Induced aggregation of culture cells by measles virus and its possible link to viral dissemination

Walter Kemp
Carroll College, Helena, MT
Induced aggregation of culture cells by measles virus and its possible link to viral dissemination

Submitted in Partial Fulfillment of the Requirements for Graduation with Honors to the Department of Biology-Chemistry
Carroll College, Helena, Montana

Walter Loren Kemp
March 29, 1993
This thesis for honor recognition has been approved for the Department of Biology-Chemistry by:

Fr. Joseph Harrington, Advisor

Dr. Tomas Graman

Dr. John Addis

March 29, 1993
# Table of Contents

1. Acknowledgments ................................................................. ii
2. Abstract ....................................................................................... iv
3. List of Tables ................................................................................ v
4. List of Figures ............................................................................... vi
5. Literature Review ................................................................. 1
6. Introduction ............................................................................... 9
7. Materials ..................................................................................... 11
8. Methods ....................................................................................... 13
9. Results ......................................................................................... 20
10. Discussions and Conclusions ............................................. 32
11. Literature Cited .......................................................................... 37
ACKNOWLEDGMENTS

I would like to thank the following people for their contributions of time, money and emotional support to me and the final copy of this Honor Thesis:

Dr. Philip Wyde, for providing me with the laboratory and patience to perform the ten weeks of research which made this thesis possible.

Nagendra, for providing me with infinite knowledge about the measles virus and for introducing me to some fine Indian food.

Alan and Miles, for their never-ending teasing and friendship, and especially Alan for his tips about New Orleans. The trip would not have been half as good, and I may not have come back alive.

Heidi and Catherine, for their never-ending patience in teaching me the methods that I needed to successfully carry out my research.

Dr. Gayle Slaughter and the rest of the people with the SMART program, for giving me the opportunity to spend ten weeks in the beautiful city of Houston.

Dr. Carol Webb, for being patient with me during my first summer of research and not turning me off to the idea.

My mother and father, for providing the financial support that I so often needed these past four years, and most of all, for teaching me to read—no one has given me a better gift than that.

My sister, for her long years of enduring support, although the road has been rough through high school and college, I know we will never go our separate ways.

Dr. Addis, Fr. Harrington, Mr. Bugni, Dr. Westwell, Dr. Christenson, Dr. Smith and the rest of the Carroll College faculty, for the tremendous education I have received over the past four years, without which, I never would have the opportunity to do the research. And, also for the letters of recommendation which secured my spot with the SMART program.

Dr. Addis, I know I've already thanked him, but one is not enough for all the time and effort he has put forward on my behalf. I know that I owe much of my success to him.

Fr. Harrington, once again, for the much-needed guidance through these past four years and for serving as my Thesis advisor.

Dr. Tomas Graman, for serving as one of my readers and for teaching me more than just Spanish in SP101.
Mrs Goyen, for showing me that writing can be enjoyable

Mr. Simpson, Mr. Chalgren, Mr. Funk and the rest of my high school teachers for providing me with the best educational foundation I could have had in order to ensure survival and success in college

Andrew, for being my first good friend at Carroll College and my first lab partner, I doubt that I would have done as good without his support

Duane, for also being a good friend and study partner--I hope that it's off to Creighton for us both next year, and also for being a big part of the best 21 birthday somebody could ever hope for

All my other friends and study partners at Carroll, without them, these four years may not have been as bearable

Don and Lachelle, for making my eight weeks in Oklahoma City some of the best eight weeks of my life

Bob, my friend from high school who has supported me like no other friend--honestly and sincerely, and, also for all the great times we had golfing because, in truth, some of the best lessons of life can be learned with a putter in your hand

Brian, for being the best friend a guy could have and for showing me how its possible to be your best when the world has given you its worst, and also for opening my eye to the world of comics

Dan, for being the best skiing partner a guy could have and for the best summer I ever will have

And, I have saved the best for last, Rachelle, for the endless support and companionship over the last three years. While the road may have been rocky at times, I know that she will probably be my source of support and companionship for the years and years to come
ABSTRACT

Measles Virus (MV) Enders and MV Edmonston-Zagreb (EZ) were grown in both U937 cells and PBLs to study their dissemination. The presence of aggregation and formation of giant cells was observed in those cultures infected with MV EZ, while it was notably absent from cultures infected with the MV Enders. Believing this difference to be a possible link in the understanding of measles virus dissemination, further studies were conducted to test the idea that MV EZ may disseminate intracellularly, while MV Enders disseminates extracellularly. Gamma Gard was added to cultures to study its effects on the dissemination of the two viral strains. Results of this experiment indicated the spread of MV Enders can be completely inhibited by antibody to measles virus, while the spread of MV EZ cannot be. Also, the two vaccine strains were grown in PBLs to ensure that results of studies in U937s could be applied to the animal model. Differences (as compared to growth in U937s) resulted for MV Enders, but not MV EZ. Apparently, the MV Enders could not infect all cells in the cultures and was dying off. So, experiments were conducted whereby aggregation was artificially induced in PBLs infected with MV Enders to see if it could facilitate the dissemination of the virus to normal lymphocytes previously unavailable to infection. While the viral quantification plates were contaminated, the aggregation and giant cell assays showed that MV Enders may disseminated intracellularly if given the opportunity.
**LIST OF TABLES**

Table 1  Cell counts for Measles Virus growth in U937 cells

Table 2  Aggregation of U937 cells when infected with Measles Virus

Table 3  Giant cell formation in U937 cell infected with Measles Virus

Table 4  Aggregation of PBLs when infected with Measles Virus

Table 5  Giant cell formation in PBLS infected with Measles Virus

Table 6  Aggregation of U937 cells when infected with Measles Virus with the addition of Gamma Gard

Table 7  Giant cell formation in U937 cells infected with Measles virus with the addition of Gamma Gard

Table 8  Viral titers for MV Enders infected PBLs with the addition of supernatant from MV Edmonston-Zagreb infected U937s to induce aggregation

Table 9  Aggregation of PBLs when infected with MV Enders plus the addition of supernatant from MV Edmonston-Zagreb infected U937s to induce aggregation.

Table 10  Aggregation of PBLs when infected with MV Enders plus the addition of supernatant from MV AC705 infected PBLs to induce aggregation

Table 11  Giant cell formation in PBLs infected with MV Enders plus the addition of supernatant from MV AC705 infected PBLs to induce aggregation
LIST OF FIGURES

Figure 1  Growth curves for MV Enders and MV Edmonston-Zagreb in U937 cells

Figure 2  Growth curves for MV Enders and MV Edmonston-Zagreb in PBLs

Figure 3  Growth curves for MV Enders and MV Edmonston-Zagreb in U937s with the addition of Gamma Gard
The measles was first recognized in the 10th Century by Rhazes, but it was not until the 14th Century when John of Gaddesden named it "measles" (1,3). And, since then, the virus which causes measles has been placed into the Paramyxoviridae class of RNA viruses under the name of Morbillivirus.

The effects of the measles virus have been apparent during periodic epidemics. As early as 1657, an epidemic hit the United States in the town of Boston (1). And, even before that, epidemics were recorded in the new world in Santo Domingo, Mexico and Ecuador (1). More recently, epidemics occur in the United States every two to five years (1). The measles virus itself infects nearly four million people each year and, during epidemic years, it is responsible for the deaths of hundreds of people each month. Most of these deaths are among young children (1).

In 1954, Drs. John Enders and Thomas Peeples successfully isolated the measles virus in culture (1,4). Using human and simian renal cells, they succeeded in isolating eight strains which all had similar cytopathogenicity (4). Cross-neutralization and complement fixation tests for the eight strains showed no difference in antigenic determinants (4). They designated one of the strains as Edmonston (4).

Through multiple passages in renal cell cultures, a weakened attenuated vaccine was produced (5). This initial vaccine had a high rate of side effects. Seventy to eighty percent of children vaccinated with it developed a febrile response; the fever ranging up to 105°F. Also, a mild measles-like rash appeared in 50% of the children vaccinated (6). Later,
Schwartz passaged the Edmonston strains through chicken embryo cells 77 times to develop a more highly attenuated virus (6). With Schwartz's vaccine, only 3.6% of those vaccinated developed a fever (of up to 100.7° ) and two in 70 developed a mild rash (6). Also, the Schwartz vaccine caused antibody production in 97.1% of children treated with it (6).

The Ender's vaccine strains were most successful when given to children over 12 months of age, in which seroconversion occurred with a 95% rate of success (2,6,7,8). However, only a 50-75% success rate was reported in children vaccinated before 12 months (2,7,8). The persistence of maternal antibodies may be responsible for this failure of the Ender's vaccine strains in children vaccinated before 12 months of age (2,8).

In response to this problem, it may seem appropriate to wait until 12-15 months of age before vaccination. However, after 6 months of age, children become more susceptible to measles (7). Early experiments were conducted in which children were vaccinated at 6 months and then re-vaccinated at 15 months (7). But, only 49% of the those re-vaccinated responded by producing antibodies to the measles virus; in the other 51%, the re-vaccination had no effect (7).

In developed countries, such as the United States, the incidence of measles is low enough that vaccination can be avoided until the child is 15 months of age, but in Africa, 15-30% of the children become infected with measles before nine months of age (9). Thus, the problem of inadequate vaccines arises again. In answer to this problem, researchers developed the EZ vaccine strain. The EZ vaccine was derived from the original Edmonston-Enders strain by passaging it through human diploid cells 19 times (10). This EZ vaccine strain underwent clinical tests in many countries including Bangladesh, Gambia, Senegal and Guinea-Bissau.
The testing in Bangladesh involved comparing the EZ vaccine to the Schwartz vaccine when applied either by aerosol or subcutaneous injection (11). The vaccines were both used at a low titer, 5000 PFU for EZ and 6300 PFU for Schwartz (11). 5000 PFU is equivalent to 5000 virus particles in one milliliter. The test subjects were vaccinated between the ages of four and six months (11). Those vaccinated with the EZ by subcutaneous injection achieved a 62% rate of seroconversion, while those vaccinated with Schwartz reached a 37% rate of seroconversion (11). When vaccinated by aerosol, both vaccines were about equal with a 35% rate of seroconversion (11). Obviously, these low-titer vaccines did not prove effective enough. Therefore, researchers thought a higher titer vaccine may work better.

Trials using a higher titer of vaccine were carried out by H.C. Whittle and associates in Gambia and by Peter Aaby and associates in Guinea-Bissau (10,14). Both groups of researchers used high titer EZ vaccine (40,000 PFU) and standard Schwartz vaccine (6000 TCID50 units) for a comparison (10,13,14). When using the EZ vaccine at 40,000 PFU, the researchers achieved 100% seroconversion, while the Schwartz vaccine at 40,000 PFU given at 18 weeks of age, still showed a 54% failure rate (10). The EZ vaccine appears to be more resistant to antibodies (10), so perhaps it is able to resist maternal antibodies in the child and provide protection from further infections. This is something which the Schwartz vaccine is obviously incapable of doing. Alternate tests were run with other levels of vaccine; 40000, 20000 and 10000 PFU (10). As the level of vaccine fell, so did the rate of seroconversion, with a 75% success rate at
10000 PFU (10). Apparently, the EZ vaccine is able to overcome the effects of maternal antibodies which may be present, but only when the EZ vaccine has a high enough titer (14).

One problem with the above clinical studies is they were only conducted for 18 months. Whittle and his associates stated that the EZ vaccine was a safe and reliable answer to the problem of vaccination before 12 months of age (10,14). However, in 1988, the World Health Organization stated that Whittle's results were encouraging, but the studies are still incomplete (15). They did not expect complete safety, immunogenicity and efficacy data to be available for at least one or two years (15).

Later studies conducted in Senegal provided some basis for the WHO's concern. Experimenters used four groups--one received a high titer (5.4 log10 TCID50 units) EZ vaccine at five months, one a high titer (5.4 log10 TCID50 units) Schwartz vaccine at five months, one the standard Schwartz vaccine (3.7 log10 TCID50 units) at ten months and one no vaccine at all (12). The high titer EZ vaccine resulted in a death rate of 75/1000 children, while the high titer Schwartz vaccine resulted in a death rate of 48/1000 (12). The group receiving the standard vaccination had a death rate of 4/1000 (12). When compared to the death rate of the unvaccinated group, the death rate of the two high titer vaccines was notable--the vaccinated children had a lower survival rate than those children who went unvaccinated (12). Although the researchers did not pinpoint a cause, the wild-type measles virus is understood to have short and long-term effects on the immune system (2,12).

Obviously, there is no easy solution to the problem of measles virus
vaccination. One problem with the EZ vaccine may be it is not as attenuated as researchers have thought. Attenuation is achieved when the virulence of the virus has been lost or greatly reduced (16). The virulence of a virus is a measure of its pathogenicity (16). Later in this paper, differences will be shown between a known attenuated virus vaccine (Enders) and the EZ strain.

During the course of measles virus pathogenesis, the virus enters the human body through the respiratory tract (2). Eventually it will enter lymphocytes either free or associated with macrophages and make its way to the lymph nodes (2). The virus then multiplies and spreads to other lymph nodes and the spleen (2). Within six days the virus will spread to all epithelial surfaces including the oropharynx, conjunctiva, skin, respiratory tract, bladder and alimentary canal (2). This migration of the virus to the epithelial surfaces causes the symptoms associated with measles. The first symptoms appear within ten to twelve days (1). The characteristic rash appears on day 14, and one to four days before this rash, Koplik spots usually appear in the mouth (1). Koplik spots result from infection of the oral epithelium with the measles virus and are also indicative of the disease (1).

While the measles itself is not primarily debilitating, the virus causes suppression of the host's immune system which allows the victim to be affected by a variety of secondary bacterial infections unrelated to the virus itself (2). Among these are pneumonia, sinusitis, otitis media, bronchitis, bronchopneumonia, subacute scleritizing panencephalitis (SSPE) and encephalitis (1,2). Infections of the middle ear, such as otitis media, often lead to deafness (2). Also, the problem of encephalitis is a major
concern as it occurs in one in 1000 patients infected with the measles virus and is itself a highly debilitating condition (2). The SSPE is the worse secondary condition that can result from measles virus infections, However, since it only affects one in a million cases, the concern over it is not as great as that for encephalitis (2). This immunosuppression and the resulting secondary infections probably result from the virus's ability to infect leukocyte subpopulations and lymphoid organs (17).

The clinical diagnosis of the measles virus is fairly easy. The presence of multinucleated giant cells indicates the presence of the virus (2). The multinucleated giant cells result from the fusion of infected mononuclear cells (2). In order to accomplish this fusion, the measles virus uses the fusion (F) protein which is an integral part of its cell surface (2). The F protein induces changes in the cell membrane of neighboring cells which results in the integration of the two (2).

Therefore, it would seem the fusion protein of the virus is useless if the cells being infected are not in close enough contact to facilitate the fusion. Thus, for the virus to spread efficiently, the cells must be brought together. Virus-induced aggregation would provide one method to bring the cells together.

To study this idea, research has been done to test the role of the Leukocyte Function Antigen one complex (LFA-1) in induced aggregation (18). The LFA-1 complex is a member of a family of cell surface adhesion molecules referred to as integrins (19). The LFA-1 complex is a heterodimer composed of two chains, designated CD11a and CD18 (19). LFA-1 has been found to play a role in cell-to-cell interactions, including those which induce immune responses involving the adherence of activated leukocytes (19.) Also, from the results of recent studies, scientists have
postulated that the Human Immunodeficiency Virus (HIV) virus can alter LFA-1 expression, thereby causing more efficient dissemination (20).

Concerning the measles virus, several experiments were carried out to test the role of LFA-1 in aggregation. Leukocyte cultures infected with the measles virus exhibited notable aggregation 24-hours post-infection; and, within four days most of the aggregates contained at least one giant cell (18). Also, immune serum globulin added to the cultures failed to stop the aggregation, indicating that the leukocyte aggregation present in the MV-infected cultures was not due to activity of viral hemagglutinin or fusion coat glycoproteins (18). In a later experiment, infected cultures were supplemented with monoclonal antibodies to CD11a and CD18. While the control showed 75-100% aggregation of cells by day four, the culture with the antibodies to CD18 showed only 10-25% aggregation and the culture with the antibodies to CD11a showed less than 10% (18). Also, in the same experiment, viral titers in the infected cultures supplemented with antibodies to the LFA-1 components declined from the first day of infection. This indicates the growth and dissemination of the virus was also being stopped (18). These results could indicate that aggregation and viral dissemination are interrelated.

In another experiment, filtered supernatant from the MV-infected cultures was added to fresh, uninfected leukocytes. While not as extensive as that seen in MV-infected cultures, aggregation resulted (18). The results of this experiment may indicate the measles virus causes the release of a cytokine(s) or other molecule into the culture medium which induces aggregation. During the experiment, no giant cell formation was observed (18). However, this result could be expected, since the fusion protein present on the measles virus surface causes the fusion and resulting giant
cells associated with measles virus infection (2).

Finally, in order to study the changes in the LFA-1 complex during measles virus infection, the cells, both uninfected and infected, were treated with monoclonal antibodies to CD11a and CD18 and then stained with an affinity-purified fluorescein isothiocyanate-conjugated F(ab’)2 goat anti-mouse antibody and analyzed with an Epics Profile flow cytometer (18). The results showed the mean fluorescence intensities of the MV-infected cultures was twice that of the uninfected controls, indicating that the MV may induce conformational changes in the LFA-1 molecule (18) which could promote intercellular adhesion processes (21). The data obtained from the experiments explained above suggest that the following sequence of events may occur subsequent to MV inoculation of leukocytes: 1) leukocyte infection, 2) release of soluble factors, 3) functional and structural alteration of leukocyte LFA-1 heterodimers, 4) leukocyte aggregation and 5) leukocyte-to-leukocyte dissemination of the virus. Incomplete information about step five above, combined with the failures of the various measles virus vaccines explained earlier served as the starting point for further research. The start of that research is the subject of this paper.
The measles is not a conquered disease. Although vaccines exist, the virus still infects millions of people every year and some of those infected die. Therefore, the existing vaccines do not adequately solve the problem. For example, the MV Enders vaccine fails to promote seroconversion when given to children under 12 months of age. In the United States where the incidence of measles is low, children can afford to wait until 12-15 months before being vaccinated. However, in Africa, where measles is a significant cause of death for children under nine months, this presents a problem. Researchers hoped to answer this problem by using a high-titer EZ vaccine. Unfortunately, long-term studies of children receiving this vaccine indicated they were worse off than those who went unvaccinated. One question is: Was the EZ vaccine attenuated to a sufficient degree?

The clinical diagnosis for measles virus is the presence of multinucleated syncytia in the patient. When grown in culture during experiments in the laboratory, the MV EZ vaccine strain produced multinucleated giant cells—one possible sign that it may not be attenuated.

Also, current knowledge of the pathogenesis of measles virus is incomplete. Questions such as how the virus moves from cell-to-cell are still not completely understood. The bulk of the experiments to be presented in this paper concern this aspect of MV pathogenesis. And, one goal was to determine if any link between the measles virus' method of dissemination and the induced aggregation of culture cells exists. As has previously been shown, inhibition of cellular aggregation also inhibits the
dissemination of the virus. However, do all virus strains follow the same path for dissemination? Notable aggregation and giant cells were present in cells infected with the EZ vaccine strain, while minimal to no aggregation and no giant cells were present in the cultures infected with MV Enders. Yet, as will be demonstrated, both of the viruses were able to disseminate equally well in U937 cells.

Based upon the above ideas and observations, a hypothesis that the EZ vaccine strain spreads cell-to-cell and MV Enders spreads by lysis of the cell and subsequent release of free viral particles developed. The virus induces aggregation of the culture cells in order to facilitate fusion. Once fusion has occurred, the virus can spread from an infected cell to an uninfected cell. However, the Enders vaccine strain is thought to spread by lysis of the cell and the resultant free virus particles. MV Enders infects one cell, proliferates, causes lysis of the cell and releases free viral particles into the medium which then infect other cells. The work was designed to test this hypothesis.
MATERIALS

The research for this paper utilized the materials which are listed and described below. The specific use for each material will be covered in the section on methods.

Cell Culture: U937, a human monocytoid cell line, Hep2 (derived from human epithelial cells), Vero (African Green Monkey kidney cells) and PBLs (Peripheral Blood Leukocytes) were utilized in the experiments. The cells were obtained from the American Type Culture Collection (ATCC). The Hep2, Vero and PBL cells were cultured in Earle's Minimum Essential Medium supplemented with penicillin/streptomycin (100 U/ml), L-glutamine (4 U/ml), sodium bicarbonate (12U/ml) and 5% Fetal Calf Serum (FCS). The cells were incubated at 35°C in 5% CO2 incubator until they formed a confluent monolayer, then they were subcultured. The U937 cells were cultured in RPMI-1640 medium supplemented as above and with 10% FCS. The cells were subcultured at a density of approximately 5x10^5 cells/ml.

Viruses: These studies utilized MV Enders, MV Edmonston-Zagreb (EZ) and MV AC705. MV AC705 is a clinical isolate obtained from a throat culture of a patient in the acute phase of measles by Dr. Gail DemmLer, Department of Pediatrics, Baylor College of Medicine, Houston, Texas, while MV Enders and MV EZ are vaccine strains. A working stock of the virus was prepared by inoculating flasks of Vero cells and
incubating the flasks in a 5% CO2 incubator maintained at 35° C. When cytopathic effects (CPE) in these cultures were evident in 80 to 100% of the monolayers (5 to 7 days), the medium and the cells in each culture were collected, placed in a Branson sonicating bath and exposed to six intermittent 15 second bursts of sonication. The sonicated suspensions were pooled, centrifuged to remove cellular debris and filtered using 0.45 μm filters to remove any excess cellular debris which may not have centrifuged out. The filtered pools were apportioned, labeled and stored at -70° C. Vesticular Stomatitis Virus (VSV) was used during interferon assays because of its sensitivity in detecting interferon production.

**Gamma Gard:** Gamma Gard is a human antibody which shows anti-measles virus activity. The antibody acts by binding to receptors on the surface of the measles virus particle thereby neutralizing it.

**Preparation of Peripheral Blood Leukocytes (PBLs):** Whole blood was drawn from the antecubital vein into a heparinized syringe. The blood was diluted 1:2 with phosphate buffered saline (PBS) and then layered onto Lymphocyte Separation Media (LSM, Organon Tenika, Durham, NC) which was then centrifuged for 30 minutes to form gradients. The blood separated into three components--plasma, PBLs and red blood cells. The PBLs formed the middle layer of the gradient. They were removed and then washed with RPMI-1640 media 2X to remove the excess gradient and finally resuspended to the desired concentration of 1x10⁶ cells/ml in RPMI
1640 medium with 10\% FCS. The PBLs consist of macrophages, monocytes and lymphocytes.

METHODS:

The general technique used to study the dissemination of the measles virus is outlined below. Following the general technique are four sections describing important aspects of the process in more detail. After those sections there are three more subheadings: two of which describe variations on the general technique (Gamma Gard and Supernatant-induced effects) and one which details a separate assay used to study another aspect of MV pathogenesis.

General technique for dissemination experiments: The culture cell type (U937s or PBLs) was infected with the appropriate virus (below). On days zero, two, four and seven post-infection, 0.4 ml samples were removed from each suspension of virus-infected cells and assessed for cell viability and virus titer (pp 13-16). Leukocyte aggregation was determined by microscopic inspection of the culture (p 14).

A flask containing uninfected culture cells was utilized as a control.

Infection of cells: U937 and PBLs were infected in 15 ml V-bottom tubes using a multiplicity of infection (MOI) of 0.1. In other words, approximately 5.0 log 10 TCID50 (10,000) units of virus were added to
$1 \times 10^6$ pelleted cells—a 1:10 ratio of virus particles to cells. After a 90
minute incubation at 35° C with occasional mixing, the cells were washed
twice with MEM to remove any free virus particles and then resuspended to
a final concentration of $10^5$ cells/ml. The suspension was then transferred
to 25 cm$^2$ flasks and incubated at 35° C in a 5% CO$_2$ incubator for 7 days.

**Cell viability:** Total counts were made using a hemocytometer and
standard counting procedures. Viability was determined using trypan blue
dye exclusion.

**Aggregation assay:** To determine aggregation, an average of ten
microscopic fields were counted using 400X magnification. An aggregate
was defined as >3 cells attached to each other. In the PBL experiments,
some spontaneous aggregation was expected from the cells, while no
spontaneous aggregation was expected in the U937 cells. Aggregation was
graded in the following manner: + 25% aggregation, ++ 50%, +++ 75%
and ++++ 100%. +/- indicates minimal aggregation of about 5-10%.

**Viral quantification:** Two methods for determining viral titers were
utilized. They were Tissue Culture Infectious Dose (TCID$_{50}$) and plaque
assays.

**TCID$_{50}$:** Quantification of the virus was done in a 96-well tissue
culture plate using Vero cells and cytopathic effects (CPE) as an end point.
The plate was first loaded with 100 μl/well of 2% MEM blanks. Then, 50
μl of the culture to be quantified was added to each well in the first row.
Two rows were used for the quantification of each culture. Therefore, 100 μl of each culture to be quantified were required.

During our experiments, both cell suspension and cell-free fractions of the cultures were quantified. To separate those two different fraction, a 0.4 ml sample was removed from the original culture flask and centrifuged for 5 minutes. The required two aliquots of 50 μl of the culture were then removed from the top in order to obtain a cell-free sample. The remaining 0.3 ml was then vortexed to resuspend the cellular fractions and then used to obtain two aliquots of 50 μl of cell suspension.

Once the culture sample to be quantified had been added to the plate, a multi-channel pipettor was used to serially dilute it 1:3 down the plate (50 μl was transferred from the first well into the second well and mixed, then, the step was repeated between the second and third wells and so on down the plate). Each dilution corresponded to a 0.5 log 10 difference in concentration. After the dilution was completed, 100 μl of 2% MEM containing approximately 2x10^3 Vero cells was added to each well. The plates were then incubated for 5-7 days in the 35°C CO2 incubator and observed daily for CPE. Virus titer of the culture was obtained by using the following formula: (Number of wells showing CPE + 1) x 0.25 + 1.3. The resultant number gives the virus concentration in log 10 of the culture.

For example, a final number of 7.3 would indicate that 10^{7.3}/ml virus particles were present in the original culture at the time the sample was removed for quantification.

Tissue controls consisting of uninfected Vero cells containing no experimental sample were utilized on the plates to ensure proper reading of the plates.
Plaque Assays: A day or two before the plaque assay was run, the plaque plate was prepared. A suspension of Vero cells of approximately $2 \times 10^3$ density was prepared in 2% MEM. One ml of this suspension was added to each well of a 12-well plaque plate and allowed to incubate at $35^\circ$ C in a 5% CO2 incubator until monolayer 60-80% confluent was formed. Before infecting the wells, viral dilutions of the culture or viral pool to be tested had to be made. Usually $10^{-3}$, $10^{-4}$, $10^{-5}$ and $10^{-6}$ dilutions were tested. After the media was removed, the monolayer was infected with 100 μl of the dilutions to be tested. Following inoculation, the plate was incubated for 2 hours with repeated swirlings every 15 minutes to keep the monolayer from drying out. After incubation, the medium was removed from the wells and 1.0 ml of 0.5% Carboxymethyl Cellulose (CMC) overlay was added to each well. The 0.5% CMC overlay was prepared by mixing 12.7 ml 2% MEM, 0.9ml FCS, 0.6 ml L-Glutamine, 0.3 ml Penicillin/Streptomycin, 0.5 ml Sodium bicarbonate and 5.0 ml 2% CMC agar, which was then autoclaved and stored in the refrigerator until use. After adding the overlay, the plaque plates were incubated for 5-7 days until CPE were observed. The plates were then stained by adding 1.0 ml 0.5% crystal violet in 50:50 formalin:methanol to each well and allowing to sit for a day. The next day, the dye was rinsed out with water and virus concentrations in PFUs were determined.

The next two sections explain changes in the general technique for studying virus dissemination in order to investigate other areas of the process.
**Gamma Gard experiments:** Seventy-two hours post-infection, cell cultures were centrifuged, washed and counted. Then, 1.0 ml containing $10^5$ cells/ml of infected U937 or PBLs was added to 9.0 ml of $10^5$ cells/ml of uninfected U937 or PBLs. 0.1 ml of the proper dilution of Gamma Gard was then added to the cell suspension which was then transferred to 25 cm$^2$ flasks and incubated for seven days. On days zero, two, four and seven, 0.4 ml samples were removed from each suspension and assessed for cell viability, leukocyte aggregation and virus levels. The sample used to determine the virus titer in the cellular fraction was washed twice with media to remove any free Gamma Gard. Flasks with uninfected culture cells and virus-infected culture cells without Gamma Gard were used as controls to ensure proper growth of the virus and cells.

**Supernatant-induced aggregation experiments:** Prior to the experiment, supernatants from MV AC705-infected and MV EZ-infected cell cultures were removed from their respective flasks and filtered with a 0.2 μm filter. No infectious virus could be detected in these filtered supernatants by using Vero cells. The filtered supernatants were stored in the refrigerator. The purpose of the supernatants was to introduce a substance into the infected cultures which could induce aggregation of the culture cells in order to observe its effect on dissemination of the MV Enders virus strain (since MV Enders produced only minimal aggregation in cells its infected). Previous work in the lab had indicated the filtered supernatants contained an unknown molecular factor which would induce aggregation of the cells (18). Two approaches were tried for this experiment.
In the first experimental approach, 1.0 ml of supernatant from MV EZ-infected U937s was added to PBLs to induce aggregation of the cells. The PBLs were then infected with MV Enders and incubated. On days zero, two, four and seven, 0.4 ml samples were removed from each suspension and assessed for cell viability, leukocyte aggregation and virus levels. The supernatant used was also added to uninfected Vero cells and incubated to ensure it was virus-free. The production of CPE in this control would have indicated the presence of a virus. Other controls in this experiment included uninfected PBLs, mock-infected cells plus supernatant from a mock-infected culture, mock-infected cells plus supernatant from MV EZ-infected cells and MV Enders-infected cells plus supernatant from mock infected cultures. Mock-infected cells are cultures which have been treated exactly the same as infected cultures, but no virus is introduced into them.

In the second experimental approach, supernatant from MV AC705-infected PBLs was used to induce aggregation. Controls included uninfected PBLs, MV Enders-infected PBLs with no supernatant added, and MV Enders-infected PBLs with supernatant from a mock-infected culture. On days zero, two, four and seven of incubation, 0.4 ml samples were removed and assayed as above. One additional test flask included MV Enders-infected PBLs with MV AC705 supernatant and anti-CD11a antibody.

Interferon assay: The supernatants used to test for interferon were obtained by filtering the infected cell suspensions through 0.2 μm filters. These samples were then concentrated 0-10x before being used in the assay. For the assay, 50 μl of 5% MEM was added to each well of a 96
well tissue culture plate. Then, 50 μl of the sample to be assayed was added to each well of the first row. Two rows were used to assay each individual sample. Using the multi-channel pipettor, serial dilutions of 1:2 were made down the plate. Then, 100 μl of MEM containing approximately $2 \times 10^3$ Hep2 cells were added to each well. The plates were then incubated at 35° C overnight. The next day, the medium was aspirated off and 100 TCID50 units of VSV virus were added to each well. Observations were made 48-72 hours later. The absence of virus in a well indicated a positive test for interferon activity. Controls consisted of untreated Hep2 cells, Hep2 cells with VSV and no interferon, and Hep2 cells with VSV and stock interferon.
Results

When MV Enders and MV EZ were grown in U937 cells, they both portrayed the same general growth curve. The viral titers of both the cell suspension and cell-free supernatant started out at a low on day zero with MV Enders-infected U937s at 2.8 and <1.3 respectively and MV EZ-infected U937s at 3.3 and 1.8. The titers increased until day four when MV Enders-infected U937s equaled 5.05 and 4.55 respectively and MV EZ-infected U937s equaled 5.8 and 5.3, then after day four, the viral titers fell off. On day seven, the titers of MV Enders-infected U937s equaled 4.3 and 2.55 respectively and MV EZ-infected U937s equaled 4.8 and 4.05 (Figure 1 on p 21). As can be seen, the titer of the cell suspension was notably higher than the titer of the cell-free supernatant in both strains, until day four, when the titer of the cell-free supernatant almost caught up to the titer of the cell suspension. Low viability of the culture cells probably caused the decline in titers on day seven (Table 1).

Table 1: Cell Counts for measles virus growth in U937 cells. The cell counts indicate the number of viable cells in the entire culture.

<table>
<thead>
<tr>
<th>Culture</th>
<th>dO</th>
<th>d2</th>
<th>d4</th>
<th>d7</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>100000</td>
<td>155000</td>
<td>335000</td>
<td>145000</td>
</tr>
<tr>
<td>MV Enders</td>
<td>100000</td>
<td>70000</td>
<td>250000</td>
<td>10000</td>
</tr>
<tr>
<td>MV EZ</td>
<td>100000</td>
<td>155000</td>
<td>150000</td>
<td>15000</td>
</tr>
</tbody>
</table>
Figure 1: Growth curves for MV Enders and MV Edmonston-Zagreb in U937 cells. Cell suspension is the cell-associated fraction and supernatant is the cell-free fraction of the culture. Titer levels below 1.3 are undetectable.
While the growth curves for the two virus strains were similar, the presence of aggregation and giant cells was notably different. MV EZ-infected U937s showed marked aggregation by day two with +/++, which increased to ++++ on day four. MV Enders-infected U937s, however, exhibited only minimal aggregation (+/-) by day four with none being seen on day two (Table 2).

Table 2: Aggregation of U937 cells when infected with measles virus. (-) indicates the absence of aggregation and NA indicates no applicable.

<table>
<thead>
<tr>
<th>Time</th>
<th>Culture</th>
<th>d0</th>
<th>d2</th>
<th>d4</th>
<th>d7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>NA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>MV Enders</td>
<td>NA</td>
<td>-</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td></td>
<td>MV EZ</td>
<td>NA</td>
<td>+/++</td>
<td>++++</td>
<td>+</td>
</tr>
</tbody>
</table>

As for giant cells, only the MV EZ-infected U937s showed any giant cell formation which reached a level of +++ by day four and was obvious as early as day two. MV Enders-infected U937s, on the other hand, showed no giant cell formation throughout the seven day incubation (Table 3 on p 23). The uninfected U937 control flask showed neither giant cells nor aggregation during the experiment.

When the MV Enders and MV EZ strains were grown in human PBLs, there was a notable difference in growth curves. The titer of the cell suspension of the MV EZ-infected PBLs started out at a low on day zero of 3.3, increased to 5.3 by day four and fell off to 4.55 on day seven. The titers of the cell-free supernatant started out undetectable and gradually increased to 3.3 by day seven. While the MV EZ growth curve followed
### Table 3: Giant cell formation in U937 cells infected with the measles virus.

(-) indicates the absence of giant cells and NA indicates not applicable.

<table>
<thead>
<tr>
<th>Culture</th>
<th>d0</th>
<th>d2</th>
<th>d4</th>
<th>d7</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>NA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MV Enders</td>
<td>NA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MV EZ</td>
<td>NA</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
</tbody>
</table>

### Table 4: Aggregation of PBLs when infected with measles virus.

(-) indicates no aggregation and NA indicates not applicable.

<table>
<thead>
<tr>
<th>Culture</th>
<th>d0</th>
<th>d2</th>
<th>d4</th>
<th>d7</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>NA</td>
<td>+/-</td>
<td>+/-</td>
<td>-</td>
</tr>
<tr>
<td>MV Enders</td>
<td>NA</td>
<td>+/-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>MV EZ</td>
<td>NA</td>
<td>++</td>
<td>+++</td>
<td>+/-</td>
</tr>
</tbody>
</table>

### Table 5: Giant cell formation in PBLs infected with the measles virus.

(-) indicates the absence of giant cells and NA indicates not applicable.

<table>
<thead>
<tr>
<th>Culture</th>
<th>d0</th>
<th>d2</th>
<th>d4</th>
<th>d7</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>NA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MV Enders</td>
<td>NA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MV EZ</td>
<td>NA</td>
<td>-</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>
the same lines when grown in PBLs as when grown in U937s, the MV Enders was notably different. The titer of the cell suspension of MV Enders-infected PBLs started out at 2.55 and gradually fell off until it was undetectable by day seven. The titer of the cell-free supernatant started out at 2.55 also and was undetectable by day two (Figure 2).

There was also a marked difference in aggregation between the two strains. MV Enders-infected PBLs showed minimal (+/-) aggregation on day two which increased to + on day four. The MV EZ-infected PBLs exhibited marked aggregation on day two (++) which increased to +++ on day four (Table 4 on p 23).

In the case of giant cells, MV Enders-infected PBLs once again showed none through the course of the incubation, while the presence of giant cells was obvious in the MV EZ-infected PBLs by day four (++) (Table 5 on p 23). The uninfected PBL control showed +/- aggregation on days two and four, and no giant cells throughout the seven days.

During the experiments in which Gamma Gard was added to the culture flasks to determine its effect on dissemination of the virus, three controls were used--uninfected U937 cells, U937s infected with MV Enders but with no Gamma Gard added, and U937s infected with MV EZ with no Gamma Gard added. The titers of the two MV-infected controls changed in a fashion similar to that observed in Figure 1.

The titers of the cell-free supernatants of the infected cultures of both strains with the addition of Gamma Gard were undetectable during all seven days. The titer of the MV EZ-infected U937s plus Gamma Gard started out at 3.55 and steadily decreased until it was undetectable by day seven. The titer of the MV Enders-infected U937s plus Gamma Gard started out at 4.05 and was undetectable by day two (Figure 3).
Figure 2: Growth curves for MV Enders and MV Edmonston-Zagreb in PBLs. Cell suspension is the cell-associated fraction and supernatant is the cell-free fraction of the culture. Titer levels below 1.3 are undetectable.
Figure 3: Growth curves for MV Enders and MV Edmonston-Zagreb in U937s with the addition of Gamma Gard. Cell suspension is the cell-associated fraction of the culture and supernatant is the cell-free fraction of the culture. Titer levels below 1.3 are undetectable.
The difference in aggregation between the MV EZ-infected and MV Enders-infected U937 controls and the MV EZ-infected and MV Enders-infected U937s plus Gamma Gard was marked. The MV Enders-infected U937 control with no Gamma Gard showed + aggregation by day two increasing to +/++ aggregation by day 4, while the MV Enders-infected U937s with Gamma Gard showed only minimal aggregation by day two (+/-) and + aggregation by day four. The MV EZ-infected U937 control with no Gamma Gard showed +++ aggregation by day two and +/- aggregation for the remainder of the experiment. However, the MV EZ-infected U937s with Gamma Gard showed only minimal aggregation (+/-) on day two and no aggregation on day four (Table 6).

Table 6: Aggregation of U937 cells when infected with measles virus. +y indicates the presence of Gamma Gard. (-) indicates the absence of aggregation.

<table>
<thead>
<tr>
<th>Culture</th>
<th>d0</th>
<th>d2</th>
<th>d4</th>
<th>d7</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>NA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MV Enders</td>
<td>NA</td>
<td>+</td>
<td>+/++</td>
<td>+</td>
</tr>
<tr>
<td>MV EZ</td>
<td>NA</td>
<td>+/-</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>MV Enders + γ</td>
<td>NA</td>
<td>+++</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>MV EZ + γ</td>
<td>NA</td>
<td>+/-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

The results of the MV Enders-infected and MV EZ-infected controls presented in Table 6 vary from those shown in Table 2, but this difference can be attributable to the fact that the cultures are living cells and sometimes subject to variations in growth from experiment to experiment.
The formation of giant cells was present only in the MV EZ-infected U937 control with no Gamma Gard. It showed +/++ giant cell formation by day two and +/- giant cell formation for the remainder of the experiment. None of the other cultures, including the MV EZ infected U937s with Gamma Gard, showed any giant cell formation (Table 7).

Table 7: Giant cell formation in U937 cells infected with the measles virus with the addition of Gamma Gard. +γ indicates the presence of Gamma Gard. (-) indicates the absence of giant cells.

<table>
<thead>
<tr>
<th>Culture</th>
<th>Time</th>
<th>Control</th>
<th>MV Enders</th>
<th>MV EZ</th>
<th>MV Enders + γ</th>
<th>MV EZ + γ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>d2</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>d4</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>d7</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

In the experiment in which supernatant from MV EZ-infected U937 cultures was used to induce aggregation in MV Enders-infected PBLs, the viral titers on day two were unattainable due to bacterial contamination of the TCID50 plates. However, on days zero, four and seven, they equaled 4.3, 4.55 and 4.05 respectively for the MV Enders-infected PBLs with mock supernatant. The titer for the MV Enders-infected PBLs with EZ supernatant equaled 4.8, 3.55 and 2.8 respectively (Table 8 on p 29).

Although day two's viral quantification assays were contaminated, the aggregation and giant cells assays were observable during the entire
experiment. The MV Enders-infected PBLs with MV EZ supernatant added had +/++ aggregation by day two and ++ aggregation by day four. However, both the mock-infected PBLs with MV EZ supernatant and the MV Enders-infected PBLs with mock supernatant (controls) showed + aggregation by day two and +/++ aggregation by day four. This indicates an approximate half step below the tested culture. The uninfected PBL controls showed +/- aggregation by day two and no further increased was seen (Table 9 on p 30). No giant cell formation was seen in any culture flask throughout the experiment.

Table 8: Viral titers for MV Enders-infected PBLs with the addition of supernatant from MV EZ-infected U937s to induce aggregation. Titers are in log 10/ml of culture and only reflect the titer of the cell suspension. (-) indicates no virus.

<table>
<thead>
<tr>
<th>Culture</th>
<th>d0</th>
<th>d2</th>
<th>d4</th>
<th>d7</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>mock infected plus EZ supernatant</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MV Enders infected plus mock supernatant</td>
<td>4.3</td>
<td>contam</td>
<td>4.55</td>
<td>4.05</td>
</tr>
<tr>
<td>MV Enders infected plus EZ supernatant</td>
<td>4.8</td>
<td>contam</td>
<td>3.55</td>
<td>2.8</td>
</tr>
</tbody>
</table>

The second experimental approach which utilized supernatant from MV AC705-infected PBLs used different controls (described in the Methods section on p 18). All of the TCID50 plates were contaminated and were unable to be ascertained for viral titer levels. However, the results of the aggregation and giant cell assays were as follow: The culture with MV Enders-infected PBLs and MV AC705 supernatant showed ++ aggregation
by day two which then continued through the experiment. The MV Enders-infected PBLs with mock supernatant control showed +/- to + aggregation on day two. This aggregation then dropped to +/- on day four and rose to + on day seven. The MV Enders-infected PBLs with no supernatant control showed + aggregation on day two which fell to +/- on day four and increased to +/- on day seven. The uninfected PBL control showed +/- to + aggregation on days two and four and increased to + aggregation on day seven. The flask with the MV Enders infected PBLs plus MV AC705 supernatant and anti-CD11a showed + aggregation on day two which decreased to +/- aggregation on days four and seven (Table 10 on p 31). Only one flask showed giant cell formation. The MV Enders infected PBLs with MV AC705 supernatant showed +/- giant cell formation on day seven (Table 11 on p 31).

Table 9: Aggregation of PBLs infected with MV Enders plus the addition of supernatant from MV EZ-infected U937s to induce aggregation.

<table>
<thead>
<tr>
<th>Culture</th>
<th>d0</th>
<th>d2</th>
<th>d4</th>
<th>d7</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>NA</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>mock infected plus EZ supernatant</td>
<td>NA</td>
<td>+</td>
<td>+/-</td>
<td>++</td>
</tr>
<tr>
<td>MV Enders infected plus mock supernatant</td>
<td>NA</td>
<td>+</td>
<td>+/++</td>
<td>+/++</td>
</tr>
<tr>
<td>MV Enders infected plus EZ supernatant</td>
<td>NA</td>
<td>+/-</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

Although several assays for interferon were carried out, the exact method (concentrating of supernatant, etc.) varied between each assay, and no reliable results were obtained. However, the assays did show the
presence of interferon production induced by both MV EZ and MV Enders.

<table>
<thead>
<tr>
<th>Culture</th>
<th>Control</th>
<th>d0</th>
<th>d2</th>
<th>d4</th>
<th>d7</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBLs + MV Enders</td>
<td>NA</td>
<td>+/- to</td>
<td>+/ -</td>
<td>+/-</td>
<td></td>
</tr>
<tr>
<td>PBLs + MV Enders + mock supernatant</td>
<td>NA</td>
<td>+</td>
<td>+/-</td>
<td>+/++</td>
<td></td>
</tr>
<tr>
<td>PBLs + MV Enders + MV AC705 supernatant</td>
<td>NA</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>PBLs + MV Enders + MV AC705 supernatant + anti-CD11a</td>
<td>NA</td>
<td>+</td>
<td>+/-</td>
<td>+/-</td>
<td></td>
</tr>
</tbody>
</table>

Table 10: Aggregation of PBLs infected with MV Enders plus the addition of supernatant from MV AC705-infected PBLs to induce aggregation.

<table>
<thead>
<tr>
<th>Culture</th>
<th>Control</th>
<th>d0</th>
<th>d2</th>
<th>d4</th>
<th>d7</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBLs + MV Enders</td>
<td>NA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PBLs + MV Enders + mock supernatant</td>
<td>NA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PBLs + MV Enders + MV AC705 supernatant</td>
<td>NA</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td>-</td>
</tr>
<tr>
<td>PBLs + MV Enders + MV AC705 supernatant + anti-CD11a</td>
<td>NA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 11: Giant cell formation in PBLs infected with MV Enders plus the addition of supernatant from MV AC705-infected PBLs to induce aggregation.

31
Discussion

One of the important results of the experiments is the finding that there is a difference in aggregation between cultures infected with MV EZ and those infected with MV Enders. The cause of the induced aggregation, whether it be a virally-induced cytokine or other virally-produced molecule, is yet unknown (18). The cause of the giant cell formation is the fusion (F) protein present on the viral surface (2). For the fusion to occur, infected and uninfected cells must be in contact. Thus, because MV Enders induced minimal to no aggregation, giant cells could not be formed. But, in the TCID50 plate, where cell contact was caused through the formation of a monolayer, syncitia were present in MV Ender-infected cells, indicating the presence of the F protein.

The contrasts in aggregation between the two strains were interpreted as a possible difference in the method of dissemination between the two viral strains. The working hypothesis, once again, is that the Edmonston-Zagreb strains spreads intracellularly, while the MV Enders strains spreads extracellularly.

If a virus spreads intracellularly, it will rarely leave the host's cells and enter the body fluids where it could be recognized and neutralized by the immune system. Thus, it is protected from the human body's first line of defense. In contrast, a virus which causes lysis and becomes free would be more easily detected and neutralized by the immune system.

If the hypothesis is correct, MV Ender's dissemination could be blocked in culture if an antibody to measles virus were added. MV Edmonston-Zagreb, however, should still be able to spread due to its
protected intracellular position. To test this fact, the Gamma Gard experiment was carried out. The results indicate MV Enders dissemination was completely inhibited from the beginning. However, the dissemination of the MV Edmonston-Zagreb was only partially inhibited. However, according to our hypothesis, Gamma Gard should not inhibit MV Edmonston-Zagreb at all. Therefore, it is possible that too high a concentration of Gamma Gard was used. Alternatively, MV Edmonston-Zagreb does not spread exclusively in an intracellular fashion. This experiment should be repeated with a lower concentration of Gamma Gard.

Although results from tissue culture provide useful information, they need to be applied to the human model. Therefore, PBLs were infected with the two measles virus vaccine strains and observed. The homotypic aggregation present in the controls indicated that PBLs aggregate spontaneously. However, the aggregation present in the MV Edmonston-Zagreb-infected cultures is significantly higher than the aggregation present in the MV Enders-infected cultures. The growth curve for PBLs infected with MV Edmonston-Zagreb paralleled the growth curve for U937s. This fact suggests the Edmonston-Zagreb strain may behave in PBLs as it does in U937s. However, the growth curve of the MV Enders-infected PBLs behaved differently from that observed in the U937s. This difference is expanded upon in the next four paragraphs.

In contrast to the U937s, a homogenous cell line, PBLs are heterogenous, composed of macrophages, monocytes and lymphocytes. Only macrophages, monocytes and activated lymphocytes carry a measles virus receptor, and thus, are capable of being infected. Unactivated lymphocytes, which comprise 75% of the PBL culture, lack the receptor and cannot be infected.
Any virus which spreads by lysing its host cell and releasing free viral particles would only be able to disseminate into the macrophages and monocytes. The unactivated lymphocytes would be free from infection. Once the virus has infected all the macrophages and monocytes (about 25% of the culture), its levels would begin to decrease due to a lack of host cells. This decrease is due to the fact that the measles virus particles degrade when they are free in the media and not associated with a host cell.

A virus which disseminated intracellularly could bypass the viral receptors. If it infected a cell and then fused to an uninfected cell, viral particles could be passed to the second cell whether or not a measles virus receptor was present. Therefore, this type of dissemination would allow the virus to spread to all of the PBLs, which would then result in a growth pattern similar to that seen with U937s. Those ideas may help explain why MV Edmonston-Zagreb titers remained normal when the virus grew in PBLs (as compared to results in U937s), while the MV Enders titers fell as the experiment progressed.

However, since MV Enders has a functional F protein, we hypothesized that MV Enders could be made to disseminate intracellularly if some outside factor made the culture cells aggregate. A normal growth curve (when compared with growth in U937s) for the MV Enders in PBLs could support the above idea and in turn, support the idea that MV Enders normally spreads through the lysis and further cellular infection by free particles.

In order to study this idea, two separate experimental approaches which utilized supernatants from MV EZ or MV AC705-infected cultures to induce aggregation were carried out. Those experiments would indicate that MV Enders is fully capable of spreading to the leukocyte population of
PBLs when it has the opportunity to spread cell-to-cell.

The high levels of aggregation present in the control flask using supernatant from a mock-infected culture seemed incorrect. Also, the supernatant from the mock-infected culture should not have caused the high titers present on days four and seven. One possible explanation is supernatants from U937 cells caused unwanted activation of the leukocytes in the PBL cultures. Perhaps, U937 cells secrete a molecule capable of activating PBLs. And, once the lymphocytes are activated, the receptor is expressed which then allows the MV Enders to spread to cells it normally could not have.

Two problems existed with the set-up of the first approach to the experiment. First, MV Enders-infected PBLs with no supernatants added should have been used as one control to ensure that the growth curve during the experiment was the same as the growth curve for MV Enders-infected PBLs observed earlier. Also, using supernatants from infected U937 cells may have introduced some new extracellular component not normally produced by the PBLs which could have induced the activation of the lymphocytes. To remedy the above two problems, the second approach to the experiment used supernatants from MV AC705-infected PBL cultures to induce the aggregation.

This second experiment failed due to bacterial contamination of all virus quantification assays. However, the results of aggregation and giant cell assays were still obtainable; and, they fit out hypothesis. Control groups without any substance added to induce aggregation showed none. However, the cultures with supernatant for MV AC705-infected cultures showed aggregation and minimal production of giant cells. Therefore, since the supernatant alone cannot induce giant cell formation (18), the
induced aggregation must have been allowing the F protein of MV Enders to work. But, in order to determine that viral dissemination was accomplished, the experiment must be repeated.

The results of the research indicated a difference between the methods of dissemination employed by the two vaccine strains, Enders and Edmonston-Zagreb, and provided a basis for further research into the problems with them. The results obtained above indicated MV Edmonston-Zagreb may spread intracellularly, while MV Enders may spread extracellularly. Further work is needed to expand upon the results. Whether or not this line of research leads to an improved vaccine strains and better immunization of children remains to be seen.
Bibliography


