THE DEVELOPMENT AND EVALUATION OF AN ATTENUATED MEASLES VIRUS VACCINE

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FROM 1749 to 1954, a period of more than two centuries, numerous attempts were made to devise an effective means for induction in man of active immunity against measles. Even the most promising reports led to eventual disappointment principally because of a lack of reliable technics for isolation and identification of the responsible agent, consistent propagation in the laboratory, and measurement of immunity. Much of this early work has been summarized elsewhere.[1] The description in 1954 by Enders and Peebles of successful growth and replication of the measles virus in tissue cultures of human and monkey kidney cells[2] provided the methods to surmount these obstacles, and the succeeding seven years have witnessed considerable progress toward attainment of the age-old goal of safe and successful vaccination against measles. In this report, attention will be directed to the Edmonston strain of measles virus which has served as the seed for nearly every vaccine currently under test. Both the history of this strain and the results obtained when it is employed as an immunizing agent will be reviewed.

History

This virus, originally isolated in human kidney cell cultures from the blood of a boy in the early stage of classical measles, was the first of eight such agents reported in 1954 by Enders and Peebles.[2] During a series of 24 passages in human renal cells it exhibited a characteristic cytopathic effect characterized by the appearance of large, multinucleate giant cells or syncytia. When inoculated into susceptible cynomolgus monkeys, the agent produced typical unaltered monkey measles. Twenty-eight passages in human amnion cells followed the initial 24 in kidney cultures. During this series a second cytopathic change, the spindle cell transformation, was first observed.[3] Pathogenicity for monkeys remained unaltered during the amnion cell passages.[4] Inoculation, via the amniotic sac, of the fertile hen's egg with fluid from the 28th human amnion passage of the Edmonston strain resulted in viral multiplication in ovo demonstrable when the harvested infected egg material was checked in tissue culture systems.[3] After six such passages in the developing chick embryo system, we were able to show that the virus could be propagated in monolayer cultures of trypsinized chick embryo cells.[5] No cytopathic changes were seen during the initial passages, but in the fifth transfer small giant cells, spindle cell transformation and degeneration appeared and have remained constant throughout subsequent passages in this cell system.

The support of viral multiplication in a host cell differing from the usual human or simian environment and the marked alteration of cytopathogenicity encouraged us to examine the effect of the chick cell virus in susceptible monkeys. Inoculation of such animals via the intravenous and intranasal, sub-

cutaneous or intracerebral and intracisternal routes showed that a definite decrease in virulence for this host had occurred during the residence of the virus in a chick system.[6] No rash or overt signs of infection were seen; no viremia was detected by the usual technics; no virus was recovered from the pharynx after parenteral administration of virus; nor was it demonstrated in the spinal fluid following combined inoculation into the cisterna magna and the cerebral hemispheres. These findings were in striking contrast to the observations recorded following infection with the virulent kidney cell progenitor via identical routes. In all cases, however, this essentially inapparent infection was followed by the development of both neutralizing and complement-fixing antibodies comparable to those measured after inoculation of virulent virus. Challenge of monkeys previously immunized with the chick cell attenuated virus revealed a solid state of immunity against the virulent strains.

This evidence for attenuation in virulence with retention of antigenicity encouraged us to assess the response of susceptible children to the chick-adapted virus. The essential steps in the preparation of vaccine from the 14th chick cell passage of the Edmonston strain have been described elsewhere.[1] After extensive safety tests in appropriate media, and animals, and finally in immune adults, trials were initiated in susceptible children. A second vaccine was prepared from Edmonston virus subjected to additional passages in chick embryos and chick cell cultures in hope of inducing further attenuation. These two vaccines were initially designated A and B, respectively. They have not differed significantly in the incidence and severity of reactions following administration and will not be considered separately in our comments on clinical experience.

Clinical Evaluation

The first study which employed the attenuated Edmonston strain as an immunizing agent in susceptible children was carried out in September, 1958.[7] Thirteen residents of a state institution for the mentally deficient were inoculated at that time. In the three years that have passed since then more than 10,000 children and a few adults have received similar vaccine in one fashion or another. Special trials have been completed to assess its effects in both well children and those suffering from a variety of disorders. These have included individuals with asthma, cystic fibrosis, congenital and rheumatic heart disease, tuberculosis, malnutrition, various endocrinopathies, epilepsy, cerebral palsy, rheumatoid arthritis, nephrosis, and leukemia. Only in the last, the leukemic children, has vaccination seemed contraindicated.[8] Subjects have ranged from four months to 32 years of age. A remarkably consistent pattern of clinical, serological, and prophylactic responses has emerged, similar to that reported previously.[9] These will now be described and illustrated in more detail.

Table 1—Major Clinical Response of 200 Seronegative Recipients of Chick Cell Vaccine

Fever (>100° F p.r.)	80 Per cent
Mean maximum	102.6
(Range)	100.2-106
Mean onset	7 days
(Range)	5-12
Mean duration	2.8 days
(Range)	1-6
Exanthem	**45 Per cent**
Mean onset	10.5 days
(Range)	7-15 days
Mean duration	2.3 days
(Range)	1-5

Table 2—Comparison of Natural Measles and Vaccination Response

	Measles	Vaccine
Incubation period	10-12 days	7 days
Catarrhal symptoms	Major	Minor
Communicability	Marked	Absent
Disability	Marked	Minimal
Duration	7-11 days	5 days
Complications		
Bacterial	5-15%	None
CNS	0.1-0.25%	None

Clinical Response

When attenuated measles virus vaccine was administered to susceptible subjects as a single subcutaneous or intramuscular injection there was no immediate or local reaction at the inoculation site. Seven to eight days later the majority of individuals were febrile for an average of three days, following which approximately 45 per cent developed an exanthem that was pink, macular, nonpruritic, discrete, and sparse in distribution. The data observed in an early study of 200 susceptible children who received this vaccine are summarized in Table 1. Fever attained a mean maximum of 102.6° F p.r. in the 80 per cent of vaccinees who manifested a temperature greater than 100° F p.r. but the range was wide with 20 per cent noted to have an individual reading of 103° F or higher. Although rash in the majority of those observed was confined to the neck, cheeks, and upper trunk, a few showed widespread involvement of the entire body. Rash and fever were the two major signs detected, but a small number of children displayed transient irritability, anorexia, cough, mild conjunctivitis, coryza, and abdominal discomfort. Previously immune individuals, as might have been anticipated, showed no clinical response at all.

In an effort to contrast the response to vaccine with that of the natural disease, Table 2 has been prepared. The shorter incubation period may permit vaccination, when instituted quite early in the course of an epidemic of the natural disease, to halt the spread by a sufficient and rapid reduction in the susceptible population at risk. Minor degrees of catarrh have been seen in a few vaccinated individuals only. Since vaccine virus cannot ordinarily be recovered from throat, urine, or blood of recipients, the absence of communicability was not surprising. Most encouraging was the opinion of both parents and physicians that even at the peak of vaccine response children pursued their usual activities and displayed a striking lack of disability. In a small number of cases there has been a report of transient "fussiness" or irritability. To date, neither secondary bacterial infections nor central nervous complications have occurred.

Serologic Response

More than 95 per cent of children who received vaccine have demonstrated a prompt antibody response beginning usually on the 15th day postinoculation, attaining a maximum level at three to five weeks and then leveling off gradually over the succeeding 6 to 12 months. Table 3 presents data relating to the serologic conversion of 311 children who had no detectable complement-fixing antibodies to measles virus in their initial serum specimens. Ninety-seven per cent showed a fourfold or greater rise in CF antibody fol-

Table 3—Serologic Response to Edmonston Chick Cell Vaccine

Number of susceptibles vaccinated (seronegative by complement-fixation test)	311
Number with antibody rise (fourfold or greater)	301
Number failing to respond	10
Per cent with antibody response	96.7%

Table 4—Antibody Titers After Attenuated Measles Virus Vaccine

Time	Complement-Fixing		Virus-Neutralizing	
	Range	Geometric Mean	Range	Geometric Mean
0 day	<2	<2	<4	<4
36 days	32-128	56	90-300	160
6 months	4-32	10	45-180	99
12 months	<2-32	7	11-105	37
18 months	4-64	10	13-128	44
30 months	<2-64	7	23-106	65
36 months	4-256	16	27-128	100

lowing vaccination. Of the ten nonresponders, seven were found to have virus-neutralizing antibodies in their first serums, evidence of previous immunity conferring resistance to reinfection with either attenuated or virulent measles viruses. In every study, there has been a small number of such children or adults who showed no detectable CF antibodies in the lowest serum dilution but who did have measurable virus-neutralizing antibodies which rendered them immune to infection.

Several other instances of immunity in the face of a paradoxical absence of detectable antibody have been encountered. Children of any age who have had injections of gamma globulin during the past six to eight weeks and infants from five to eight months of age showed no clinical or serologic response to Edmonston vaccine, presumably because of passive immunity protective in its effect though no longer quantitatable by our usual technics. Active immunity is assumed to persist in patients with known agammaglobulinemia after a single attack of measles and in a few rare adults whose serums failed to reveal any antibody despite a past history of measles and resistance to challenge with vaccine or natural disease.

Table 4 lists the range and geometric mean titers of both complement-fixing and virus-neutralizing antibodies persisting over a three-year period in the serums of 11 children vaccinated in 1958. These titers are quantitatively comparable to those acquired after an attack of measles[10,11] and qualitatively are indistinguishable in vitro. This strict parallelism to the patterns seen after the natural disease encourages us to anticipate indefinite persistence of such vaccine-induced antibodies.

Prophylactic Effect

Only a limited amount of data has accumulated to date regarding the protective effect of Edmonston vaccine. After intimate exposure to known cases of measles, 95 children who had been vaccinated 2 to 21 months previously failed to manifest any sign or symptom of this illness. As shown in Table 5, their unvaccinated colleagues suffered a 50 to 100 per cent attack rate on simultaneous exposure. Family and nursery school exposures accounted for 39 and intimate institutional contact for 56. That attenuated measles virus vaccine produced highly effective protection against natural measles is apparent from this data.

Other Programs

The use of Edmonston vaccine in conjunction with human immune globulin will be discussed by other contributors to this session.[12] In addition it is proper to call attention to the investigations of Zhdanov and Fadeeva who employed both Edmonston virus and a strain isolated in Moscow,[13] Smoro-

Table 5—Prophylactic Effect of Edmonston Vaccine (2 to 21 Months Postimmunization)

Locale	Vaccinees at Risk	Measles Rate on Exposure	
		Vaccinated	Control
Boston families	26	0	50%*
Cleveland families	13	0	100%
New York institution	32	0	76%
Baltimore institution	24	0	74%

* Gamma globulin given exposed controls.

dintsev and his colleagues who have reported on the use of vaccine originating from their Leningrad strain,[14] and Okuno and his co-workers who described trials of a vaccine consisting of their Toyoshima strain of measles virus.[15] In those instances where successful results were obtained they have not differed markedly from those reported here. However, most of these studies carried additional disadvantages, including the use of vaccines prepared in monkey kidney tissues or in malignant cell lines or a diminished antigenicity necessitating two or three injections rather than a single inoculation. The most promising recent report has been that of the preliminary tests by Schwarz[16] of a vaccine prepared from the Edmonston virus carried through 77 further passages in chick embryo tissue culture. Tested in 70 susceptible children, this material retained its antigenicity but showed a diminished reactivity with a mean maximal rectal temperature postvaccination of only 100.7° F. Further trials of this vaccine are awaited eagerly.

Summary and Conclusions

The steps in the development and evaluation of an attenuated measles virus vaccine have been reviewed. Three years' experience with such a vaccine has been briefly recounted. The results of these investigations demonstrate that adaptation of the Edmonston strain of measles virus to chick embryo cell tissue culture has decreased its pathogenicity for man while retaining its antigenicity. Whether complete attenuation can be achieved has not yet been shown, but further research is in progress with this as one objective. However, at this time we do not feel unduly optimistic in looking forward to the day when measles may be effectively eradicated as a cause of morbidity and mortality in this and other nations.

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This study was supported in part by the Office of the Surgeon General, Department of the Army, and sponsored by the Commission on Viral Infections of the Armed Forces Epidemiological Board, and in part by Research Grant E-1992, Public Health Service, U. S. Department of Health, Education, and Welfare.