HUMAN HISTOCOMPATIBILITY DETERMINANTS AND VIRUS ANTIGENS: EFFECT OF MEASLES VIRUS INFECTION ON HLA EXPRESSION*, ‡

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The genes of the major histocompatibility complex code for the histocompatibility antigens as well as control the immune response to certain antigens. The human histocompatibility (HLA) antigens are glycoprotein components of the plasma membrane. These antigens are the major recognition sites of immunological identity and are responsible for the induction of allograft rejection. Certain HLA antigen types are known to correlate with an individual's susceptibility and immunological responsiveness to virus infection (1–5). Examples are the elevation of measles virus antibody levels (1, 4) and the enhanced risk of developing paralytic poliomyelitis (2) in individuals of HLA-A3 and HLA-B7 specificities. Theoretically, viruses can induce an autoimmune response either by modifying HLA antigens during the course of virus infection or by incorporating altered HLA antigens into the envelope of maturing virions (5). Recent interest has focused on the major histocompatibility (H-2) antigens in the mouse in a variety of virus infections. Evidence has accumulated that H-2 compatibility between the effector thymus-derived (T) cytotoxic lymphocyte and the infected target cell is a prerequisite for destruction of virus-infected cells (6–10). The mechanism of this restricted cell-cell recognition is not clear. On the basis of co-capping experiments, it has been suggested that there is a physical association between oncornavirus cell surface antigens and H-2 determinants (11).

HLA antigens are host cell surface markers that can be followed easily during the course of virus infection. Therefore, we studied the interaction of HLA antigens with measles virus antigens expressed on the surfaces of a variety of cells. The concentration of HLA antigens on the surfaces of cultured human lymphoblastoid cells was unchanged by infecting cells with measles virus. The

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large amounts of virus antigens inserted into the cells' membranes remained physically independent from HLA antigens. HLA antigens appear neither to serve as receptors for measles virus nor to be incorporated into the maturing virus particle. In contrast to measles virus infection, infection of cultured human lymphoblastoid cells with poliomyelitis virus and vesicular stomatitis virus (VSV)[1] was found to decrease the concentration of HLA antigens.

Materials and Methods

Virus. Wild-type Edmonston measles virus originally obtained from the American Type Culture Collection, Rockville, Md. was maintained in this laboratory by passage through HeLa cells. The virus was harvested by sonic oscillation and clarified by low speed centrifugation. The virus was assayed on BSC-1 cell monolayers as previously reported (12). The VSV (Indiana serotype) and poliovirus type 1 were obtained from Dr. John Holland, University of California at San Diego, La Jolla, Calif. The VSV was grown and assayed on L-929 cells, while the poliovirus was grown and assayed on HeLa cells. The techniques were adapted from those used for measles virus (12).

Infection of Human Lymphoblastoid Cells. Daudi (no detectable HLA), WI-L2 (HLA-A1, HLA-A2, HLA-B5, and HLA-B17), and RPMI-8866 (HLA-A2, HLA-A3, HLA-B7, and HLA-B12) cells were grown in suspension in RPMI-1640 medium supplemented with 10% fetal calf serum, glutamine, and antibiotics. Raji (HLA-A3 and HLA-BW35) cells were grown in Eagle's minimal essential medium with the same additives. To initiate infection the cells were suspended in the virus stock and infected with measles virus [multiplicity of infection (MOI) = 1], VSV (MOI = 10), or poliovirus 1 (MOI = 100) by incubation at 37°C for 2 h on an inverted shaker. After virus adsorption the cells were diluted with medium to 2×10^5 cells/ml.

HLA Antisera. The alloantisera Gaulier 1-01-8-12-22-02 (anti-HLA-A1), Bizot 1-01-8-12-22-03F (anti-HLA-A2), Stackenburg 1-04-0-02-10-02 (anti-HLA-A2), Storm 2-50-6-02-22-01 (anti-HLA-A3), Victor 1-01-9-07-17-04 (anti-HLA-B5), Melnikoff 2-57-9-03-14-08 (anti-HLA-B7), and Willet 2-51-2-04-27-01 (anti-HLA-B8) were obtained from the serum bank at the National Institute of Allergy and Infectious Diseases, Bethesda, Md. The anti-HLA-A9 serum Grubisch was obtained from Dr. Edmond Yunis, Harvard Medical School, Boston, Mass.

Surface Immunofluorescence. Approximately 1×10^6 infected and uninfected viable cells, suspended in 20 μl of antiserum, were agitated for 30 min at room temperature. After two washes of the cell pellet, the cells were then incubated in an appropriate fluorescein isothiocyanate (FITC)-conjugated antiserum. Each cell pellet was then washed three additional times. Measles virus antigens were detected by direct immunofluorescence, while VSV and HLA antigens were demonstrated by the indirect method. The techniques and specificity of the various reagents have been previously reported (13, 14).

Microcytotoxicity. Human alloantisera were titrated for cytotoxic antibody to HLA antigens by a modification (15) of the microcytotoxicity assay of Mittal et al. (16). Briefly, approximately 1×10^3 human peripheral lymphocytes were incubated at room temperature for 30 min with diluted antisera in Møller-Coates plates. After the addition of rabbit complement (C) the plates were incubated at room temperature for an additional 3 h. After the addition of eosin and formalin, the percent of C-lysed (eosin-stained) cells was determined by phase microscopy. The highest dilution of antiserum to lyse over 90% of the target cells was defined as the cytotoxic end point.

Quantitation of Surface HLA Antigens. Surface HLA antigens were quantified, as previously reported (17) by a microabsorption technique. In brief, various concentrations of washed cells (300–50,000) were incubated for 1 h at room temperature with an equal volume of antiserum at twice the cytotoxic end-point concentration. After centrifugation, the residual cytotoxic antibody in the supernate was again determined. The percent absorption was defined as: [1 − (% cell lysis by absorbed serum)/(% cell lysis by unabsorbed serum)] × 100.

Depletion of Measles Antibody. Anti-HLA-A2 (Bizot) serum was incubated overnight at 4°C

[1] *Abbreviations used in this paper:* AD_{50} adsorption of 50% antibody; EMC, encephalomyocarditis; FITC, fluorescein isothiocyanate; MOI, multiplicity of infection; PFU, plaque-forming units; VSV, vesicular stomatitis virus.

with approximately 1×10^8 measles virus-infected Raji (HLA-A3 and HLA-BW 35) or Daudi (no detectable HLA type) cells and then centrifuged at 5,400 *g* for 5 min. The procedure was repeated until the antiserum was depleted of measles antibody, as detected by indirect immunofluorescence. No decrease in the titer of cytotoxic antibody directed against HLA-A2 was observed after these absorptions.

Co-Capping of Surface Antigens. WI-L2 cells (HLA-A2), 48 h after infection with measles virus (MOI = 3), were incubated for 30 min at 37°C with anti-HLA-A2 serum previously depleted of measles antibody. After two washes of this cell pellet, the cells were incubated at 37°C for 30 min with rabbit anti-human Ig conjugated with FITC. The cells were washed four times with cold medium and then incubated at 4°C with rhodamine-labeled human anti-measles serum for 30 min. After thorough washing, the cells were examined for patterns of rhodamine and fluorescein fluorescence with a Zeiss fluorescence microscope (Carl Zeiss, Inc., N. Y.) (18).

Electron Microscopy. The surfaces of RPMI-8866 and WI-L2 cells were examined for measles virus antigens 48 h after infection (MOI = 1), after incubation with normal human serum for 30 min at room temperature. Human anti-HLA-A2 serum, depleted of detectable measles antibody, was used to detect HLA-A2 antigen. The cells were washed twice and then incubated with ferritin-labeled goat anti-human Ig. The cell pellet was washed seven times and the cells were fixed with 2.5% phosphate-buffered glutaraldehyde and processed for electron microscopy.

Treatment of Measles Virus with HLA-A2 Antibody and C. Measles virus, grown in RPMI-8866 cells, was diluted with medium to 1.4×10^4 plaque-forming units (PFU)/ml. 100 μl of human anti-HLA-A2 serum, previously depleted of detectable measles antibody, and 400 μl of a 1:2 dilution of guinea pig C in medium were mixed with 400 μl of virus. As a control, fetal calf serum was substituted for the HLA-A2 antisera. After incubation at 37°C for 30 min, the residual infectivity was assayed on BSC-1 cell monolayers.

Measles Virus Infection of WI-L2 Cells Pretreated with HLA-A2 Antibody. WI-L2 cells (HLA-A2) were incubated at room temperature for 30 min with an excess of human anti-HLA-A2 serum previously depleted of detectable measles antibody. Control cells were incubated with fetal calf serum. The cells, in the presence of HLA-A2 antibody, were infected with measles virus (MOI = 10) and agitated for 1 h at 37°C. The preparation was monitored by immunofluorescence for excess HLA-A2 antibody as well as for retention of cell membrane-bound antibody after virus adsorption. The supernate was also assayed for residual infectious measles virus. Similarly, Daudi cells which express Ig on their surfaces were incubated with rabbit antibody to human Ig and then infected with measles virus. The cells were washed twice, suspended in medium at 4×10^5 cells/ml, and incubated at 37°C. After 18 h, yields of infectious virus were determined by plaque assay and the proportion of cells expressing measles virus antigens on their surfaces was determined by direct immunofluorescence.

Results

The Effect of Measles Virus upon HLA Expression. WI-L2, Raji, and RPMI-8866 cells were infected with measles virus at an MOI of 1. 48 h after initiation of infection, measles virus antigens were detected on the surfaces of greater than 90% of the infected cells, while the viabilities of virus-infected and uninfected cells were similar (greater than 90%). The number of infected or uninfected cells required to absorb 50% (AD_{50}) of the cytotoxic antibody was determined using the appropriate human alloantisera. As seen in Fig. 1, equal numbers of measles virus-infected and uninfected cells were required to absorb 50% of the HLA-directed antisera. The cytotoxic antibody directed against HLA-A2 was reduced by 50% after absorption with 450 measles virus-infected or uninfected WI-L2 cells; the AD_{50} for HLA-A3 serum was 1,100 uninfected and 1,400 measles virus-infected Raji cells. Absorption with 420 uninfected or 480 measles virus-infected RPMI-8866 cells reduced the anti-HLA-B7 cytotoxic antibody titer by 50%. Thus, the amount of HLA antigen expressed on the surfaces of these lymphoblastoid cells was not significantly changed after infection with measles virus.

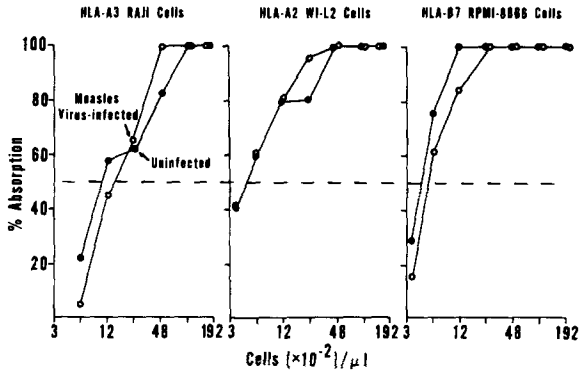

FIG. 1. The absorption of cytotoxic HLA-directed antibody by measles virus-infected and uninfected lymphoblastoid cells. Cell surface HLA antigens were quantified by a microabsorption technique as described in the Materials and Methods. Cells infected with measles virus were assayed 48 h after infection at a MOI of 1. The percent absorption was defined as [1 − (% cell lysis by absorbed serum)/(% cell lysis by unabsorbed serum)] × 100.

To determine whether alterations in HLA specificity may occur during infection, Raji cells were infected with measles virus and then incubated with sera directed against six other HLA specificities. Absorption of these sera with 100,000 measles virus infected Raji cells failed to reduce the cytotoxic antibody titers (Table I). Hence, infection of Raji cells with measles virus did not reduce the ability of these cells to absorb HLA-A3 antibodies or induce the appearance of six other HLA antigens.

The Effect of VSV or Poliovirus Infection and Puromycin Treatment upon HLA Expression. WI-L2 cells infected with either VSV (MOI = 10) or poliovirus type 1 (MOI = 100) or incubated with puromycin (50 μg/ml) were assayed for surface HLA-A2 antigen after 12 h. The viability of the VSW-infected and puromycin-treated cells were comparable to the uninfected control cells (greater than or equal to 90%) while the viability of the poliovirus-infected cells was about 70%. As shown in Table II, both these virus infections or puromycin treatment reduced the amount of HLA-A2 50–70% by 12 h. Inhibition of cellular protein synthesis, either by virus infection or treatment with puromycin produced similar decreases in HLA-A2.

Physical Relationship between Measles Virus and HLA-A2 Antigen on the Surface of Infected Cells. WI-L2 cells infected with measles virus were incubated under conditions favoring capping with human anti-HLA-A2 sera (previously depleted of measles virus antibody), goat anti-human Ig conjugated with FITC, and rhodamine-labeled human antibody to measles virus. As seen in Fig. 2, considerable divergence in the localization of the rhodamine-labeled measles antigens and fluorescein-labeled HLA-A2 antigens on the surface of WI-L2 cells occurred. Next the localization of measles virus and HLA surface antigens were mapped by immunoelectron microscopy. Measles virus antigens were found on the outer plasma membrane of infected cells above aligned nucleocapsid-like structures (Fig. 3 A). HLA-A2 antigens, detected by human alloantiserum depleted of measles antibody, were located at membrane sites distinct from the maturing measles virions on several cell lines (Figs. 3 B and 3 C). No HLA-A2 antigens were detected on the surface of budding measles virions.

TABLE I

The Effect of Measles Virus Infection upon Expression of HLA Antigens on the Surfaces of Raji Cells

Specificity of HLA allo-antiserum	Number of Raji cells to absorb 50% cytotoxic antibody	
	Measles-virus infected*	Uninfected
HLA-A1	>100,000	>100,000
HLA-A2	>100,000	>100,000
HLA-A3	1,400	1,600
HLA-B5	>100,000	>100,000
HLA-B7	>100,000	>100,000
HLA-B8	>100,000	>100,000
HLA-A9	>100,000	>100,000

* Raji cells (HLA-A3 and HLA-BW35) were infected with measles virus (MOI = 1) and examined 48 h later for absorption of cytotoxic antibody as described in the Materials and Methods.

TABLE II

The Effect of Puromycin Treatment or Measles Virus, Poliovirus, or VSV Infection on Surface HLA-A2 of WI-L2 Cells

Treatment	Exp. no.	AD_{50} ratio*	% Reduction‡
Measles virus§	1	800/800	0
	2	300/300	0
	3	1,000/1,200	17
	4	300/300	0 (4.3)‖
Poliovirus type 1¶	1	600/1,500	60
	2	500/1,500	67
	3	500/1,500	67
	4	500/1,150	57 (63)
Puromycin**	1	800/2,250	64
	2	300/1,000	70
	3	1,000/2,000	50
	4	300/900	67 (63)
VSV‡‡	1	800/1,800	56
	2	300/600	50
	3	1,000/2,100	52
	4	300/900	67 (56)

* The number of treated and control cells required to AD_{50} of the anti-HLA-A2 cytotoxic antibody as described in the Materials and Methods. The numerator of the ratio is the AD_{50} for the control cells while the denominator is the AD_{50} of the treated cells. WI-L2 cells express HLA-A1, HLA-A2, HLA-B5, and HLA-B17 on their surfaces.

‡ (1-AD_{50} ratio) × 100.

§ Measles virus-infected (MOI = 1) cells were assayed 48 h after infection.

‖ Average reduction.

¶ Poliovirus-infected (MOI = 100) cells were assayed 12 h after infection.

** Puromycin (50 μg/ml) was incubated with cells for 12 h.

‡‡ VSV-infected (MOI = 10) cells were assayed 12 h after infection.

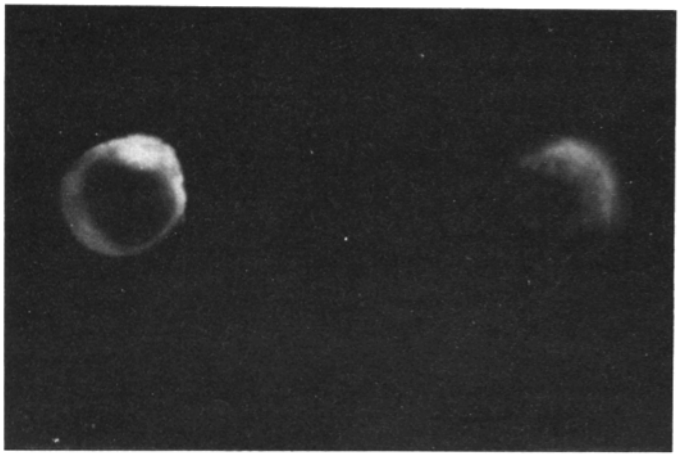

FIG. 2. The immunofluorescent localization of measles virus and HLA antigens on the surfaces of WI-L2 cells. WI-L2 cells, 48 h after infection with measles virus (MOI = 3), were incubated with human HLA-A2 alloantiserum depleted of detectable measles antibody and rabbit anti-human IgG labeled with fluorescein. The cells were then exposed to human anti-measles serum labeled with rhodamine. The dissimilar localizations of HLA-A2 antigens and measles virus antigens on the same cell are seen clearly when viewed through rhodamine-(right) and fluorescein-sensitive (left) filters.

To determine whether HLA-A2 antigens occurred on the surfaces of infectious measles virions, measles virus grown in RPMI-8866 cells (containing HLA-A2) was incubated with HLA-A2-directed alloantiserum (previously depleted of measles antibody) and guinea pig C. No significant differences in plaque numbers were observed between virus treated with HLA-A2 antibody (average = 64) and virus treated with control (fetal calf) serum (average = 69). Thus, utilizing several different techniques, HLA antigens were not detected in association with measles virus antigens on either the surface of infected cells or on the envelope of mature infectious virions.

The Effect of Pretreating WI-L2 Cells with Antibody to HLA-A2 Determinants and Daudi Cells with Antibody to Human Ig upon Measles Virus Adsorption. WI-L2 cells contain HLA-A2 and Daudi cells contain human Ig determinants on their surfaces, respectively. Initially WI-L2 cells were incubated with an excess of antibody to HLA-A2 and Daudi cells with rabbit antibody directed against human Ig. As a control, WI-L2 and Daudi cells were treated with fetal calf serum. After 1 h incubation, the cells were infected with measles virus. At that time 100% of the WI-L2 cells and 85% of the Daudi cells exhibited anti-HLA and anti-human Ig antibodies, respectively, bound to their surfaces. No cell clumps were observed after the addition of measles virus to the antibody-treated cells. Sufficient infectious virus remained after virus adsorption to be able to infect a similar number of cells at a MOI of 1. After virus adsorption, the majority of the cells were found to have retained the appropriate antibody. 18 h after infection, 54% of the WI-L2 cells pretreated with HLA-A2 antibody expressed measles virus antigens, whereas 69% of control cells did. Yields of infectious measles virus from HLA-A2 antibody-treated WI-L2 cells was 0.12 PFU per viable cell while control cells synthesized 0.18 PFU per viable cell. Similarly, 79% of Daudi cells initially treated with anti-human Ig antibody

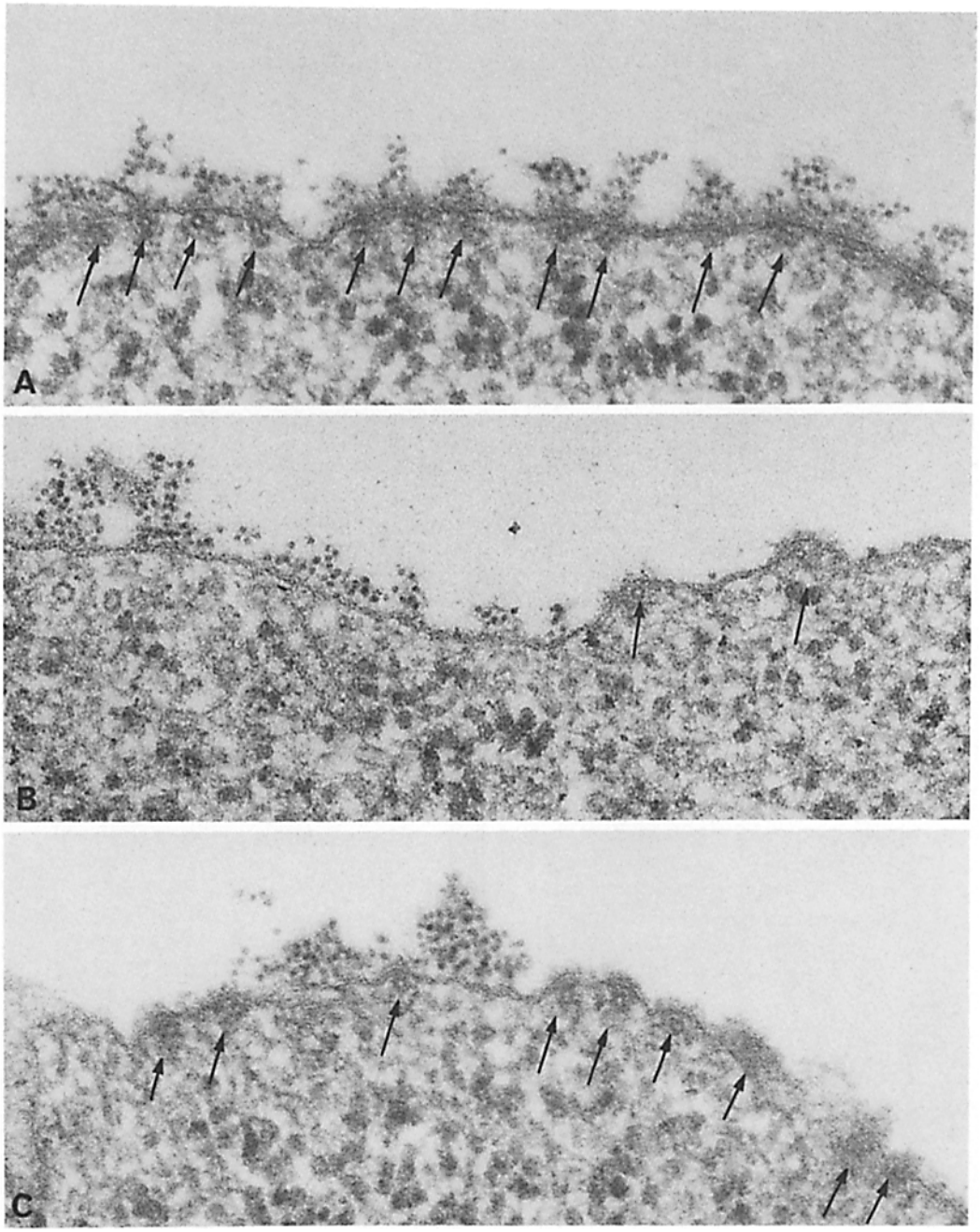

FIG. 3. Measles virus antigen and HLA-A2 antigen localization on the surfaces of RPMI-8866 (A and B) and WI-L2 (C) cells. Cell preparation A was exposed first to human antibodies against measles virus, then to ferritin-labeled goat anti-human IgG, whereas preparations B and C were exposed first to human anti-HLA-A2 serum depleted of detectable measles antibody, then to ferritin-conjugated goat anti-human IgG. Arrows point to fuzzy nucleocapsids that have aligned under those areas of the plasma membrane that have been modified by the insertion of measles virus antigens. HLA-A2 and measles surface antigens are localized at distinctly different membrane sites on both RPMI-8866 (B) and WI-L2 (C) cells (× 120,000).

expressed measles virus surface antigens as compared to 95% of control Daudi cells. Antibody-treated Daudi cells synthesized 0.74 PFU per viable cell as compared to 1.1 PFU per viable cell for control cells. Hence, cells coated with anti-HLA-A2 antibody were as susceptible to productive measles virus infection as noncoated cells or cells coated with antibody to human Ig.

Discussion

In this paper we describe the relationship between measles virus antigens on the cell's surface and the expression of surface HLA antigens. Using three lymphoblastoid cell lines of different HLA phenotypes, we found that there was no significant reduction in the quantity of HLA determinants during infection with measles virus. In contrast, WI-L2 cells infected with VSV or poliovirus type 1 exhibited a 50–70% decrease in surface HLA antigens. Puromycin treatment (50 μg/ml) was associated with a similar decrease in HLA expression. In addition we noted no alteration in the specific binding of HLA-directed antibody during measles virus infection. Measles virus-infected Raji cells and uninfected Raji cells absorbed antibody directed to the same HLA specificities. These results contrast with the report that some human cells transformed by SV40 virus expressed an HLA specificity (HLA-B5) not present on the surfaces of uninfected cells (19). There are conflicting reports of altered H-2 specificities after infection with vaccinia virus (reference 20 and R. M. Zinkernagel, unpublished data). Thus, results with one virus infection cannot be generalized to other virus infections.

Hecht and Summers reported that after infection with VSV, a budding RNA virus that inserts virus glycoproteins into the cell's membrane, murine L-929 cells exhibited decreased amounts of surface H-2k antigens (21). They also studied the effects of actinomycin D or cyclohexamide treatment and encephalomyocarditis (EMC) virus, a nonbudding RNA virus, on H-2k expression in L-929 cells. They found no significant decrease in H-2k despite the inhibition of cell protein synthesis of over 95% (21). These results have been interpreted to mean that the decrease of H-2k antigens during VSV infection is a result of virus-induced modification of the cell membrane. In contrast, our data suggest an alternative explanation. The decrease in H-2 observed by Hecht and Summers and the decrease in HLA observed by us with VSV and poliovirus infection most likely reflects an inhibition of host protein synthesis and not virus-induced modification of the cell membrane. First, we found that despite the insertion of large amount of measles virus antigens into the cell membrane, quantities of HLA antigens remained at pre-infection levels. Other experiments not presented here demonstrated that infection of these cells with measles virus did not significantly inhibit host protein synthesis (less than 15%) (M. V. Haspel and M. B. A. Oldstone, unpublished data). Second, poliovirus, which is related to EMC virus, inhibited host protein synthesis and reduced the amount of HLA-A2 antigen expressed at the cell's surface without the insertion of virus-specified antigens into the cell membrane. Third, treatment of WI-L2 cells with cyclohexamide or actinomycin D did not affect the expression of HLA-A2, whereas puromycin treatment decreased the amount of surface HLA (22). Fourth, other viruses such as Newcastle disease virus (23, 24) and vaccinia virus (20), which inhibit host protein synthesis, also decrease H-2 antigens on the cell's surface.

Although large amounts of measles virus antigens were inserted into the plasma membrane of infected cells, the virus antigens were physically independent of the HLA antigens. In tests for co-capping, labeled virus antigens and labeled HLA localized separately on the same cell. These experiments showed the independence of virus antigens from HLA since if they were linked, movement of one antigen in a membrane would pull the other antigen with it.

Measles virus antigens were observed by immunoelectron microscopy at plasma membrane sites devoid of detectable HLA antigens. This separation of HLA and measles virus antigens was consistently observed in over 100 cells studied in two different lymphoblastoid cell lines. These findings were further confirmed when the infectious titer of measles virus grown in HLA-A2 cells was not significantly reduced after incubation with antibody directed against HLA-A2 and C. In concert with our findings, HLA antigens are not detected by electron microscopy on the envelope of Epstein-Barr virus (25), nor are H-2 antigens frequently (4-5%) observed on the surface of murine leukemia virus particles (26). Although murine leukemia virus particles seem to be independent of the histocompatibility complex during the maturation of virus through the plasma membrane, the co-capping of Friend leukemia virus gp69 and antibodies directed towards the major *H-2* complex has recently been reported (11). These discrepancies should be resolved in future experiments. Hecht and Summers have reported that H-2k antigens were associated with purified preparations of VSV (27). In this latter case it is not clear whether this association is with a mature infectious virion or rather with some aberrant uninfectious particles.

It seemed possible that although HLA antigens do not appear to be involved in the maturation of measles virus, HLA antigens could play an integral role in the adsorption of measles virus (i.e., serve as or be spatially related to measles virus receptor sites). The incubation of WI-L2 cells with an excess of HLA-A2-directed antibody did not abrogate measles virus infection of these cells. The differences observed between HLA-directed antibody-treated cells and control serum-treated cells probably reflects a steric hindrance by the large quantities of cell membrane-bound antibody, since Daudi cells, which bear surface Ig, behave similarly when treated with rabbit antibody directed against human Ig before measles virus infection.

Our studies demonstrate that HLA antigens neither serve as the receptor sites nor maturation sites for measles virus. HLA antigens remain quantitatively unaltered during the course of measles virus infection. Previous studies demonstrated that measles virus replication is not influenced by a specific HLA phenotype expressed on the surfaces of a variety of cultured cells (28). Hence, the enhanced immune response of individuals of certain HLA types to measles virus is unlikely to be explained on the basis of measles virus replication and its control by HLA antigens. Possibly, the reported differences in susceptibility to disease associated with specific HLA haplotypes may in fact be related to immune response genes closely linked to HLA antigens.

Summary

Histocompatibility antigens on the surface of human lymphoblastoid cells were quantified by a microadsorption technique. During the course of measles

virus infection, no quantitative or qualitative alterations in surface HLA antigens were observed. In contrast, infection with poliovirus type 1 or vesicular stomatitis virus, or treatment with puromycin (50 μg/ml) resulted in a significant decrease in surface HLA. These experiments suggest that an inhibition of host protein synthesis rather than the insertion of virus-specified antigens into the membrane results in a net decrease in amounts of this cell surface antigen. The HLA antigens also appear to be both functionally and structurally distinct from measles virus surface antigens. Pretreatment of cells with HLA-directed antibody did not prevent the infection of these cells by measles virus, thus HLA antigens appear unrelated to the measles virus receptor site on the plasma membrane. Electron microscopic studies revealed that measles virus maturation occurs at membrane sites devoid of demonstrable HLA. Furthermore, HLA antigens could not be detected on the surfaces of mature infectious virions.

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