Measles Virus-Specific Cellular Immunity in Patients with Vaccine Failure

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The cytotoxic T-lymphocyte (CTL) response to measles virus (MV) was studied in blood samples from 13 acute- and early convalescent-phase patients with measles infection despite previous vaccination with the live-MV vaccine. MV CTL responses were also measured in six healthy peer controls who had live-MV vaccination during childhood and in five healthy adults who had a remote history of natural measles. All patients recovered from illness without complication. Acute MV infection was diagnosed on the basis of the Centers for Disease Control criteria and by measuring MV-specific immunoglobulin G (IgG) and IgM antibodies. Elevated IgG titers occurred in 80% of the patients at 1 to 2 weeks and in 100% at 4 weeks postinfection. IgM antibodies were detectable in all patient tested and were elevated in 60% of the patients at 1 to 2 weeks postinfection. The MV-specific CTL response was enhanced in 10 of the 13 patients tested, with a mean maximal lysis of $48.5\% \pm 13.3\%$, compared with that of healthy peer controls who had had live-MV vaccinations during childhood (mean lysis, $14.6\% \pm 12.9\%$; $n = 6$) and healthy adults with a remote history of natural measles (mean, $30.8\% \pm 12.2\%$; $n = 5$). Three patients had low MV CTL levels at two time points following measles, with a mean lysis of $12\% \pm 1.7\%$. It is concluded that while there is no evidence for a deficiency in the generation of cellular immunity to MV in the majority of patients with MV vaccine failure, a small number of individuals may fail to develop an enhanced T-cell response following infection.

Prior to the introduction of mass vaccination policies, measles was an endemic and epidemic disease in the United States. Presently, with more than 95% of school children immunized, the incidence of measles has fallen to 1 to 2% of prevaccination levels but the disease continues to exist in the form of small (5 to 1,000 cases) outbreaks and is becoming an increased public health concern. Measles cases typically occur in two distinct cohorts, preschool children less than 5 years of age and school children aged 5 to 19. In the former group, failure to obtain recommended vaccination is prominent, while in the latter, the majority of patients are infected despite previous vaccination (16).

Vaccine failure has been attributed in part to technical aspects of administration (15). Under carefully controlled test conditions, live-virus vaccines are nearly 100% effective in eliciting humoral responses from immunized subjects and titers remain for over 15 years. Under conditions of actual use, estimates of efficacy are somewhat lower and storage of the vaccine, injection technique, and coadministration with immune globulin have been identified as possible causative factors (13, 15). Epidemiologic studies of patients with vaccine failure have found age at vaccination and number of doses received to be significant risk factors (2, 8, 18).

Deficient host response to properly timed and administered vaccination might also contribute to failure to respond initially or failure to maintain a response. However, patients with vaccine failure have not been reported to have recurrent, prolonged, or severe clinical courses, and previous serologic studies have shown that these patients manifest

antibody responses at appropriate times during illness (3, 19).

While generally believed to be important in controlling and eliminating viral infections (4), cellular responses to measles virus (MV) have not been studied in patients with vaccine failure. In healthy, normal late-convalescent-phase adults, in vitro generation of MV-specific cytotoxic T lymphocytes (CTL) occurs (9). This response, as measured in vitro, is mediated largely by CD4⁺ T cells and is restricted by class II HLA molecules (10), although CD8⁺ class I-restricted cytotoxicity has also been reported in acute MV infection (11, 24, 25). The objective of the present study was to determine whether patients with measles vaccine failure can generate an adequate CTL response to MV.

MATERIALS AND METHODS

Patients. An outbreak of measles occurred in Virginia during the summer and fall of 1988. Twenty-five patients aged 15 to 30 years who met the Centers for Disease Control criteria for the diagnosis of MV infection (16) were recruited (Table 1). All patients gave informed consent to participate in this study, and the guidelines of the U.S. Department of Health and Human Services were followed in the conduct of this research. The patients had a relatively mild illness consisting of fever, the characteristic rash, and a cough, coryza, or conjunctivitis in the setting of a measles outbreak.

Patients older than 20 years were excluded because of uncertainties regarding the nature of the vaccine they might have received during childhood. Of the 25 patients, 13 had been vaccinated after introduction of the live vaccine and were therefore studied. All 13 patients reported a history or produced records of measles vaccination: 7 were vaccinated

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TABLE 1. Patients' characteristics and immune responses to MV

Patient no.	Age (yr), sex ^a /vaccination age(s) (mo)	Anti-MV antibody titer(s) ^b		CTL response(s) ^c to:	
		IgM	IgG	MV	Influenza virus
1	20, F/24	3.78 (1) ^d	2.0 (1), 4.56 (8)	48 (8)	NT ^e
2	18, M/24	3.99 (1)	3.08 (1), 4.28 (30)	7 (1), 10 (30)	56 (1), 81 (30)
3	19, M/36, 48	2.30 (1)	4.78 (1), 4.63 (8)	22 (8)	26 (8)
4	18, M/15	1.40 (1)	2.90 (1), 4.67 (8)	56 (8)	57 (8)
5	18, M/24	4.08 (1)	2.0 (1), 4.41 (8)	3 (1), 33 (5)	1 (1), 29 (5)
6	18, F/16	2.6 (2)	4.89 (2), 4.64 (5)	11 (2), 13 (6)	17 (2), 24 (6)
7	18, M/15, 16	4.18 (1)	4.11 (2), 4.61 (6)	28 (2), 38 (6)	25 (2), 33 (6)
8	17, F/15	1.40 (2)	4.30 (2), 4.61 (13)	13 (2), 4 (13)	51 (2), 22 (13)
9	18, F/72	1.40 (2)	5.08 (2), 4.90 (6)	65 (2), 21 (6)	20 (2), 18 (6)
10	18, F/12	1.40 (2)	4.94 (2), 4.88 (6)	55 (2), 44 (6)	32 (2), 39 (6)
11	18, M/12, 96	NT	4.94 (5), 4.66 (8)	34 (5), 40 (8)	30 (5), 32 (8)
17	15, M/13	NT	4.90 (8), 4.78 (20)	61 (8), 24 (20)	56 (8), 38 (20)
13	16, M/11, 72	NT	4.88 (8), 4.41 (20)	52 (8), 8 (20)	22 (8)

^a F, female; M, male.

^b Measles antibody titer is expressed as the negative log of the highest dilution that produced cutoff points of 1.1 for IgM and 1.7 for IgG, as determined by ELISA.

^c CTL response is expressed as percent specific lysis at an effector-to-target cell ratio of 40:1.

^d Numbers in parentheses indicate time (weeks) of sample collection after rash onset.

^e NT, not tested because early convalescent-phase (1 to 2 weeks) blood samples were not available.

between 11 and 16 months of age, and 6 were vaccinated between 2 and 6 years of age.

Blood samples were obtained from seven patients during the first 2 weeks after onset of the rash and from six patients 5 to 8 weeks after onset. Follow-up samples were obtained from 10 patients (cases 2 and 5 to 13) 5 to 30 weeks post rash onset. At the time of follow-up, all patients reported recovery from illness without complications.

Controls. A peer group of healthy young adults aged 16 to 17 years with a history of measles vaccination but without recent exposure to MV was selected to establish baseline values of MV-specific antibody and cytotoxicity. These are termed vaccinated peer controls. A second control group of healthy adults (aged 27 to 59) whose childhood preceded routine measles vaccination was studied to provide a quantitative comparison for MV-specific antibody and cytotoxicity. Serum samples from nine unvaccinated Peruvian patients obtained 0 to 5 days after onset of acute measles rash were studied to establish antibody levels that occur during acute infection.

MV-specific antibody. Enzyme-linked immunosorbent assay (ELISA) was performed as previously described (5). Briefly, lysates were prepared from MV (Edmonston strain)-infected or mock-infected Vero cells. Ninety-six-well plates (Immulon; Dynatech) were coated with lysate and stored overnight at 4°C. After the plates were rinsed with phosphate-buffered saline-0.05% Tween 20, 100 µl volumes of serially diluted serum samples were added and incubated for 2 h at room temperature. The plates were rinsed again, 100 µl of a goat anti-human immunoglobulin G (IgG) or IgM F(ab')₂ fragment-alkaline phosphate conjugate (Sigma) dissolved in 10% diethanolamine buffer (pH 9.8) was added, and the color reaction optical density was read on an MR580 Micro ELISA reader (Dynatech) at 60 min. Titers were determined graphically as the point of intersection between test sera and control negative sera collected from measles-naïve donors as previously described (5). For statistical analysis, we computed the negative logarithm of the titer and used Student's *t* test for analysis.

Rheumatoid factor detection. Rheumatoid factor detection was performed to exclude the possibility that antimeasles IgM antibodies as determined by ELISA might have been

related to an IgM response directed against IgG (rheumatoid factor), which has been described in acute viral infections (1). The Becton Dickinson Macro-Vue card test was used to detect and quantitate the rheumatoid factor.

MV-specific cytotoxicity. Cytotoxicity of MV-stimulated peripheral blood lymphocytes (PBL) was assayed by using autologous Epstein-Barr virus-transformed B-cell targets as previously described (9, 20). Briefly, PBL were isolated from heparinized whole blood by Ficoll-Hypaque gradient separation and frozen in liquid nitrogen until used. For in vitro generation of cytotoxic effector cells, PBL were infected with MV (Edmonston strain) at a multiplicity of infection of 0.1 for 90 min at 37°C. Cells were then cultured in RPMI 1640 containing 5% human AB serum at a concentration of 4 × 10⁶/2-ml well in a 24-well plate (Costar) for 7 days in a 5% CO₂ humidified incubator at 37°C. Generation of influenza virus-specific effector cells was performed similarly, except that PBL and influenza virus (A2/Japan/305/57 strain) were incubated for 60 min prior to addition of serum.

MV-infected target cells were prepared by infecting 4 × 10⁶ autologous Epstein-Barr virus-transformed B cells with MV (Edmonston strain) at a multiplicity of infection of 0.5 for 90 min at 37°C and culturing them in 40 ml of RPMI-20% fetal bovine serum for 4 days. Influenza virus-infected targets were generated in a similar fashion, except that the B cells were infected for 60 min and cultured overnight before use.

On the day of assay, effector cells were washed and plated in triplicate wells at 2 × 10⁵ per well in 96-well U-bottom plates (Costar). Target cells were pelleted and suspended in 0.2 ml of complete RPMI-5% fetal bovine serum (CTL medium). A 0.2-ml volume of sodium [⁵¹Cr]chromate (Amersham) was added at a final concentration of 100 µCi/ml, and the cells were labelled for 90 min. Target cells were washed twice in CTL medium and then plated at 5 × 10³ per well with effector-to-target cell ratios of 40:1, 10:1, and 2.5:1, each in triplicate. The plates were incubated for 4 h at 37°C in a 5% CO₂ humidified incubator and then spun at 1,000 rpm (Sorvall RT 6000B) for 5 min. Supernatant fluids were harvested by an absorbent filter system (Skatron) and assayed in a gamma counter for ⁵¹Cr content. Percent lysis was calculated as follows: [(mean experimental release - mean

TABLE 2. MV-specific CTL in healthy controls who had natural measles during childhood

Donor no.	Age/sex ^a	% Specific lysis ^b	
		MV CTL	Influenza virus CTL
1404	35/F	24	35
1719	59/M	52	42
3360	27/M	26	39
0646	46/M	22	NT ^c
2607	28/F	30	NT

^a F, female; M, male.^b Effector-to-target cell ratio, 40:1.^c NT, not tested.

spontaneous release/mean detergent release – mean spontaneous release)] × 100. Virus-specific lysis was calculated as percent lysis of virus-infected targets by virus-stimulated PBL minus percent lysis of virus-infected targets by mock-infected PBL.

RESULTS

MV-specific antibody. MV-specific IgM and IgG levels were measured in the patients with vaccine failure (Table 1). All 10 patients with vaccine failure tested had detectable MV IgM antibodies at a ≥1:25 dilution (Table 1). Four of 10 patients with vaccine failure seen in the first week after rash onset had MV IgM antibody levels comparable to those of 9 unvaccinated Peruvian patients with acute natural measles infection (mean titer, 3.11 ± 0.53). MV IgM in the patients with vaccine failure was not detected in samples taken after week 4, with the exception of patients 5 and 7, who had the highest levels of MV IgM initially. Rheumatoid factor was not detectable in any of the 10 patients with vaccine failure. By comparison, none of seven healthy adult controls tested had detectable MV IgM at a serum dilution of 1:12.5.

The group mean MV IgG titer for patients with vaccine failure at 4 weeks post rash onset was 4.64 ± 0.20, significantly higher than those for seven healthy adults (4.10 ± 0.27) ($P < 0.001$) and nine Peruvian patients with acute measles (3.78 ± 0.25) ($P < 0.005$). Two of 13 patients (cases 1 and 4) seen during weeks 1 and 2 had IgG titers below measurable levels (log titer, ≤2.0). Two additional patients (cases 2 and 4) had low MV IgG titers at weeks 1 and 2. Both of these individuals had substantially increased MV IgG titers in blood samples obtained during subsequent follow-up.

MV-specific cytotoxicity. Previous studies in this laboratory have demonstrated the ability to generate an HLA-restricted cytotoxic T-cell response to MV by using PBL of healthy adults with normal childhood exposure to measles. Nine of 13 patients with vaccine failure (Table 1) had MV-specific lysis greater than the previously reported norm for healthy adults (26.8% ± 10.4%; reference 8) and the mean for the five adult controls in this study (30.8% ± 12.2%; Table 2) at at least one time point after rash onset. Considerable variability among patients was observed in weeks 1 and 2. Four patients (no. 2, 5, 6, and 8) had MV-specific lysis well below the norm, while two had substantial elevations (no. 9 and 10). In weeks 4 to 8, all patients except 2, 6, and 8 had MV-specific lysis comparable to or greater than the norm. The mean MV-specific lysis for the 13 patients in early convalescence was 35% ± 22%, significantly greater than that for the peer vaccinated con-

TABLE 3. MV-specific CTL in healthy peer controls with a history of live-MV vaccination

Donor no.	DOB ^a /sex ^b	Vaccination date(s)	% Specific lysis ^c
1	12-19-72/F	3-75	6
2	4-30-72/M	5-73	0
3	9-2-71/M	2-73	10.6
4	2-6-72/F	8-73, 7-90 ^d	37.6
5	9-24-72/M	2-73, 7-90 ^d	17
6	6-24-72/F	12-72, 7-89 ^d	16.5
7 (positive control)	12-3-56/M	None (natural measles)	59.1

^a DOB, date of birth.^b F, female; M, male.^c CTL assay was performed on cells obtained between July and December 1991. Effector-to-target cell ratio, 40:1.^d Revaccination.

trols shown in Table 3 (mean lysis, 14.5% ± 12.9%; $P < 0.025$).

Influenza virus-specific cytotoxicity. Influenza virus-specific lysis was measured in the same PBL samples studied for MV-specific lysis (Table 1). Cytotoxicity of effector cells was virus specific, that is, MV-specific effector cells caused lysis of MV-infected target cells but not influenza virus-infected or uninfected target cells. Similarly, influenza virus-stimulated effector cells lysed influenza virus-infected target cells and not MV-infected or uninfected target cells. Despite an increase in MV-specific cytotoxicity, 6 of 13 patients had influenza virus-specific cytotoxicity (Table 1) lower than the mean for the three healthy adults studied (38.7% ± 3.5%) (Table 2), suggesting that nonspecific mechanisms were not responsible for their increased MV-specific cytotoxicity. Interestingly, patients 2 and 8, each of whom had low MV-specific lysis at two time points after rash onset, also had intact responses to influenza virus. By contrast, patient 5 had decreased responses to both MV and influenza virus during week 1 and substantial increases in both by week 5, suggesting that acute MV infection may have suppressed the response to influenza virus. Mean influenza virus-specific lysis for patients with vaccine failure at 4 to 8 weeks was 37.1% ± 14.2% and did not differ significantly from that of healthy adults (38.7% ± 3.5%).

DISCUSSION

The occurrence of small outbreaks of MV infection in children and young adults previously vaccinated with live attenuated measles vaccine has stimulated interest in the mechanisms of vaccine failure. The results of the present study demonstrate that the majority of individuals infected with MV despite previous vaccination had a normal capacity to generate a humoral and cellular immune response to MV. Thus, vaccine failure in these individuals was not due to inability of the host to respond to the virus. However, 3 of the 13 patients (cases 2, 6, and 8) failed to generate an enhanced MV CTL response at two time points postinfection despite an adequate antibody response, suggesting that in these 3 patients inability to generate an enhanced cellular immune response to MV might have increased their susceptibility to MV infection.

The ability to synthesize virus-specific antibody is thought to confer protection from infection (8, 20). Although the pre-exposure MV antibody titers of the patients were not known, the low levels of antibodies found in 4 of the 13

patients (cases 1, 2, 4, and 5) early after infection, suggest that antibody titers were insufficient to provide protection. However, the fact that the patients in this study developed substantial levels of MV-specific IgG by weeks 4 to 8 after onset of rash suggests that the early low titers were not due to inability of the hosts to respond to the virus but rather to inability of the vaccine to induce persistent high levels of humoral immunity.

Previous studies of vaccine failure (3, 16, 17, 19) have distinguished primary failure (failure to seroconvert upon vaccination and subsequent illness accompanied by a primary immunologic response to MV) from secondary failure (successful seroconversion followed by loss of protection and illness accompanied by a secondary immunologic response). In this study, three patients (cases 1, 2, and 5) had serologic patterns consistent with a primary humoral response to MV (high levels of MV IgM and low levels of MV IgG at weeks 1 to 2, with increases in MV IgG at weeks 4 to 8). Three other patients (cases 3, 6, and 7) had high IgM and IgG antibody titers. Since a rise in IgM antibody titer can occur during a secondary immune response, and since it is uncertain whether these individuals were seronegative pre-infection, it cannot be ascertained whether these cases represent primary or secondary failure. Primary vaccine failure could be related to improper vaccine storage or administration or failure of the host to mount an immune response to MV. Four patients (cases 4, 8, 9, and 10) had low IgM and high IgG titers during weeks 1 to 2, indicating a secondary serologic response. Secondary failure is likely to be related to failure to maintain adequate antibody levels over a long period of time (12).

The cellular immune response is thought to be important in recovery from viral infections, since patients with cellular immune deficiencies are susceptible to overwhelming viral illnesses (21). All of the patients in the present study recovered from infection. In addition, most of the patients (10 of 13) had elevations in MV-specific cytotoxicity at 2 to 8 weeks after rash onset, including those with elevated IgM antibodies. In contrast to the general pattern of increased MV-specific cytotoxicity, patients 2, 6, and 8 did not show elevated MV CTL responses. All three of these patients were vaccinated at 15 months of age or greater, and all developed MV IgG titers at 4 to 8 weeks after acute infection. Two of the three patients (cases 2 and 6) had IgM levels consistent with primary vaccine failure, suggesting that failure to generate MV CTL is not due to a generalized defect in the cellular immune response. Moreover, influenza virus-specific cytotoxicity was intact in those three patients. Thus, the possibility of selective hyporesponsiveness in the generation of MV CTL cannot be ruled out in those three patients. Another consideration, however, is that the peak in cytotoxicity occurred in the interval between sample collections. Patient 2 was not studied between weeks 1 and 30, and patient 8 was not studied between weeks 2 and 13. If an interval peak had occurred, these individuals would be remarkable for their failure to maintain MV-specific cytotoxicity at the level expected in healthy adults, which might have contributed to their increased susceptibility to measles.

Infection with MV strains that have undergone mutations in the HA protein has been documented in isolates from three patients who developed measles between 1989 and 1990 in the United States (22). Theoretically, such antigenic drift could be related to the variable MV CTL and antibody responses among the patients in this study. However, this is unlikely for a number of reasons. (i) The cases with both unenhanced and enhanced CTL occurred during the same

outbreak on a college campus and, therefore, were likely to have resulted from the same strain of MV. (ii) Studies done in our laboratory indicate that the CTL response to MV is directed against at least four MV proteins (6). Thus, an antigenic drift would need to affect multiple MV proteins to have a significant impact on the generation of MV CTL. (iii) Point mutations are less likely to affect the CTL response than the antibody response, since CTL recognizes processed antigen while immunoglobulin recognizes conformational epitopes that could be altered by point mutations.

Our results differ from those of Kreth et al. (11) and Sissons et al. (24), who showed MV cytotoxicity in acute-but not convalescent-phase patients. While CD8⁺ HLA class I-restricted CTL may be present in patients with acute MV infection (14, 25), MV-specific CD4⁺ HLA class II-restricted CTL have been shown to be the predominant cell population in late convalescence (10). Thus, HLA classes I and II bearing Epstein-Barr virus-transformed B cells are more appropriate targets for MV-specific CTL than the class I-bearing phytohemagglutinin-stimulated lymphoblasts used by Kreth and Sissons (9). In addition, the assay employed in this study used PBL stimulated *in vitro* with MV for 7 days, rather than unstimulated PBL as in the studies by Kreth et al. and Sissons et al. (11, 24). Thus, expansion of memory CTL populations, the presence of class II-restricted killing, or both may explain the detection of MV CTL in convalescent-phase patients.

The vaccinated peer controls in this study (Table 3) had lower indices of MV-specific CTL than did the older, healthy adults, who most likely had natural infections (Table 2). Although the vaccinated peer control sample was small, it suggests that the MV CTL response is not maintained for years after vaccination. The fact that the three individuals who had been vaccinated twice (donors 4 to 6 [Table 3]) had higher MV CTL levels than the three controls who had been vaccinated once (donors 1 to 3 [Table 3]) supports the notion that measles vaccine may not result in maintenance of long-term cellular immunity to MV. Despite the low MV CTL levels, it is uncertain whether the vaccinated peers are truly susceptible to infection with MV. It has been claimed that many of the individuals who become seronegative after vaccination are nevertheless protected from infection. This is based largely on the data of Krugman et al. (14), who found that upon exposure to virus, a number of these individuals developed secondary MV-specific antibody responses but did not become symptomatic. However, a recent report suggests that secondary vaccine failure with negative IgM measles antibodies can be associated with a mild illness (7). Interestingly, it has been proposed that cellular immunity may play a protective role in seronegative individuals (23). If this were the case, then a subset of vaccinated peers with low levels of MV-specific cytotoxicity and antibodies would be at risk for infection. These findings indicate the need for more complete examination of the cellular immune response to MV and its role in protection from infection.

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