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Measles Virus-Induced Changes in Leukocyte Function Antigen 1 Expression and Leukocyte Aggregation: Possible Role in Measles Virus Pathogenesis

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Measles virus (MV) infection of U937 cell or peripheral blood leukocyte cultures was shown to induce changes in the expression of leukocyte function antigen 1 (LFA-1) and cause marked aggregation of these cells. Addition of selected monoclonal antibodies specific for LFA-1 epitopes that did not neutralize MV in standard neutralization assays were found to block both virus-induced leukocyte aggregation and virus dissemination. These data suggest that MV modulation of LFA-1 expression on leukocytes may be an important step in MV pathogenesis.

Measles virus (MV)-leukocyte interactions appear to play a prominent role in the pathogenesis of MV. The marked immunosuppression and secondary infections associated with MV infections are probably a result of this virus's ability to infect leukocyte subpopulations and lymphoid organs (4, 5, 13, 19, 21, 23). In addition, infected leukocytes probably provide the major means by which MV translocates from the respiratory tract (2, 4) to distant organs, where the virus can cause an array of debilitating disorders, including those involving the pancreas, the liver, and the immune and central nervous systems (7, 8, 12–15, 23).

Integrins constitute a family of cell surface adhesion molecules that are involved in a number of important biological responses (16). One integrin, leukocyte function antigen 1 (LFA-1), is a heterodimer composed of two chains, designated CD11a and CD18 (16). LFA-1 has been shown to be important in mediating diverse heterotypic and homotypic cell-cell interactions, including those responsible for the induction of cognate and noncognate immune responses involving or requiring adherence and transendothelial migration of activated leukocytes and localization of leukocytes into various somatic tissues (16, 20). Because many of the functions mediated by LFA-1 are essential for the development of a robust host immune response, virus-induced modulation of LFA-1 activity could effectively alter the course of disease. In fact, recent studies using human immunodeficiency virus (HIV) suggest that HIV can modulate LFA-1 expression, possibly fostering the dissemination of this virus (6, 18).

In this study, we examined MV interactions with U937 human monocytoid cells and purified peripheral blood leukocytes (PBL). Evidence is presented that infection of these leukocytes with MV leads to altered LFA-1 expression and increased cell-cell interactions, resulting in efficient dissemination of virus among these leukocytes.

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MV infection and leukocyte aggregation. Two minimally passaged clinical isolates and three multiply passaged live attenuated MV strains (Enders, Edmonston, and Edmonston-Zagreb) were tested in human U937 monocytoid cells (ATCC CRL1593) or mononuclear leukocytes separated from peripheral blood on Ficoll-Hypaque gradients (1) for their ability to grow in these cells. To infect the leukocytes, each MV strain was added to pelleted cells (400 × *g*) at a multiplicity of infection (MOI) of 0.1. After a 90-min adsorption at 35°C with occasional mixing, the cells were washed twice with RPMI 1640 medium (GIBCO) supplemented with 2% fetal calf serum, penicillin (100 U/ml), streptomycin (100 μg/ml), and sodium bicarbonate (0.2%). The washed leukocytes were resuspended in 10 ml of RPMI 1640 medium at a concentration of 10^5 cells per ml and added to 25-cm^2 tissue culture flasks (Falcon 30133). These flasks were incubated upright in a 35°C, 5% CO_2 incubator for 7 days. Samples of 0.2 ml were removed from each suspension on days 0, 2, 4, and 7 and centrifuged at 400 × *g*. Each of the resulting supernatants and cellular fractions was assayed for virus by serially diluting the sample in 0.5 50% tissue culture infectious dose ($TCID_{50}$) increments in 96-well tissue culture plates (Falcon 3072) with 2% fetal calf serum–RPMI 1640. Approximately 2×10^3 Vero cells (ATCC CCL81) in 100 μl of minimal essential medium were then added to each well. All plates were incubated for 7 days at 35°C and observed daily for syncytia.

All of the MVs tested grew to $>10^4$ $TCID_{50}$ per ml in cultures of U937 cells. Determination of titers of both the cell-free supernatant and leukocyte fractions resulting from centrifugation of the samples taken from these cultures indicated that regardless of the time interval assayed, ≥90% of the infectious virus in infected leukocyte cultures remained leukocyte associated and was not readily released into the medium (data not shown).

The most consistent and striking feature observed following MV infection of either U937 cells or PBL was a conspicuous aggregation of the cells in the infected cultures. This

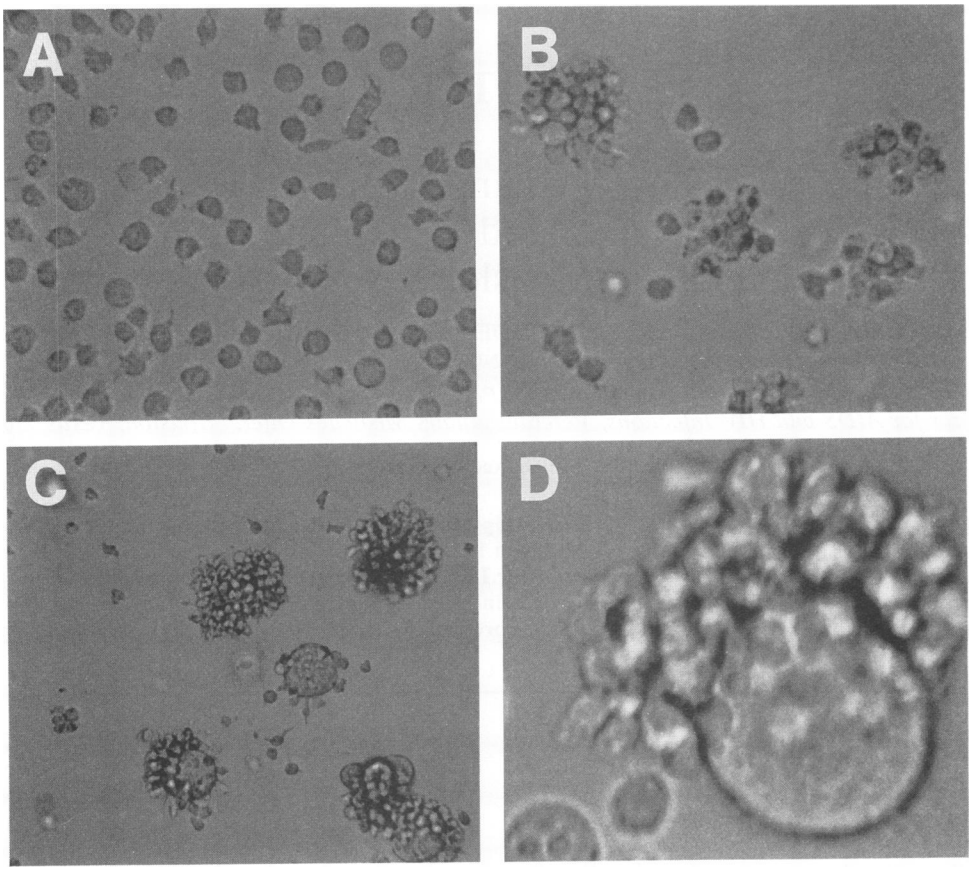

FIG. 1. MV-induced aggregation of U937 cells. (A) Uninfected U937 cells (magnification, ×200). Note the single-cell suspension and absence of any aggregation. (B) U937 cells 24 h after infection with MV (magnification, ×200). Note the presence of small aggregates and absence of giant cells. (C) U937 cells 72 h after infection with MV (magnification, ×100). Note the presence of large aggregates with giant cells. (D) U937 cells 72 h after infection with MV (magnification, ×1,000).

aggregation was semiquantified by using the method of Kansas and Tedder (9), with the exception that the values obtained were expressed as percentages of cells aggregated. In these assays, 10 microscopic fields of each suspension were observed with a 40× objective. An aggregate was defined as more than three attached cells. In uninfected cultures, such aggregates were rare (Fig. 1A).

Aggregation was first discernible 24 h after virus inoculation (Fig. 1B) and was most evident on day 4, when numerous, very large aggregates (≥20 cells) were evident (Fig. 1C) and MV titers were maximal. By day 4, most aggregates contained at least one giant cell (Fig. 1C and D). These giant cells exhibited marked cytoplasmic fluorescence and numerous nuclei following indirect immunofluorescence staining (3) with MV-specific antisera (Whittaker Bioproducts; catalog no. 30-206N). In contrast, virtually no aggregation or MV-specific fluorescence was observed in uninfected or mock-infected leukocyte cultures, in leukocyte cultures infected with parainfluenza virus type 3, or in cultures exposed to MV inactivated with UV light (22) (data not shown). Moreover, aggregation was not significantly reduced when immune human serum globulin (Armour Pharmaceuticals Co.; lot 17-305) was present in the MV-infected leukocyte cultures at final hemagglutination inhibition and fusion inhibition titers of >20/0.05 ml and final MV-neutralizing titers of >100/0.05 ml. These data suggest that the leukocyte aggregation seen in these MV-inoculated cultures was not due to the activity of viral hemagglutinin or fusion coat glycoproteins. In these and all subsequent experiments, the antibodies were added within minutes of the addition of the infected leukocytes.

Effects of anti-integrin MAbs on leukocyte aggregation. In

TABLE 1. Differential inhibition of measles virus-induced aggregation of U937 cells by different antibodies[a]

Antibody	% of cells aggregated on day[b]:		
	2	4	7
None	50–75	75–100	25–50
M1020 (anti-CD11a)	<10	<10	<10
BD7950 (anti-MAb CD18)	10–25	10–25	<10
R7.1 (anti-CD11a)	50–75	75–100	25–50
BD7328 (anti-CD4)	50–75	75–100	25–50
Immune serum (Armour)	50–75	75–100	25–50

[a] U937 cells infected for 72 h with a clinical MV isolate (AC705) were added to cultures of uninfected U937 cells. Cultures were observed for 7 days.

[b] An aggregate was defined as more than three attached cells. Numbers indicate percentage of cells in each culture in aggregates. The aggregation seen on day 0 (10 to 25% for all samples) was due to the addition of aggregated MV-infected cells to culture; on day 7, cell viability was markedly decreased and aggregation was less evident.

contrast to the marked aggregation seen in MV-infected cultures of leukocytes containing immune serum with high levels of MV-specific antibodies (Table 1), virtually no leukocyte aggregation was observed during the 7-day observation period in MV-infected cultures containing monoclonal antibody (MAb) M1020 (≥20 μg of total protein per ml; Sanbio Corp., Uden, The Netherlands; catalog no. M1020), a MAb that reacts with an epitope on the CD11a chain of LFA-1 (10). Leukocyte aggregation in MV-infected cells was also significantly inhibited by MAb BD7950 (Becton Dickinson; catalog no. 7950), a MAb specific for an epitope on the CD18 chain of LFA-1, at a final concentration of 0.07 μg/ml. However, not all antibodies reactive with LFA-1 epitopes suppressed leukocyte aggregation. No significant reduction of aggregation was observed in MV-infected leukocytes with MAb R7.1 at final concentrations of up to 100 μg/ml. This MAb is specific for a different epitope of CD11a than is M1020 (18). (R7.1 was obtained from C. Wayne Smith, Department of Pediatrics, Baylor College of Medicine.) Reduced aggregation was also not evident in control cultures containing a 20-μg/ml final concentration of BD7328, a MAb specific for a CD4 epitope (Becton Dickinson; catalog no. 7328).

Effects of anti-integrin MAbs on MV growth and dissemination. The effects of MAbs specific for integrin epitopes on MV growth and dissemination were also studied. Dissemination experiments were initiated by adding 1 ml (10^5 cells) of U937 cells or PBL infected with MV for 72 h to 9 ml of uninfected U937 cells or PBL (10^5 cells per ml). Various concentrations of the appropriate antibody were then added to the suspensions, and the leukocyte-antibody mixtures were transferred to a 35°C, 5% CO_2 incubator for 7 days. The suspensions were observed daily for aggregation. In addition, on days 0, 2, 4, and 7, a 0.2-ml sample was removed from each suspension and centrifuged (400 × *g*). The virus titer of each resulting supernatant and leukocyte fraction was determined.

We found that the same antibodies that inhibited aggregation of MV-inoculated leukocytes also inhibited growth and dissemination of MV in both U937 cell and PBL cultures. Indeed, suppression of aggregation correlated with virus growth inhibition. Thus, as indicated in Fig. 2A and 3, virus was virtually eliminated from U937 cell and PBL cultures containing a 20-μg/ml final concentration of M1020, the MAb with the most marked antiaggregation effect (Table 1). Virus was only partially inhibited (reductions of 1 to >2 $\log_{10}$ units in virus titers compared with virus titers in the control cultures) in cultures containing MAb BD7950 (Fig. 2B), the CD18-specific MAb that was only partially effective in inhibiting MV-induced leukocyte aggregation. Furthermore, no inhibition of MV replication and dissemination was evident in cultures containing either of the two MAbs that did not block leukocyte aggregation (i.e., MAb R7.1 and the anti-CD4 MAb, BD7328; data not shown). The inhibition of MV growth and dissemination observed with either MAb M1020 or BD7950 was not apparently due to direct interaction of either MAb with MV, since neither MAb had any detectable MV-inhibiting activity when tested at dilutions of ≥1:4 in neutralization assays utilizing Vero cells.

Medium from virus-leukocyte cultures induces aggregation. To determine whether the observed aggregation was due to a factor released into the culture medium following infection, culture fluids from mock-infected and virus-infected U937 cells were collected 72 to 96 h after MV inoculation, centrifuged to remove cellular debris, and passaged through a 0.2-μm-pore-size filter (Acrodisc; catalog no. 4192). No

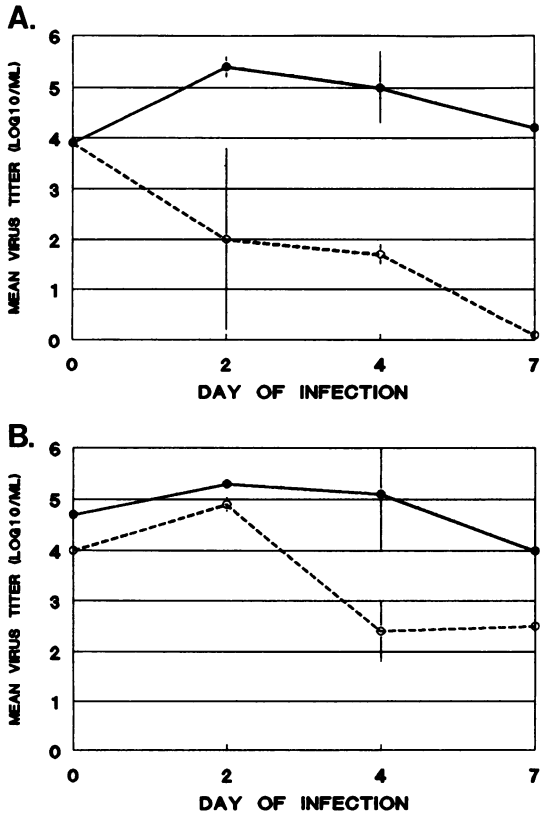

FIG. 2. (A) Levels of MV in cultures of U937 cells containing MAb M1020 specific for CD11a (LFA-1) epitopes (20 μg of protein per ml, final concentration; dashed line and in control cultures lacking this MAb (solid line). (B) Levels of MV in cultures of U937 cells containing MAb BD7950 specific for CD18 (LFA-1) epitopes (0.07 μg of antibody per ml; dashed line) and in control cultures lacking this MAb (solid line). Data are from two experiments; markers indicate geometric mean virus titers obtained in duplicate experiments; vertical lines indicate standard deviations of the mean. Mean virus titers in cultures containing antibodies and control cultures were significantly different on days 4 and 7 ($P < 0.01$ by Student's *t* test).

infectious virus could be detected in these filtered supernatants by using Vero cells and a 96-well microassay (24). Nonetheless, addition of the filtered supernatants to fresh U937 cells induced discernible aggregation. This aggregation was less extensive than that seen in MV-infected cultures, in that fewer aggregates and smaller numbers of cells (i.e., 4 to 10) per aggregate were evident in these cultures. Moreover, in contrast to MV-infected cultures, no giant cells were seen (data not shown). No aggregation was seen when similarly treated medium from mock-infected cultures was tested.

The fact that filtered medium from 72- or 96-h MV-infected U937 cell cultures, lacking detectable viable MV, also induced leukocyte aggregation suggests that a virus-induced cytokine(s) or virus products could mediate the observed aggregation. Release of soluble mediators following initial MV infection may be an important amplification step in inducing leukocyte aggregation and promoting virus dissemination. This was most evident in experiments using low MOIs. When an MOI of 0.1 was used, overt aggregation of leukocytes was observed by 24 h, a time when cell-free virus was minimal and levels of infectious virus were not sufficient

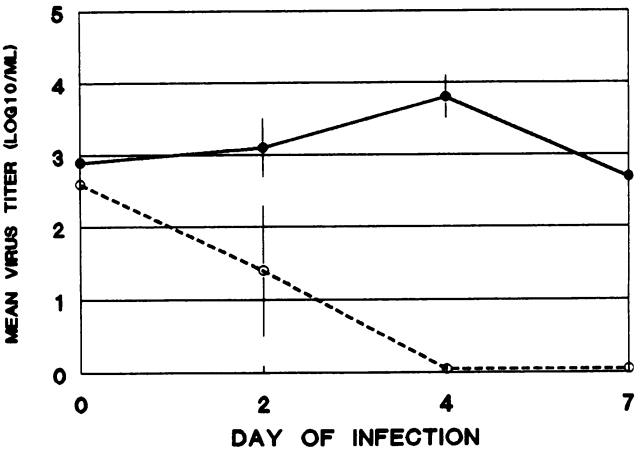

FIG. 3. Levels of MV in PBL cultures of in the presence of M1020 specific for CD11a (LFA-1) epitopes (20 μg of protein per ml, final concentration; dashed line) and in control cultures lacking this MAb (solid line). Data are from two experiments; markers indicate geometric mean virus titers obtained in duplicate experiments; vertical lines indicate standard deviations of the mean. Mean virus titers in cultures containing antibodies and control cultures were significantly different on days 4 and 7 ($P < 0.01$ by Student's t test).

to allow infection of a large percentage of leukocytes. The release of soluble mediators (cytokine or viral product) at these early times could be critical in promoting close cell contact, facilitating the activity of the virus fusion protein.

Flow cytometry analysis of MV-infected cells. Expression of LFA-1 epitopes on U937 cells was measured by using indirect immunofluorescence labeling and flow cytometry. In these assays, mock- and virus-infected cells were incubated at 4°C for 30 min with an MAbs specific for CD11a, CD18, or CD4. The cells were then washed and stained at 4°C for 30 min with affinity-purified fluorescein isothiocyanate-conjugated $F(ab')_2$ goat anti-mouse antibody (Cappel). Analysis of stained leukocytes was made on an Epics Profile flow cytometer (Coulter Cytometrics, Hialeah, Fla.) by gating on the respective populations, using forward and side light scatter. In all experiments, the cursor for the negative population of cells was set by using a mouse isotype (immunoglobulin G1) control MAb (Coulter Cytometrics). The results were expressed as mean fluorescence intensity.

Results of flow cytometric studies performed on uninfected and MV-infected (72 h) U937 cells by using MAbs to CD11a and CD18 are shown in Table 2. As indicated, compared with the fluorescence intensity on uninfected cells, significantly increased mean fluorescence intensity was observed on MV-infected U937 cells stained with anti-CD11a MAb M1020 (6.55 to 11.99) or anti-CD18 MAb BD7950 (5.63 to 11.44). In contrast, the mean fluorescence intensity on uninfected and MV-infected U937 cells did not change significantly (11.84 versus 11.63) when MAb R7.1 (also specific for LFA-1 heteroduplex epitopes) was used to stain these cells. These data and the temporal relationship between them suggest that MV can induce conformational changes in the LFA-1 molecule which may promote intercellular adhesion processes (11). MAbs M1020 and BD7950, in contrast to R7.1 and BD7328 (the anti-CD4 MAb), appeared to bind to epitopes that specifically participate in conformation-dependent adhesive interactions stimulated by MV infection.

TABLE 2. Expression of LFA-1 epitopes on uninfected U937 cells and U937 cells infected with MV for 72 h as measured by flow cytometry[a]

Antibody	Uninfected		MV infected	
	% Cell	MFI	% Cell	MFI
Immunoglobulin G1 control	1.3	0.34	2.3	0.25
M1020 (anti-CD11a)	93	6.55	99.1	11.99
R7.1 (anti-CD11a)	98.9	11.84	99.3	11.63
BD7950 (anti-CD18)	89.8	5.63	98.9	11.44

[a] The percentages of positive-fluorescing cells (% Cell) and their mean fluorescence intensities (MFI) were obtained by an indirect fluorescence staining procedure. The indicated MAbs were used in the primary staining step, and fluorescein isothiocyanate-conjugated goat anti-mouse antibody was used in the secondary staining step.

The data obtained suggest that the following sequence of events may occur subsequent to MV inoculation of leukocytes: (i) leukocyte infection, (ii) release of a soluble factor(s), (iii) functional and structural alteration of leukocyte LFA-1 heterodimers, (iv) leukocyte aggregation, and (v) leukocyte-to-leukocyte spread of virus. Although it is not known whether these events occur in vivo, the parallel findings in PBL and U937 cells suggests that it may. Moreover, the data are consistent with what is known about natural MV infection; i.e., MV infection of the pulmonary tract precedes infection of leukocytes, which in turn occurs prior to dissemination from the lungs and localization of virus to dispersed organs (2, 4, 5, 13, 19, 21, 23).

The finding that MV may directly or indirectly alter CD11a/CD18 expression may be the key to understanding several aspects of MV pathogenesis. The CD11a/CD18 (LFA-1) complex has been reported to mediate a number of diverse adherence-related functions, including those necessary for the induction of inflammation, for the induction of humoral and cell-mediated immune responses (16), and for leukocyte emigration and localization to various organs (e.g., the liver, pancreas, lymph nodes, and brain) (2, 4). Thus, alteration of LFA-1 expression by MV could account, at least in part, for the marked immunosuppression and virus dissemination often associated with MV infection.

At least two other viruses have been shown to infect leukocytes and induce alterations in leukocyte integrins: Epstein-Barr virus and HIV (6, 17, 18). Interestingly, leukocyte infection and leukocyte-mediated transport and distribution of virus appear to be important aspects of the pathogenesis of Epstein-Barr virus, HIV, and MV. As more data are acquired, it will be of interest to compare and contrast the events that occur in leukocytes infected by these different viruses.

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