decrease in percentage of viable cells in the infected cultures but a considerable fraction of cells was not destroyed by virus replication. A persistent low grade replication of virus was observed and demonstrable during 56 days after inoculation in one LBL cell line. Presumably a small fraction of cells supporting virus replication is continuously recruited from refractory cells. No virus propagation was demonstrable in 4 Burkitt lymphoma (BL) cell lines. Measles virus grew efficiently in both types of cell lines. Using indirect immunofluorescent technique poliovirus and measles virus antigen could be demonstrated in the cytoplasm of LBL cells in parallel with disappearance of F-actin containing microvilli.

Introduction

Poliovirus as well as measles virus are usually propagated in monolayer cultures of primate fibroblasts or epithelial cells grown in monolayer. However, in some experiments suspension cultures have been used, e.g. HeLa cell cultures for propagation of poliovirus (12). Poliovirus was also found to replicate in *in vitro* stimulated peripheral lymphocytes but not in non-stimulated lymphocytes (7, 19). Furthermore, a lymphoblastoid cell line derived from B-cells transformed *in vitro* by Epstein-Barr virus (EBV) has been found susceptible to poliovirus (16). Myxoviruses including measles virus can infect mononuclear cells from human

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peripheral blood when mitogen stimulated (2, 8, 9, 17, 18, 20). Infection of Burkitt lymphoma cell line with measles virus gives a high yield of virus but in spite of this a persistent infection.

The object of this investigation was to follow the replication of poliovirus and measles virus in human lymphoblastoid (LBL) and Burkitt (BL) cell lines. The difference between several properties of these two types of EBV-associated cell lines is well known (3, 5, 10). The effect of virus infection on the morphology and actin cytoskeleton of cells has also been examined.

Materials and Methods

Cell Lines

Eight human lymphoid cell lines were used. Four of these were lymphoblastoid lines and four were lymphoma lines. Two lymphoblastoid lines were established from young mononucleosis patients, U-1450 kindly supplied by Dr. K. Nilsson at the Wallenberg Laboratory in Uppsala, and Mo 1 established in this laboratory. Two cell lines were initiated by infection *in vitro* with EBV-virus. The Robinson lymphoblastoid cell line was provided by Dr. G. E. Moore, Roswell Park Memorial Inst., Buffalo. The fourth EBV-transformed lymphoblastoid cell line PsBi as well as all four Burkitt lymphoma lines (Namalva, Raji, Ramos and Daudi) were made available to us by Dr. G. Klein, Dept. of Tumor Biology, Karolinska Institute, Stockholm. The cell lines were maintained at 37° C in 50 ml volumes in stationary bottles without stirring. The medium was RPMI-1640 supplemented with 8 per cent foetal calf serum and 100 IU penicillin and 50 μg streptomycin per ml. The optimal cell concentration for cell growth was 0.3×10^6 per ml. The viability of cells in repeated passages varied between 85—98 per cent. During experiments taking 6—7 days the cells were kept in the same bottles and not fed with new medium. Number of viable cells were determined by the trypan blue exclusion method.

Virus Strains

Polio Viruses

Single seed lots of polio virus strains A-Stockholm 53 (Type 1), MEF (Type 2) and Saukett (Type 3), prepared in green monkey kidney cell line (GMK) were used. Infectivity titres of these three lots were for type 1—$10^{8.7}$ $TCID_{50}$/ml, for type 2 to $10^{7.4}$ $TCID_{50}$/ml and for type 3—$10^{7.2}$ $TCID_{50}$/ml.

Measles Virus

The Lec strain of measles virus, originally isolated from a patient with subacute sclerosing panencephalitis, was used. The virus was propagated in Vero cells and the titer of seed material was 10^6 $TCID_{50}$/ml.

Propagation of Virus in Suspension Cultures

Immediately after the adjustment of the cell density to 0.3×10^6 cells per ml and passage to new bottles, the LBL or BL cell suspension cultures were inoculated with seed virus at concentrations giving multiplicities of infection (MOI) varying from 27 to 0.027. After adequate mixing of virus and cells, the culture bottles were incubated at 37° C. Non-inoculated control bottles were incubated in parallel. Specimens were taken from the cultures for virus infection titrations and for determinations of cell viability. Preparations of cell smears were made following a technique that has been described in detail (4). The virus titers were determined in monolayers of GMK cells for polio virus and of Vero cells for measles virus determinations. 0.1 ml amounts of serial tenfold dilutions of each specimen were inoculated into groups of 5 roller tubes of the appropriate cell line. Final readings of the specific cytopathogenic effect for both poliovirus and measles virus were made after 7 days.

Immunofluorescent Technique (IFL)

Two human sera containing antibodies against poliovirus type 1, 2 and 3 and one human serum containing antibodies to measles virus were used. The human serum containing antibodies against actin (5, 6) was used as whole serum or as the eluate after two precipitations with purified actin. Hyperimmune rabbit anti-actin serum was used. Antisera against human and rabbit immunoglobulin were produced in sheep. Purified gammaglobulin fractions of the respective antisera were absorbed with globulin preparation of the other species involved and then conjugated with fluorescein isothiocyanate (FITC) or tetrametylrhodamine isothiocyanate (TRITC) as described earlier (1). The FITC-conjugated anti-IgG and anti-IgM were a gift from Mrs. I. Batty, Wellcome Research Laboratories, Beckenham, England. The human sera, containing antibodies to poliovirus or measles virus were mixed with rabbit anti-actin serum and added to the cell smears followed by a mixture of the species specific FITC- and TRITC-conjugates. The stained smears were examined in a Zeiss universal microscope using incident light and filter sets 44 77 10 for FITC and 48 77 15 for TRITC.

Results

Poliovirus Type 1 Replication in Lymphoblastoid Cell Lines

Replication of poliovirus type 1 was readily observed in all LBL lines, Robinson, U-1450, Mo1, PsBi, each tested in 2—5 experiments. The multiplicity of infection (MOI) varied between 27 to 0.027. Maximum titers in 3 of the 4 lines tested, varied between 7.1—7.9 $\log_{10}$/ml. The line PsBi consistently gave 10 times lower titers. Virus replication could be shown in all cell lines one day after inoculation. The maximal virus yield was obtained on day 2—4 and the titers diminished on day 5—6. The increase in virus titers correlated with a decrease of relative numbers of viable cells as compared to the non-infected cells. The cells usually increased again in parallel to the decreasing yield of virus (Fig. 1, Table 1). As can be seen in Fig. 1

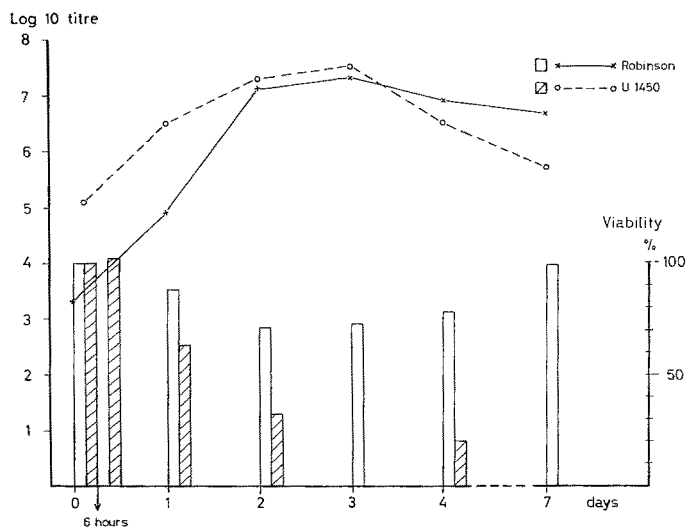

Fig. 1. The unbroken line refers to titers of Robinson cell cultures (MOI 0.27) and the dashed line to the titers of U-1450 (MOI 2.7). The nonfilled and the striped bars represent the cell viability of Robinson and U-1450 respectively

there was a difference in the cell viability between the Robinson and U-1450 lines, the destruction of cells being more moderate in the Robinson cells than in the U-1450 cells. But also this last cell line showed an increase in cells as virus production was restricted (Table 1). In one experiment the Robinson cell line was followed during 56 days and a continuous virus production at low level of about 5 $\log_{10}$ could be demonstrated (Fig. 2). The cell viability did not change markedly during this time. The newly isolated line, called Mo1, showed the same picture as U-1450.

Table 1. *Multiplication of poliovirus type 1*

	U-1450		Raji	
Day	$\log_{10}$ titre	Viability %	$\log_{10}$ titre	Viability %
0	4.3	100	4.5	100
1	6.7	112	3.9	114
2	7.5	59	3.7	92
3	7.1	73	3.5	97
4	6.7	54	3.1	83
7	6.5	76	ND	112

Virus yield obtained in U-1450 (LBL-line) and Raji (BL line) infected in parallel (MOI 0.27). The viability figures are given as the percentage ratio of numbers of live cells in infected and non infected cell cultures

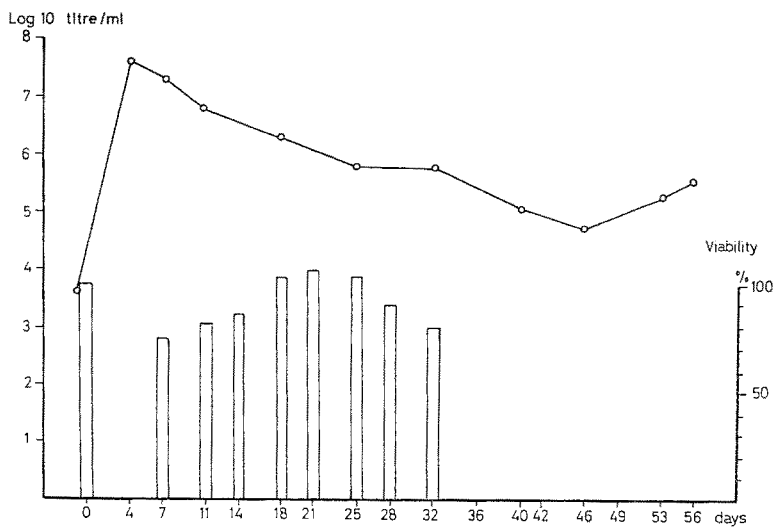

Fig. 2. Poliovirus replication in Robinson cell cultures followed for 56 days. The bars indicate the cell viability of the culture followed during 32 days only

Poliovirus Replication in Burkitt Lymphoma Cell Lines

Multiplication of poliovirus in the four BL-lines tested could not be demonstrated even when a multiplicity as high as 27 was used. Instead virus titers showed a steady decrease reflecting the termal inactivation of virus (Table 1). The inability

of the BL-lines to propagate poliovirus was mirrored by the viability tests that did not reveal any cytopathogenic effect on the cells in the cultures inoculated with poliovirus.

Measles Virus Replication in Lymphoblastoid and Burkitt Lymphoma Cell Lines

Multiplication of measles virus was documented in both the LBL- and the BL-lines. Increase in virus titers was readily registered in the culture medium two days after inoculation and maximal titers were reached on day 4—6 (Table 2).

Measles virus replication caused strong cytopathic effects in 3 out of 4 LBL lines (PsBi also here being more resistent) as well as in the 4 BL-lines tested, leaving rather few cells in the cultures alive after 4—6 days. No attempts were made to study measles virus production after this time.

Table 2. *Yield of measles virus grown in Robinson (LBL) and in Raji and Namalva (BL) (MOI 0.17)*

Day after	Virus titre $\log_{10}$/ml		
virus infection	Robinson	Raji	Namalva
1	ND	1.5	1.6
2	$\geqq$6.5	1.8	2.6
3	$\geqq$6.5	3.8	4.4
4	7.3	ND	ND
6	ND	6.4	$\geqq$6.5

Titration in Vero cells. The percentage of live cells in infected culture on day 4 is for Robinson 26, on day 6 for Raji 41 and in Namalva 50

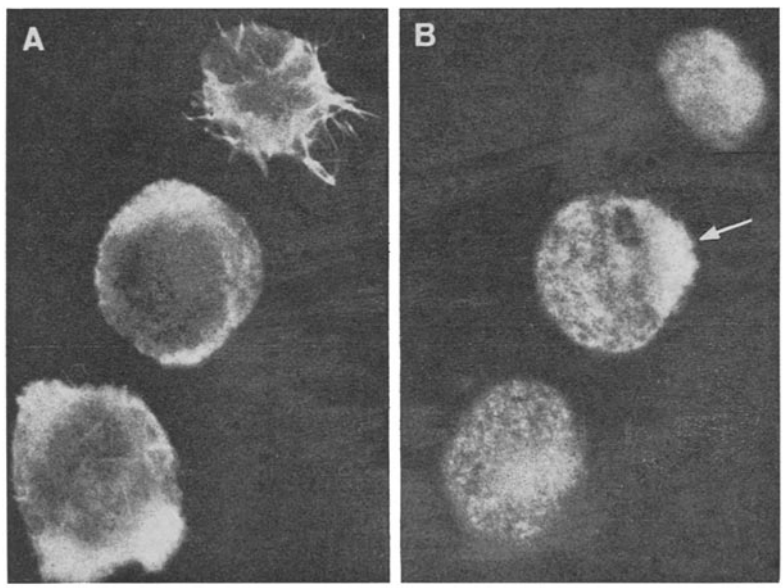

Fig. 3. IFL-pattern of LBL cells (U-1450) 24 hours after infection with poliovirus type 1 (MOI 2.7). Double staining technique is used. *A* shows the cells stained for actin and *B* for poliovirus antigen. The disappearance of the F-actin containing microvilli in the poliovirus containing area of the infected cell (arrow) is obvious. Remnants of stainable actin are seen in other parts of this cell *(A)*. Magnification $\times$1000

Morphological Investigations

Immune fluorescence studies were made on smeared cells using a double staining technique with a human serum containing antibodies against poliovirus and rabbit hyperimmune serum against actin and species-specific conjugates. Studies of smeared preparations made 3—24 hours after inoculation of the cultures revealed focal accumulation of poliovirus antigen in the cytoplasm of <1 per cent of the cells (Fig. 3). In contrast to the non-infected cells these virusantigen containing cells were devoid of microvilli. The relative number of villous cells decreased in the infected cultures and this pattern followed approximately the viability of the cells. BL cells in cultures inoculated with poliovirus showed no morphological changes or structures in the cytoplasm stainable with poliovirus antibodies.

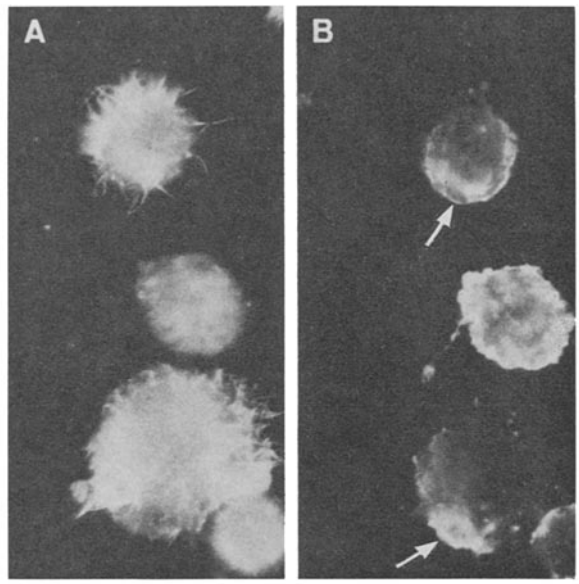

Fig. 4. Robinson cells double-stained for actin in microvilli *(A)* and measles virus in the cytoplasm *(B)*. The arrows indicate measles virus inclusions close to the nucleus in B_a (top cell), or close to the cell membrane B_c (bottom cell). The corresponding parts of the cells show little or no surface microvilli. The cell in the middle, B_b stains for measles throughout the cytoplasm and is devoid of microvilli. Magnification × 700

Double-staining of the infected LBL cells with anti-measles virus antibodies and anti-actin serum showed typical cytoplasmic staining for measles virus. Fig. 4 shows examples of the measles virus multiplication in cells and the influence on microvilli containing actin filaments. In the cell B_a in Fig. 4 the remaining villi were located in the part of the cell which did not contain virus inclusions. When measles virus was found close to the membrane the surface was devoid of villi in that region (Fig. 4 c). The appearance of measles virus was also followed in cultures of the BL-lines, Raji and Namalva. On two days after infection Raji cells showed a strong cytoplasmic staining with a more general occurrence of virus antigen than what was seen in the LBL cells. Syncytial formation could be seen in both LBL and

BL lines 2—3 days after measles infection. The giant cells showed typical measles virus inclusions.

In the smears of the LBL cell line PsBi 2—5 per cent cells were strongly positive when stained directly with anti-IgG, and negative with anti-IgM. After infection with measles virus these cells increased two to three times on the third day and many IgG positive mitoses could be seen. When the anti-IgG conjugate was combined with staining for measles virus no measles virus antigen was found in the IgG positive cells. The giant cells were all IgG negative. No other cells of the LBL of BL lines showed IgG positive cells.

Discussion

The primary intention of this study was to determine whether LBL-cells could be used for propagation of poliovirus. Studies on multiplication of poliovirus in lymphocytes have been summarized by Wheelock and Toy (18). While no production was found to occur in resting cells virus production was found after stimulation of lymphocytes conditions (7) or by addition of phytohaemagglutinin (PHA) (19). An increase in the number of poliovirus adsorbing and producing cells was found during the blastogenesis following PHA stimulation. The same effect of blast transformation on the propagation of mumps virus was found by Wheelock. It was suggested (17) that the increase in metabolism 2—3 days after mitogen stimulation might favour virus replication.

In the present study a multiplication of poliovirus in cultures of lymphoblastoid cell lines was found. These cells are B-blasts, obtained from cases of infectious mononucleosis or lymphocytes transformed *in vitro* by EBV. LBL and BL-cells have been extensively studied during the last decade (10, 3, 5). The LBL-cells can be maintained as rapidly dividing polyclonal cell lines. When infecting different lines a somewhat different behaviour was observed as regards the extent of cytopathogenic effects of the poliovirus. Robinson cells repeatedly showed a better viability of the cells than U-1450 and Mo1. However, approximately the same virus titers were reached in different lines (Fig. 1). Robinson, the only LBL-line followed for a longer time was capable of virus production for at least 56 days (Fig. 2). This finding is in agreement with previous observations (16). The other cell lines were not followed for more than 7—10 days. Difference in clonal composition of the cell lines might be the explanation of the differences in cell viability between the LBL-cell lines after poliovirus inoculation. A less likely explanation seems to be the difference in interferon production by the LBL-cells, known to appear after poliovirus infection (14, 15).

All attempts to get poliovirus type 1 to grow in the four BL cell lines failed. As is shown in Table 1 the titers in the cell line Raji decreased 1.4 $\log_{10}$ in 4 days apparently due to thermoinactivation. The titer curve of the LBL-line U-1450, inoculated at the same time, rose in the typical way. The good viability of the BL lines after polio inoculation and the absence of any cytoplasmic material stainable with antipolio antibodies supported the negative results obtained in titrations of culture fluid.

Interferon production might be one reason for the lack of polio replication after inoculation of BL cell lines since e.g. the Raji cell line is known to be a good

interferon producer (14). However, since Daudi cells, which do not produce interferon were also found not to propagate poliovirus, effects of interferon production does not seem to provide a full explanation for the documented resistence of the BL lines to polio infection. The question if this resistence is due to absence of poliovirus receptors on the surface of cells or to incapacity of these less differentiated cells to synthesize virus so far is not possible to answer.

Measles virus replicated well in both LBL and BL cell lines confirming earlier reports (9). Using the IFL-technique measles virus antigen was readily detected in the cytoplasm of LBL-cells as well as in BL-cells. Also syncytial cell formation were seen in both types of cell lines. Following the extent of the virus infection of the LBL-cells a gradual disappearance of the surface microvilli could be observed. Thus both poliovirus and measles virus were found to affect the organisation of cytoskeletal actin. Similar results were described for mesales virus infecting fibroblasts grown in monolayer (6). Cytoskeletal changes in poliovirus infected HeLa cells have been reported (11).

Poliovirus has thus been found to replicate in LBL-cells but not in BL-cells. Only results of tests with poliovirus type 1 have been reported here but in a series of experiments with poliovirus types 2 and 3 it has been shown that these types show a corresponding behaviour.

The results here reported have shown that poliovirus readily replicates in LBL cell lines. The possible use of such cell lines for the production of killed poliovaccine might be considered.

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