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Human Cellular Immune Response to Measles Virus Polypeptides

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Measles virus polypeptides were separated by polyacrylamide gel electrophoresis and electroeluted from gel sections. The antigenicity of the polypeptides was determined by enzyme-linked immunosorbent assays. The ability of these measles virus antigens to stimulate lymphoproliferation was measured in both high- and low-responder individuals. In contrast to the low-responder lymphocytes which did not proliferate when stimulated with measles virus antigens, the high-responder lymphocytes proliferated when challenged with hemagglutinin, nucleocapsid-associated phosphoprotein, nucleocapsid protein, matrix protein, and fusion protein.

Measles virus is an infectious agent of humans which usually produces a limited disease followed by lifelong immunity. The virus has been shown to cause subacute sclerosing panencephalitis, which reflects the ability of the virus to produce a persistent infection (11, 13, 15). In addition, measles has been implicated as a possible causative factor in multiple sclerosis (1, 4, 6, 10). Acute infection is followed by a substantial antibody response which is initially immunoglobulin M (IgM) and subsequently IgG (7, 8, 10, 14). The IgG response persists for years without apparent reinfection. The parameters of the cellular immune response are not as well understood (4). A moderate cellular response, as measured by lymphocyte proliferation, has been demonstrated during the early convalescent period, but 3 to 4 months after infection only very low T-cell responses can be demonstrated in most individuals (5). Similarly, specific T-cell killing of measles virus-infected target cells has not been demonstrated years after natural infection or immunization with the virus (4). In contrast to the majority of low-responder (LR) normal individuals, a few seropositive individuals who are high responders (HR) have been identified. The HR individuals have substantial responses long after measles virus infection and have been previously studied in an effort to examine the nature of this persistent response and to provide some insight into the normally low response.

In a previous study from our laboratory, a substantial cellular response in two HR individuals (HR_1 and HR_2) was demonstrated using affinity-purified hemagglutinin (HA). Cellular immune responses to fusion protein (F_1), nucleocapsid protein (NC), nucleocapsid-associated phosphoprotein (P), and matrix protein (M) have not been assessed. In the present study, milligram quantities of infectious virus were obtained (3) and used to isolate relatively pure measles virus proteins. The characteristics of the measles virus proteins and the lymphoproliferative responses in HR and LR patients to these viral components are described.

Measles virus was prepared from supernatants of infected Vero monolayers as previously described (9). The supernatants were centrifuged at 10,000 × *g* and filtered through a membrane (Pellicon PSVP, Millipore Corp., Bedford, Mass.) with a nominal molecular weight exclusion of 10^6. The material retained was centrifuged at 4°C for 3 h in an SW27 rotor (Beckman Instruments, Inc., Palo Alto, Calif.) at 25,000 rpm through 20% sucrose onto a 60% (wt/wt) sucrose cushion. Virus was collected at the interface and then sedimented through 35% (wt/wt) sucrose onto a 60% cushion. One to two milligrams of virus was suspended in 2 ml of sample buffer (0.0625 M Tris-hydrochloride, 10% [wt/vol] glycerol, 10% [wt/vol] sodium dodecyl sulfate, 5% 2-mercaptoethanol; pH 6.8) and immersed in a water bath at 100°C for 4 min. This preparation was placed on a polyacrylamide slab gel consisting of a 5% stacking gel and a 7.5% running gel. Electrophoresis was conducted at 150 V until the tracking dye reached the bottom of the running gel. The vertical edges of the gel were cut off and stained for 45 min in 0.2% Coomassie brilliant blue R-250 dissolved in destaining solution (45% methanol, 10% acetic acid, 45% water). The vertical gel strips were placed in destaining solution for 30 min and then used to locate measles virus polypeptides. Most of the running gel was stored at 4°C and subsequently sliced into horizontal bands containing a viral protein(s) of restricted molecular weight. Horizontal sections were made based on the migration of the viral polypeptides (HA, P, NC, F_1, and M) demonstrated in the stained vertical gel strips. The horizontal sections were cut into fragments and electroeluted at a 1-W constant power with a sample concentrator (ISCO, Lincoln, Nebr.) over a 16-h period. Protein determinations were made with a protein assay (Bio-Rad Laboratories, Richmond, Calif.) or an automated Lowry assay. The protein composition of the electroeluted fractions was analyzed on a 9% slab sodium dodecyl sulfate-polyacrylamide gel.

Figure 1A demonstrates the migration of fractions 1 to 5 from preparation 1. Proteins with molecular weights of 77,000, 59,000, and 37,000 were present as single bands in fractions 1, 3, and 5, respectively. These molecular weights correspond to the HA, NC, and M polypeptides of the virus. Fraction 2 contained a doublet with proteins of 71 and 69 kilodaltons, consistent with the presence of P protein. Fraction 4 also consisted of two polypeptides whose molecular weights of 43,000 and 39,000 are equivalent to those of the polypeptides actin (A) and F_1. Figure 1B characterizes the molecular weight and composition of the proteins in fractions 1 to 5 of preparation 2. The molecular weights of the proteins in fractions 1, 2, and 3 are consistent with those of HA, P, and NC, respectively. Fraction 4 proteins probably represent F_1 and M on the basis of molecular weights. The composition of fraction 5 is not known, but this fraction may be a breakdown product of a high-molecular-weight protein.

The antigenic properties of the eluted proteins were determined by enzyme-linked immunosorbent assay (ELISA). To

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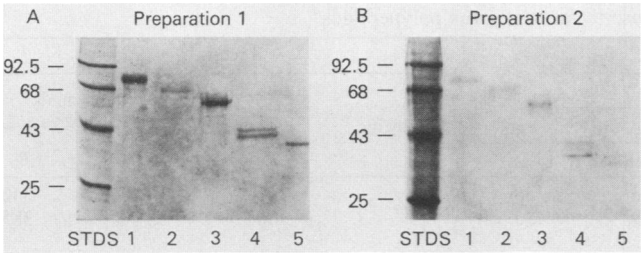

FIG. 1. Coomassie brilliant blue-stained analytic 9% sodium dodecyl sulfate-polyacrylamide gel of eluted fractions from (A) preparation 1 and (B) preparation 2 compared with high-molecular-weight standards (STDS). Fractions 1 to 5 from preparation 1 contain proteins with molecular weights of 77,000, 71,000 and 69,000, 60,000, 43,000 and 39,000, and 37,000, respectively. Fractions 1 to 5 from preparation 2 contain proteins with molecular weights of 77,000, 71,000, 61,000, 41,000 and 37,000, and 36,000, respectively.

use the eluted fraction in ELISA, it was necessary to remove detergents as follows. A 200-μl volume of polypeptide or bovine serum albumin was combined with 20 μl of 3 M sodium acetate, 2 μl of 1 M magnesium chloride, 2.4 μl of RNA from *Escherichia coli* (10 mg/ml; lot 1421448, Boehringer Mannheim Biochemicals, Indianapolis, Ind.), and 625 μl of absolute alcohol. After a 1-h incubation at −70°C, the solutions were centrifuged at 4°C in a Beckman Microfuge B for 15 min at 9,000 × *g*. The precipitates were washed with 625 μl of 80% ethanol and centrifuged at 9,000 × *g* for 10 min at 4°C. The supernatant was discarded, and the precipitates were dried by centrifugation in a vacuum for 20 min. The dried precipitates were dissolved at a concentration of 1 μg/100 μl in carbonate-bicarbonate coating buffer (pH 9.6). Sonic extracts of uninfected and Edmonston strain measles virus-infected Vero monolayers were diluted 1:20 in coating buffer. These solutions were incubated on 96-well plates (Immunol 1, Dynatech Laboratories, Inc., Alexandria, Va.) with 100 μl per well at 4°C for 16 h. The plates were washed three times in phosphate-buffered saline containing 0.05 Tween 20 after this initial incubation and each subsequent incubation. The monoclonal antibodies developed in our laboratory were the HA-specific (366), the P-specific (153), and the NC-specific (168) immunoglobulins. Monoclonal antibody specific for M protein (C6-5) was a gift from Wolfgang Bohn.

ELISA (Table 1) confirmed that the predominant proteins in fractions 1, 2, 3, and 5 of preparation 1 were HA, P, NC, and M. Fraction 4 of this preparation was reactive with polyspecific antimeasles sera; however, a monoclonal reagent specific for F_1 was not available. Similarly, in preparation 2 the proteins in fractions 1, 2, and 3 were HA, P, and NC. Significant reactivity to M was present in fraction 4 but not in fraction 5 of this preparation.

Proliferative assays were performed with lymphocytes from two twin sets (twin set 1 dizygotic haploidentical and twin set 2 monozygotic) discordant for multiple sclerosis. In each twin set the individual with multiple sclerosis was an HR and the unaffected twin was an LR (16). Mononuclear cells were obtained from heparinized peripheral blood by Ficoll-Hypaque density gradient centrifugation. A lymphoproliferative assay with virus polypeptides, Edmonston strain measles virus-infected Vero cell lysates, and control lysates as the antigens was employed (9). Lymphocytes were cultured in 96-well microtiter plates. Triplicate wells containing 3×10^5 cells and each antigen were cultured for 96 h in RPMI 1640 with 1% AB serum, penicillin, and streptomycin. The cultures were then pulsed with 1 μCi of [^{3}H]thymidine for 4 h and harvested on a MASH II cell harvester.

Lymphocyte proliferation in response to the measles virus proteins obtained from the individual fractions was measured in two sets of twins (Table 2). In twin set 1 there was a significant lymphoproliferative response to the HA, NC, A + F_1, and M proteins (fractions 1, 3, 4, and 5) in the HR_1 twin but not in the LR_1 twin. Similarly, HR_2 had significant proliferation in response to all of the proteins, and LR_2 had a minimal response to A + F_1 (fraction 4) but not to the other measles virus protein fractions.

An interesting difference was noted in comparing the pattern of responses in the HR twins: HR_1 lymphocytes proliferated best in response to HA and NC and had modest response to A + F_1 and M, but HR_2 cells showed the highest proliferative response to A + F_1 and M and had a relatively low response to HA. HR_1 and HR_2 lymphocytes were equivalent in capacity to proliferative in response to NC.

After storage for ca. 4 weeks, the proteins no longer stimulated lymphocytes even though the antigens reacted with antibody in ELISA. Since the cellular response to P had not been studied, a second preparation of protein fractions was required.

Lymphoproliferative responses to the measles virus protein from the fractions of preparation 2 were determined after the antigens had been precipitated by the alcohol-tRNA method to remove detergent. The cellular respones from twin set 1 were similar to those obtained with preparation 1 (Table 2). The cells from HR_1 responded not only to fraction

TABLE 1. ELISA of protein fractions[a]

Anti-serum	Specificity	Absorbance value													
		Prepn 1							Prepn 2						
		Fraction					Vero	Edmonston	Fraction					Vero	Edmonston	BSA
		1	2	3	4	5			1	2	3	4	5			
366	HA	0.785	0.098	0.047			0.027	0.318	0.214	0.040	0			0.019	0.264	0
153	P	0.072	0.698	0.303			0.011	0.251	0.098	0.769	0.090			0.016	0.435	0.044
168	NC	0.008	0.062	1.331	0.239	0.076	0.013	0.481	0.016	0.066	1.500			0.015	0.813	0.006
C6-5	M				0	0.440	0.013	0.330				506	0.067	0.013	0.330	0.019
X63		0.030	0.013	0.027	0.038	0.039	0.017	0.021	0	0	0	0	0.004	0.015	0.014	0

[a] Monoclonal antibodies were diluted 1:10,000. Secondary antiserum (diluted 1:1,000) was goat anti-mouse IgG conjugated with alkaline phosphatase (lot 62F-8920, Sigma Chemical Co., St. Louis, Mo.). Disodium *p*-nitrophenyl phosphate (1 mg/ml) dissolved in 10% diethanolamine buffer was used to develop the plates. The reaction was stopped by adding 50 μl of 3 M NaOH to each well. The degree of substrate catalysis was determined in optical density units at 405 nm with a microtiter spectrophotometer (Dynatech Laboratories, Inc.). An absorbance value above a threshold level of 0.200 is considered positive. BSA, Bovine serum albumin.

TABLE 2. Lymphoproliferative response to measles virus polypeptides[a]

Fraction	Prepn 1						Prepn 2			
	Specificity	μg/well	Δcpm				Specificity	μg/well	Δcpm (twin set 1)	
			Twin set 1		Twin set 2					
			HR_1	LR_1	HR_2	LR_2			HR_2	LR_2
1	HA	0.1	6,574	493	1,226	86	HA	0.1	3,018	−340
		0.01	1,243	107	339	430		0.01	1,540	385
2	P		NT	NT	NT	NT	P	0.1	2,275	−724
								0.01	2,959	−233
3	NC	0.1	6,173	446	6,759	815	NC	0.1	2,866	57
		0.01	1,321	119	990	208		0.01	2,876	193
4	A + F_1	0.1	4,798	−150	9,521	246	F_1 + M	0.1	3,146	−434
		0.01	1,350	−346	5,076	1,375		0.01	1,033	−4
5	M	1.0	3,624	703	1,397	368	?	0.1	624	−424
		0.1	101	344	8,390	39		0.01	484	394
		0.01	31	99	1,972	176				

[a] Background counts ranged from 1,000 to 1,500 with bovine serum albumin, tRNA, and Vero cell lysates. NT, Not tested.

2 (P) but also to fraction 1 (HA), fraction 3 (NC), and fraction 4 (F_1 + M). LR_1 cells failed to respond to any of the fractions.

Generally, the lymphoproliferative responses to the individual measles virus polypeptides of the HR twin cells are consistent with previous studies of these individuals in which virus-infected monolayers were used as the antigen (2, 4). In addition, the responses to HA and NC by HR_1 and to NC, A + F_1, and M by HR_2 are of a degree similar to that reported for affinity-purified HA (2). The spectrum of responses by HR_1 and HR_2 lymphocytes suggests that their cellular proliferation in response to sonic extracts of measles virus-infected cells is the result of a combined response to several measles virus polypeptides.

HA and F_1 are located on the surface membrane of measles virus and infected cells and consequently are targets for immune cells. In the present study, the greatest lymphoproliferative responses by HR_1 and HR_2 were to HA and A + F_1, respectively. Although ELISA evaluation of fraction 4 from preparation 1 suggested minor contamination with a component produced by the degradation of NC, it is unlikely that the lymphoproliferative response was directed at the contaminant because it was demonstrated that HR_2 lymphocytes proliferate significantly better in response to fraction 4 (A + F_1) than to fraction 3 (NC) over a range of antigen concentrations. HA is expressed in dimeric form on the cell surface, which may contribute to the antigenicity of this glycoprotein. For example, the response of HR_2 lymphocytes to HA antigen previously observed was greater than that observed with monomeric HA used in this study. However, it was also observed that HR_2 lymphocytes had a significantly higher response to membranes from measles virus-infected cells than to HA alone (2). These results suggested that another membrane-associated antigen (F_1) was an important stimulus for lymphoproliferation. This hypothesis is supported by the present findings which demonstrate that the F_1 protein stimulates significant proliferation of both HR_1 and HR_2 lymphocytes.

In these studies of HR individuals, there is a significant lymphoproliferative response not only to the surface antigens (HA and F_1) but also to the other major structural proteins of the virus (P, NC, and M). This suggests that measles virus has been processed by antigen-presenting cells in vivo which leads to the expansion of lymphocytes sensitized to these virus components. Antigens prepared by these techniques will allow better definition of immune responses to measles virus, including the delineation of both cellular and humoral specificities (12). This approach should be particularly useful in the study of clinical conditions, including natural measles virus infection, subacute sclerosing panencephalitis, and multiple sclerosis.

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