The Mode of Action of Gamma Globulin in the Prevention of Measles

By

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It was Degkwitz [4] who broke ground for the prevention of measles by recommending the use of convalescent serum and, later, of the serum of adults. Subsequently, the effectiveness of passive immunization has been improved by the introduction of gamma globulin.

Administration of adequate quantities of gamma globulin at a proper time modifies the clinical and immunological consequences of measles infection. Modification of the clinical course may mean fully symptomless infection or various degrees of mild measles. The amounts of antibody required for complete protection and those required for modification have been determined by Csapó [3]. According to his data, which were calculated for 10 per cent gamma globulin preparations, minute amounts (0.02 ml per kg body weight) are sufficient when given before exposure. In this case the antibodies prevent the virus to penetrate into susceptible cells, *i.e.* prevent the infection. If gamma globulin had been administered immediately after exposure, the antibody requirement was found to be strikingly increased, but requirement increased slowly with the postponement of passive immunization to later stages of the incubation period.

Up to some years ago the immunological problems of measles could only be investigated by epidemiological methods. The value of such studies was questionable because the lack of clinical symptoms after challenge might have been due to insufficient exposure as well as immunity. It was the cultivation *in vitro* of the measles virus by Enders and Peebles [5] that resulted in the neutralization test, a method allowing to investigate the immunological problems of measles with proper accuracy.

It is generally accepted that the immunity following natural measles is life-long. According to epidemiological observations, the immunity that follows modified measles is also life-long [1, 7, 8]. Subsequent serological studies have confirmed these early observations. In contrast, the passive protection resulting in a lack of clinical symptoms was generally regarded as pure passive immunity, which thus expires when the passive antibody has been excreted, although Degkwitz had anticipated that the

virus while multiplying in the organism before the administration of convalescent serum might induce antibody production. Evidence of this concept was only obtained after proper serological tests had been developed.

Black [2] was the first to publish exact data concerning the question. Thirty-eight children living in an institution were exposed to measles. Administration of 0.2 ml 16 per-cent gamma globulin to these children on the 5th day of incubation secured full symptomlessness. In spite of this, 19 children produced antibodies up to an average titre of 1 : 22. Five of these proved to be immune against natural infection seven months later. These observations drew special attention to the possible existence of "inapparent measles".

In the present studies we wished to re-examine these surprising findings, on the one hand, and to obtain data on the duration of the immunity acquired during the period of passive protection, on the other.

Materials and Methods

Children. Twelve children about two years of age with no history of measles were exposed to massive measles infection at an exactly known time. From this time on the children were under careful clinical control. They were examined physically, and their temperature was measured, several times every day and, from the 8th day on, the leucocyte count was determined daily. We wished to allow the virus to multiply as long as possible without consequent clinical symptoms. For this purpose gamma globulin was administered later than usual; to six children on the 7th day, to the other six on the 8th day, of incubation. In order to achieve full protection, the dose of 10 per cent gamma globulin was raised to 2.0 ml per kg body weight, i.e. four times the minimal dose calculated by Csápó for these days of incubation. To demonstrate the degree and duration of passive immunity brought about in this manner, three seronegative children, who had not been exposed to measles, were given the same relative dose of gamma globulin.

Blood was taken (i) on the day of exposure; (ii) on the 4th day after the injection of gamma globulin; (iii) one month and (iv) one year later. A 5th sample was taken after 21 months if it seemed to be necessary. From the three uninfected controls blood was taken immediately before the administration of gamma globulin, and four and 30 days later.

Neutralization tests. The serum samples were titrated against 30 to 100 $TCID_{50}$ of the Leningrad 4A virus in the AM-57 continuous line of human amnion cells, except the 21-month samples, which were titrated in HeLa cell cultures. In general, serial fourfold serum dilutions were prepared from 1:4 to 1 : 1024 (final dilutions with virus). The serum-virus mixtures were kept at $+4^{\circ}$ C overnight. Three tube cultures were inoculated with each of the mixtures, 0.1 ml per tube. The results were read, in general, 7 days after inoculation. The highest dilution that neutralized the virus in one or two of the three tubes was accepted as the neutralization titre. When a dilution causing neutralization in all three cultures was followed by another showing no neutralization, the geometric mean of the two dilutions was regarded as the titre. In Table I $<1 : 4$ means that the 1 : 4-diluted serum definitely delayed and moderated the cytopathic changes without causing complete neutralization in any of the cultures; 0 titre means no sign of neutralization at 1 : 4 dilution.

TABLE 1

Clinical symptoms, leucopenia and neutralization titres shown by the children under study

No.	Initials	Fever	Rash	Leuco-penia	Neutralization titres+				
					0 day	4 days	1 month	12 months	21 months
		after exposure			after administration of gamma globulin*				
1.	M. E.	—	+	—	0	32	64	64	4
2.	N. É.	+	+	+	0	32	128	128	64
3.	B. J.	—	—	—	256	256	256	256	n. t.
4.	M. J.	—	—	—	256	256	256	256	n. t.
5.	B. E.	—	—	—	0	128	256	64	8
6.	B. Em.	—	—	+	0	64	128	128	8
7.	J. K.	—	—	—	0	32	256	0	n. t.
8.	M. M.	—	—	—	0	128	64	0	n. t.
9.	H. A.	—	—	—	0	64	128	<4	n. t.
10.	K. E.	—	—	—	0	64	32	0	n. t.
11.	B. G.	—	—	—	0	64	62	<4	n. t.
12.	L. F.	—	—	—	<4	32	64	n. t.	n. t.
C 1.	B. G.	unexposed children			0	64	4	—	—
C 2.	G. E.				0	64	4	—	—
C 3.	O. K.				0	64	4	—	—

+ Reciprocals.
* Gamma globulin was administered on the 7th or 8th day after exposure.
n. t. = Not tested.

RESULTS

During the period of incubation all children were symptomless, except for a slight rise in temperature for several hours after the injection of gamma globulin. The clinical and laboratory data obtained after the 8th day of incubation are summarized in Table 1.

Rash was observed in two cases. On Child 1 the pale pink, non confluent, maculo-papular rash appeared on the face and the trunk 12 days after exposure. The rash was not preceded by fever or catarrhal symptoms, nor accompanied by leucopenia. It disappeared on the 14th day. The temperature of Child 2 rose to 38.2 on the 10th day after exposure. Next day it was normal, but a pale pink, maculopapular rash appeared on her trunk and face. The rash was observable for two days. Catarrhal symptoms were not observed. The leucocyte count fell to 3900 on the 11th day. Child 6 had leucopenia (3200) on the 9th day of incubation. The other children were symptomless during the whole period of observation.

Table 1 also shows the serological data. On the basis of the clinical and serological results the exposed children were classified into four groups. (i) Children 1 and 2 showed clinical symptoms and gave remarkable serological responses, which seemed to be long-lasting; (ii) Children 3 and 4 had had serum antibodies in spite of the negative clinical history for measles; their antibody levels did not change after exposure; (iii) Children 5 and 6 showed no clinical symptoms, but produced antibodies still demonstrable 21 months after exposure. (iv) Children 7 to 12 also gave antibody responses, but antibody could not (or hardly) be detected in the sera of Children 7 to 11 one year after exposure. From Child 12 no one-year sample was available. The neutralization titres of the three control children were relatively high on the 4th day after the injection of gamma globulin, but the antibody level fell to the limit of detectability by the end of the first month.

Discussion

The present serological data have brought evidence that all the 10 susceptible children became infected by the measles virus, although the large amounts of gamma globulin prevented the clinical symptoms to develop, except in Children 1 and 2, who showed some slight symptoms.

Ten exposed children and the three controls had no measles antibodies in their first serum samples. Four days after the injection of gamma globulin all these children had titres ranging from 1 : 32 to 1 : 128, irrespective of previous exposure. These levels of antibody are thus attributable to the injected gamma globulin. One month later the titres of the exposed children showed an obvious divergence from the control values. At that time the passive immunity of the control children was hardly demonstrable (1 : 4) while the titres of the children who had been exposed to measles ranged from 1 : 32 to 1 : 256. These levels exceeded 8 to 64 fold those of the control children. It can therefore be concluded that the passive component was negligible in these titres, *i.e.* all the 10 exposed children gave antibody responses.

According to the clinical course and the serological response, the clinical pictures shown by Children 1 and 2 may be regarded as modified measles, though one might object that the period of incubation was unusually short in contrast to that of modified measles which, as a rule, is prolonged. The shortness of incubation may be attributed either to the injected gamma globulin, the fixation of which to the virus might have caused the early clinical symptoms, or to the unusual intensity of infection.

Similar conclusions may be drawn from the serological data. The titres developed by Children 1 and 2 (1 : 64 and 1 : 128) conform to the average titres observable after modified measles (1: 70, according to literary data) and the relative loss of antibody during the 21 months was in the range observable after natural measles. The

epidemiological observations concerning long-term immunity following modified measles have been confirmed by KARELITZ [6, 7], who found the subjects who had had modified measles within a period of nine years to be immune, while those whose clinical symptoms had fully been suppressed by gamma globulin showed no immunity at the end of the nine-year period. It may thus be expected that the immunity of Children 1 and 2 will persist.

In Children 3 and 4, who had had antibodies in spite of their negative clinical history, the repeated exposure resulted in neither clinical nor serological consequences. Numerous authors have published similar experiences. Antibody titres as low as $< 1:4$ were found to prevent natural infection. Furthermore, these cases suggest that the immunity resulting from natural infection cannot be enhanced by antigenic stimuli from infection by the natural route.

Children 5 to 11 behaved, at the beginning, like BLACK and YANNET's [2] cases, *i.e.* showed no clinical symptoms, but developed active immunity. Even the titres were approximately equal to those reported by BLACK and YANNET. After one year, however, the serological behaviour of these children became inconsistent. The immunity of Children 5 and 6 remained at a considerably high level while that of the other fell to, or below, the threshold of detectability.

Although Children 5 and 6 had shown no clinical symptoms and only one of them had leucopenia (3900) on a single day, one month after exposure their antibody titres agreed with those usually observed after modified measles. It can be concluded that the immunity of these two children resulted from inapparent measles. We expect that their protection will persist.

Inapparent measles may be considered a fortunate form of the modified disease; the symptoms are so mild that they escape the most careful attention; nevertheless, antibody is produced. It should be added that inapparent measles has never been observed to the be source of infection.

Children 7 to 11 lost their detectable antibodies by the end of the first year although the conditions of infection had been the same as for Children 5 and 6. The traces of antibody in the sera of Children 9 and 11 suggest that one or two months earlier antibodies could have been detected even in the other three children.

Our findings raise the question why the virus induced life-long antibody production in some cases and transient one in others. Supposedly the factor that induces an alteration in the immune apparatus, resulting in life-long immunity, is closely related to the illness. When passive immunization prevents the manifestation of illness, the virus, which had not been hindered in its multiplication before the passive immunization, may induce antibody production, but this is only transient in a considerable number of cases. The cause of the qualitative difference between the two types of antibody response cannot be revealed until the corresponding pathophysio-

logical processes have been recognized in detail.

The practical conclusion to be drawn is that gamma globulin should be administered in a late stage, on the 6th or 7th day of the incubation period, as first suggested by CSAPÓ [3] on epidemiological and immunological evidences. The present serological data have confirmed CSAPÓ's conception. Accordingly, a proper dose of gamma globulin, even if given as late as on the 8th day of incubation, may afford full protection. Susceptible children may in this manner develop an active immunity of variable duration, in addition to the passive protection. Thus, late administration of large doses of gamma globulin exerts a double effect, *viz.* provides direct passive protection and induces indirect active immunity. Actually, the risk of modified measles is increased in the case of late administration of gamma globulin, but modified measles will never cause contact cases in passively immunized communities since the children who have failed to develop active immunity are protected by the passive antibodies at the time when contact infection would be possible. The excretion time of the minimal protective dose calculated for the 7th day (0.5 ml of 10 per cent gamma globulin per kg body weight) is approximately 21 days. Thus, this dose is expected to supply protection till the 28th (7+21) day after exposure.

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SUMMARY

Twelve children were given 2.0 ml gamma globulin per kg body weight on the 7th day after exposure to measles. Clinical and serological observations allowed the following conclusions to be drawn.

1. Measles can be prevented with proper amounts of gamma globulin given as late as on the 8th day of incubation, even if the infection was massive.

2. Modified measles may appear under experimental conditions after normal or even prolonged periods of incubation.

3. On the 7th and 8th days of incubation the amount of virus in the organism is sufficient to induce antibody production even when the manifestation of the illness is prevented by gamma globulin.

4. The antibody-indicated immunity that follows inapparent measles may be persistent, but more frequently expires within one year.

5. Exposure to measles failed to raise the pre-existing high antibody level, *i.e.* hyperimmunization in the natural way seems to be impossible.

6. The active immunity following late administration of large doses of gamma globulin renders this method of prevention preferable to the early administration of small doses.

7. Until mass vaccinations will be introduced, late administration of large doses of gamma globulin seems to be the only procedure providing lasting protection against measles.

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