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Development of Antibody to Measles Virus Polypeptides During Complicated and Uncomplicated Measles Virus Infections

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Immune precipitation of 181 sera from 152 patients with natural measles was studied to determine the temporal course and frequency of antibody responses to nucleocapsid, fusion, hemagglutinin, and matrix proteins of measles virus. Large amounts of antibody to nucleocapsid protein developed in all patients by day one of the rash. Antibody to hemagglutinin and fusion proteins developed in all patients over the next 3 weeks, the former to high levels and the latter to low levels. Antibody to matrix protein developed to very low levels and was detectable in only 41% of the patients; this poor response to matrix protein was not correlated with the age of the patient or the acute neurological complications of measles.

Natural measles virus infections are associated with a number of complications. The most common of these are secondary bacterial infections which result in pneumonia or otitis media and may be related to virus-induced depression of immune responses (23). In addition, two neurological complications may occur at different times relative to the primary infection. Postinfectious encephalomyelitis occurs in ca. 1 in 1,000 measles cases usually within 10 days of the exanthem. This is an acute demyelinating disease in which the role of virus infection of the central nervous system is uncertain, and an immunopathological mechanism has been suggested (13). Subacute sclerosing panencephalitis (SSPE) is a rare complication which occurs many years after the exanthem in less than 1 in 100,000 cases. SSPE is caused by a persistent infection of the central nervous system with measles virus and is characterized by large amounts of measles antibody in the serum and cerebrospinal fluid of affected individuals.

In patients with SSPE, antibodies are present to all measles virus proteins except the matrix (M) protein (3, 6, 9, 15, 24). Antibody to this protein is undetectable or present in small quantities, and this observation has led to the hypothesis that the virus is persistent because of a lack of production of adequate amounts of M protein by the infected brain cells (6, 7). A problem in the interpretation of these data has been a paucity of information on the normal antibody response to this and other viral polypeptides during the acute infection and normal recovery period. To determine how frequently antibody to M protein is made by children with natural measles virus infections and whether this differs either with time after infection, age, or with complications of encephalomyelitis or pneumonia, antibodies to measles virus polypeptides were measured by immunoprecipitation at various times after infection.

MATERIALS AND METHODS

Patients. We studied 181 blood samples from 152 patients with natural measles. Patients with uncomplicated measles (N = 68, 33 female, 35 male; age range 6 months to 25 years, mean 5.6 years) were referred from the emergency room at the Hospital of Universidad Peruana Cayetano Heredia or from a community health center at Canto Grande. The patients with measles pneumonia (N = 57, 25 female, 32 male; age range 8 months to 8 years, mean 2.5 years) and other non-neurological complications (N = 7, 4 female, 3 male; age range 5 months to 22 years, mean 8.5 years) were seen at Hospital del Niño. Patients with pneumonia were not known to be immunocompromised before measles and probably represent mild viral pneumonitis complicated by superinfection with a variety of bacterial and viral pathogens (2). Patients with postinfectious encephalomyelitis (N = 20, 9 female, 11 male; age range 2 to 13 years, mean 6.3 years) were seen at several hospitals in Lima. Serum was obtained at different times during disease, ranging from 1 or 2 days before the rash, when Koplik spots were present, to 3 years after infection. Blood was collected in preservative-free heparin (20 U/ml of blood), and plasma was separated from the cells and frozen at −20°C until thawed for assay.

Immune precipitation. This analysis was carried out as previously described (5). Briefly, an extract of ^{35}[S]methionine-labeled measles virus-infected HeLa cells was used as a source of radiolabeled virus proteins. Serum and cell extract were incubated for 3 h on ice, and immune complexes were absorbed on *Staphylococcus aureus* protein A-Sepharose beads, washed, and analyzed on 10% polyacrylamide slab gels (5). The viral polypeptide bands were visualized by exposure to Kodak XR-5 X-ray film and quantitated by densitometry with an LKB model 2202 scanning laser densitometer linked to an integrator (Hewlett-Packard model 3390 A). The amount of each viral polypeptide band was calculated by multiplying the densitometer reading by a factor which included the proportion of isotope decay, the sensitivity factors of the instrument, the ratio of ^{35}S cpm to densitometer units (determined by solubilizing and counting bands which were previously scanned), and the specific activity of the proteins in the extract (5). An expression for each viral polypeptide was obtained as μg of protein precipitated per μl of serum, and values are expressed in units of 10^{-4} μg of viral protein precipitated per μl of serum. A series of dilutions was assayed for several sera to assess the linearity

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of the relationship of serum added to antigen precipitated for each polypeptide band, since antigen-to-antibody excess is required to accurately quantitate antibody for each polypeptide. These experiments indicated that a ratio of 5 μl of serum to 500 μl of extract would underestimate the value for nucleocapsid protein (NP) antibody in the higher-titered sera but that lower ratios of serum-to-extract would fail to detect M antibody present in small amounts. Reference sera with antibody to viral polypeptides were: (i) a convalescent serum provided by Henry McFarland, (ii) a monkey hyperimmune measles serum from Julie Milstein, (iii) an SSPE serum from Michel Philippart, and (iv) a monoclonal antibody directed against M protein from T. F. Wild.

The smallest amount of M protein which produced a visible band on the autoradiograph was calculated to be 0.1 $\times 10^{-4}$ μg per μl of serum. On each gel, reference sera known to precipitate 25×10^{-4} to 35×10^{-4} μg of M protein were included. The extracts did not contain adequate amounts of the measles virus phosphoprotein (P), since only small amounts were precipitated by reference sera known to immune precipitate this protein. Presumably P was proteolytically degraded in the extracts as discussed by Norrby et al. (18) and, like these authors, we chose not to report data on this protein.

RESULTS

The appearance of antibodies to measles virus polypeptides NP, hemagglutinin (HA), fusion (F), and M was first examined according to the time during the infection that the sample was taken (Fig. 1). Antibody to the NP protein appeared coincident with the rash (day 0) and increased rapidly thereafter. Antibody to the HA protein increased slowly over the first and more rapidly during the second and third weeks after infection. Antibody to the F protein also increased slowly over the first week but then remained essentially stable at a low level. Small amounts of antibody to M protein were present in some patients during the first week, showed a modest increase over the next two weeks, and then stabilized at a low level.

The same data were analyzed for the percentage of patients at a given time after infection having detectable antibody to each of the proteins (Fig. 2). Antibody to the NP protein was not only present in the largest amounts (Fig. 1)

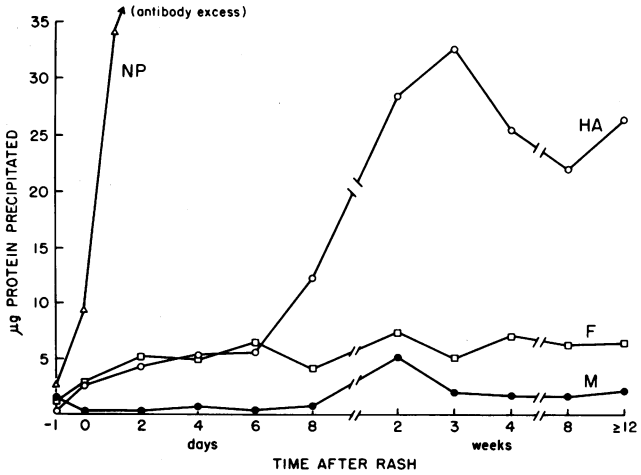

FIG. 1. Mean level of immune precipitation of each viral protein as a function of time after appearance of measles rash. Units are 10^{-4} μg of viral protein precipitated per μl of serum.

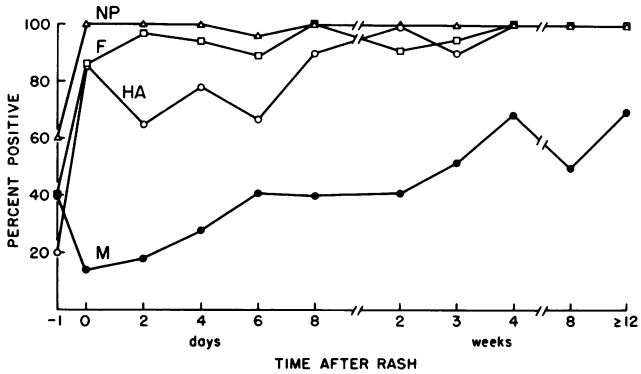

FIG. 2. Percentage of serum samples positive for antibody to viral polypeptides as determined by immune precipitation, as a function of time after onset of rash.

but also appeared earliest, so that all patients had some antibody to this protein at the onset of the rash. Antibody to the F and HA proteins was present in most patients when the rash appeared and was present in all at 4 weeks. In contrast, antibody to the M protein was present in less than half of the patients for the first 2 to 3 weeks of infection and at maximal production (1 to 6 months after infection) was present in only 50 to 70%.

In 22 patients more than a single sample was obtained during the course of the study. In 6 of these patients, no antibody to M protein was detected, and in 13, levels increased or were stable with time. In two patients the antibody was initially present and was subsequently lost, and in a third patient the level of the antibody significantly fell during the study period. These last three cases demonstrate that individual patients may lose antibody to M protein with time.

The groups of patients with and without detectable antibody to M protein were compared with respect to their levels of antibody to other viral proteins. Some patients with no detectable antibody to M had high levels of antibody to other viral proteins, suggesting that a poor overall humoral immune response does not explain the absence of antibody to M protein in many patients.

To determine whether the presence of complications of the infection influenced the pattern of antibody production to measles virus polypeptides, patients with measles complicated by pneumonia or encephalomyelitis were compared with children with uncomplicated infections for presence and amount of antibody to each of the proteins at various times after infection. Table 1 presents the data for M protein. No significant differences were found between the disease groups for antibody to this or any of the other measles virus polypeptides studied.

Since SSPE is significantly associated with measles virus

TABLE 1. Antibody to M protein in patients with complicated and uncomplicated measles virus infections

Infection	Wks after rash				
	No. positive/no. tested (%)				
	1	2	3	4	≥5
Uncomplicated	11/41 (27)	5/10 (50)	4/9 (44)	5/6 (83)	3/5 (60)
Pneumonia	11/38 (29)	5/15 (33)	4/8 (50)	1/2 (50)	
Encephalomyelitis	1/3 (33)	3/7 (43)	4/4 (100)	3/5 (60)	9/15 (60)

infection before 2 years of age (11), the presence of antibody to M protein 6 days of more after the rash was also examined according to the age of the child at the time of infection (Table 2). There was no significant difference ($P < 0.1$) in the presence or absence of antibody to M protein between children 2 years of age or less and children older than 2 at the onset of measles.

DISCUSSION

The appearance of antibody to HA, NP, F, and M polypeptides after natural measles virus infection has been characterized. Antibody to NP protein appears first along with the rash. Antibody to this protein accounts for most of the complement-fixing antibody (17) and is thus consistent with previous data that complement-fixing antibody appears coincident with the rash (1). Our data also confirm those of Norrby et al. (18) that antibody to NP protein is the predominant antibody made during natural infection. The second most abundant antibody is directed against the HA protein. This antibody is responsible for virus neutralization (16), and all patients developed antibody to this protein within 2 to 4 weeks after the appearance of the rash.

Only small amounts of antibody are produced to the F protein, but the protein is consistently recognized by the immune system so that all patients have antibody within 1 month. The only protein which is not recognized by 100% of the patients during the convalescent phase of natural measles virus infection is M. Previous studies with small numbers of sera have indicated that some measles patients produce detectable levels of M protein antibody but that others do not (15, 18, 21). In our study this did not correlate with age, complications of the infection, or antibody levels to other proteins or with the extent of depression of cellular immune responses (R. L. Hirsch, D. E. Griffin, R. T. Johnson, S. J. Cooper, I. Lindo de Soriano, S. Roedenbeck, and A. Vaisberg, Clin. Immunol. Immunopathol., in press). The large number of sera in the present study documents the time course of M antibody production and indicates that about half of the patients produce this antibody after natural measles.

The first observations of lack of antibody to the measles virus M protein were made in studies of SSPE (9, 15, 24). These reports encompass about 50 SSPE sera, but 2 SSPE sera which appear to have antibody when studied by precipitation techniques have been reported (4, 20). Using a sensitive solid-phase assay, Trudgett et al. (22) reported antibody to M protein in all of five SSPE sera which suggests that small amounts are present in some SSPE sera. The paucity of M protein antibodies that is characteristic of humans with SSPE is not observed in dogs with chronic neurological disease caused by canine distemper virus (8) or in mice with Sendai virus encephalitis (14).

Subsequent to the initial observation in SSPE, other clinical conditions have been described with a similar lack of antibody to M protein. A mentally retarded patient who had measles encephalomyelitis 24 years previously (10) and a child with measles inclusion body encephalitis, an opportunistic infection which occurs in immunodeficient individuals (19), were both reported to lack antibody to M protein. In addition, sera from patients with chronic active hepatitis, multiple sclerosis, and normal adult individuals who had measles in childhood generally lack M antibody (12, 18; M. C. Graves, unpublished data). Indeed, the only reported condition with uniform presence and relatively high titers of antibody to M protein is atypical measles (18; M. C. Graves, unpublished data). Our data indicate that even in initial measles virus infections, many individuals do not make detectable levels of antibody to M protein, and this cannot be totally ascribed to poor overall antibody response to the virus, nor can it be explained by other factors such as age or severity of clinical disease.

TABLE 2. Patients less than or greater than 2 years old producing antibody to M protein during complicated and uncomplicated measles virus infection

Infection	Age	
	No. positive/no. tested (%)	
	≤2 yrs	>2 yrs
Uncomplicated	9/14 (64)	8/18 (44)
Pneumonia	8/25 (32)	6/10 (60)
Encephalomyelitis	2/4 (50)	15/27 (56)

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