THE USE OF IMMUNE BODIES IN THE TREATMENT OF CERTAIN INFECTIOUS DISEASES (VIRUS AND RICKETTSIAL DISEASES) CAUSED BY INTRACELLULAR PARASITES

With Emphasis on the Need for Early Diagnostic Criteria of Infection*

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In the diseases caused by intracellular parasites, among which one may in general include the virus and rickettsial diseases, the passive transfer of immune bodies for treatment of the exposed susceptible has received scant attention in comparison with other studies related to these diseases and their agents. Such a paucity of studies has resulted, to some extent, from the widely accepted premise that signs and symptoms of these groups of diseases in general appear only after the intracellular causative agent has become established in the parasitized cell, relatively safe from the action of circulating antibodies. In such diseases treatment, even in the earlier stages, has often been regarded as merely closing the barn door after the horse has been stolen. The present discussion suggests that such a premise may not be correct in certain virus diseases and that in the light of such findings as are here recorded, further study is required of the value of the passive transfer of antibodies in the diseases caused by intracellular parasites. It is also suggested, in view of these data, that the development of earlier diagnostic criteria of disease is essential for the lengthening of the period in which parenterally injected antibodies may be considered as treatment; and further, the suggestion is made that the use of immune bodies at any time following exposure of the susceptible individual

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and during the incubation of the disease should be considered as treatment.*

The use of human immune bodies in measles

Although the passive transfer of human immune bodies to the exposed susceptible in measles has received intensive study, until recently the lack of large quantities of convalescent serum and of concentrated preparations of immune bodies has prevented studies of the use of such amounts of immune bodies following the onset of fever.[4, 6] Also, up to the present time conclusive evidence of the presence of measles cannot be obtained until the Koplik spots have appeared, although fever, cough, coryza, and conjunctivitis all may have preceded the Koplik spots. The difficulty, therefore, of selecting a suitable time for the parenteral injection of large amounts of immune bodies in the treatment of measles is very great. This difficulty is well illustrated in Chart 1, which indicates the chronologic order of appearance of the first signs and symptoms, the Koplik spots and the rash, in a group of 44 children within a single enclosed space, 43 of whom developed severe measles. (This group of children was studied in conjunction with Dr. Elizabeth P. Maris, who was chiefly responsible for their care.)

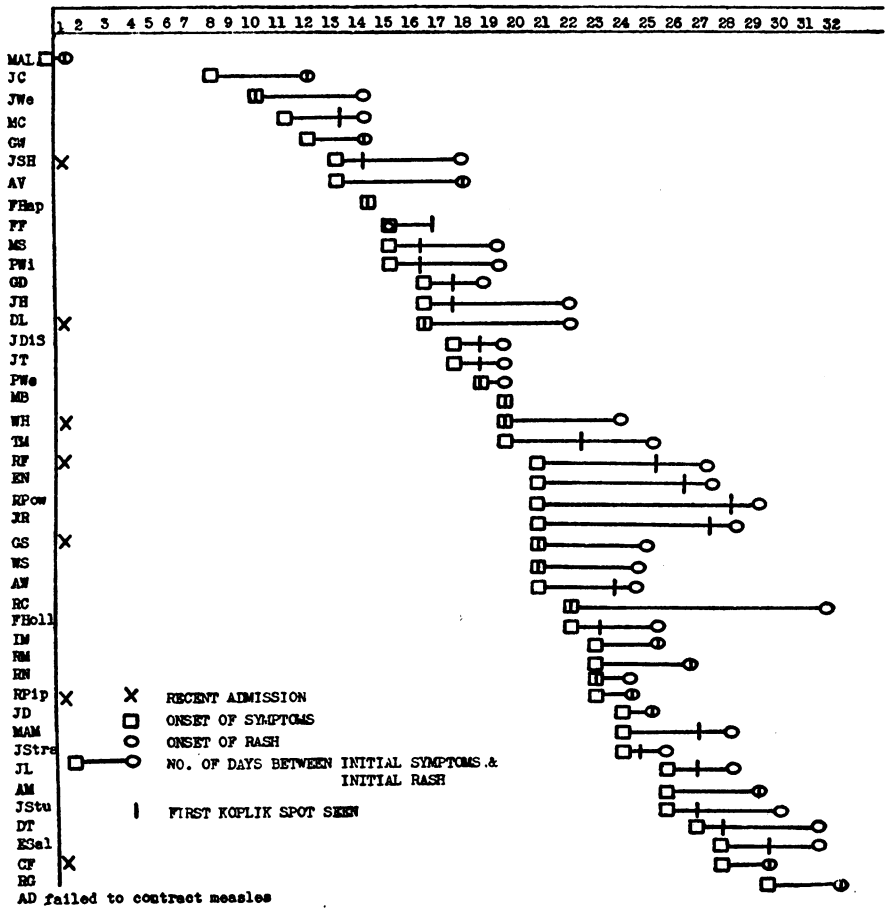
It will be noted that if the diagnosis of measles and therefore the injection of immune bodies in large amounts for treatment had depended upon the time of appearance of the Koplik spots, great variations would necessarily have occurred in the interval between the onset of fever and the time of treatment. Such a variation did actually occur in a group of children injected intramuscularly, following the appearance of Koplik spots, with the gamma globulin fraction II of pooled plasma, fractionated according to the method of Cohn.†

* In measles, as in other diseases with long incubation periods, the use of immune bodies during the early incubation stage has been termed prophylaxis, at least by clinicians. This term should probably be limited to the use of antibodies before incubation has occurred. It may be felt that the term treatment should not be used unless one can be certain the virus is present in the individual. In reply to such a premise it may be stated that the term prophylaxis as applied to injection of antibodies after exposure, is ordinarily used with equal uncertainty as to whether or not the virus is present. After exposure and during incubation, therefore, it would appear to be accurate and less confusing to use the term treatment for the parenteral injection of immune bodies, particularly in view of the findings herein described.

† More complete data concerning these children are to be published shortly by Stokes, Maris, and Gellis.

CHART 1

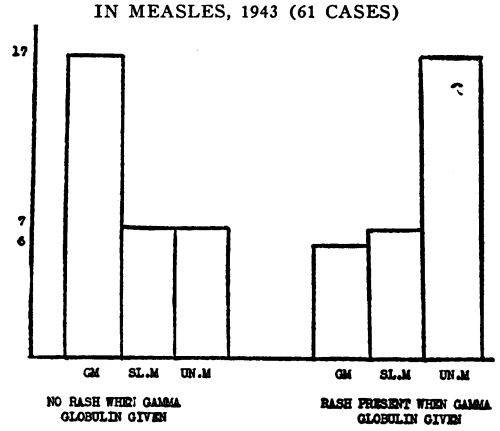
PROGRESS OF MEASLES EPIDEMIC IN A HOME FOR PRE-SCHOOL CHILDREN
PHILADELPHIA—1943



In this group, treatment with large amounts of the globulin containing approximately a 15- to 30-fold concentration of immune bodies, as compared to pooled normal adult serum, was withheld until Koplik spots appeared, but given prior to any rash. This group of children had a striking modification of the disease as compared with a group of cases first treated just after the rash had appeared about the ears and face. The modification obtained in the first group was most clearly evident in those children in whom the Koplik spots appeared more than two days before the appearance

of the rash. In such cases the rash was usually sparse, light in color, and evanescent, and the cough and fever were greatly modified. On the other hand, modification of the disease by gamma globulin was not so consistently obtained in those children in whom the Koplik spots appeared within approximately 24 hours of the appearance of the rash. In two injected children, despite the appearance of fever, conjunctivitis, cough, coryza, and florid Koplik spots, the rash failed to appear and the fever dropped by crisis. As far as we are aware, this phenomenon has not been reported previously in measles and probably affords conclusive evidence of the value of such therapy.* Chart 2, from the same group of cases, illustrates the difference between the value of therapy before the rash appeared and after the earliest evidence of rash.

CHART 2
THERAPEUTIC USE OF GAMMA GLOBULIN IN MEASLES, 1943 (61 CASES)



There were 2 cases where no rash appeared.
GM = greatly modified
SL.M = slightly modified
UN.M = unmodified

The use of immune bodies in epidemic influenza

Little evidence is as yet available in human beings concerning the use of immune bodies in the treatment of epidemic influenza type A, while in influenza type B evidence concerning such therapy is entirely lacking. In man the uncomplicated disease is usually short-lived and specific therapy is extremely difficult to evaluate. Smorodinzew and his co-workers[8] have reported favorable results from the use of homologous convalescent sera by inhalation in the treatment of epidemic influenza type A in large groups of individuals. No confirmation of these findings has been obtained. In mice, however, when they are injected intranasally under anesthesia with dosages of influenza A and B viruses which ordinarily would

* Certain cases occur in which mild symptoms, including Koplik spots, are seen without subsequent rash, but these do not appear to be of a florid type of measles with severe cough and catarrhal signs.

kill in four to six days, death may be prevented by the subsequent intranasal or intraperitoneal injection of homologous immune sera.[9] Such sera are more effective in the prevention of death when administered within 24 hours of the time of intranasal injection of virus, but they may also be effective in influenza A virus even after 48 hours have elapsed from the time of infection and when the mouse is obviously ill with severe influenza. It has been demonstrated also[9] that the amount of serum required for such treatment and protection by the inhalation route is approximately one-tenth of the amount required for similar results by the intraperitoneal route. In the ferret, protection against the more severe complication of pneumonia following intranasal infection could be obtained by similar inhalation of immune sera under anesthesia. It is also worth indicating that in both mice and ferrets, similar injection of immune sera by the respiratory route before infection of these animals with influenza virus type A was highly effective in preventing the development of the disease.

The use of immune bodies in poliomyelitis

Satisfactory experiments for the determination of the effect of homologous antibodies in poliomyelitis have not been carried out under natural conditions of infection and with recently isolated strains of virus. Previous experiments conducted by Schultz and his co-workers[7] have included the difficulties inherent in the use of a virus (M. V. strain) long adapted to monkeys and injected intracerebrally or by intranasal instillation. Similar difficulties are inherent in the use of the Lansing strain of virus injected intracerebrally in mice and with a variable incubation period ranging in control mice from approximately 4 to 14 days. Nevertheless, protection from immune bodies injected intraperitoneally has been obtained in test mice when the immune gamma globulin is thus injected in amounts as small as 0.1 cc. at the time of intracerebral injection of the virus.* A certain degree of protection is afforded by the same amount of gamma globulin injected as long as 96 hours after intracerebral injection of the virus. In these experiments the amount of virus used was 0.1 per cent and the control mice were all dead from poliomyelitis in 15 days. Kramer,[5] in similar experiments in mice with the Lansing strain of virus, has also obtained protection by

* This work has been carried out in conjunction with Dr. Werner Henle, Dr. J. H. Jones, and Miss Claire Foster.

immune gamma globulin after the virus has been injected intracerebrally.

Discussion

Over a period of years a small number of data similar to those above outlined have accumulated which indicate that the use of immune bodies for the treatment of specific diseases caused by intracellular parasites should receive increasing attention.

(a) The use of immune bodies in measles differs from their use in many other virus diseases primarily because of the different group of cells parasitized and secondarily because of the long incubation period of the disease. Accurate knowledge of the development of the virus of measles during its incubation phase and its spread to the cells of the skin and mucous membrane has not been obtained, but in general it may be said that the virus is multiplying in the blood stream during the prodromal phases of the disease while the Koplik spots and early rash are appearing. As the rash becomes more pronounced, it apparently leaves the blood stream and is probably present almost entirely in the cells of the mucous membranes and skin. What the activity of the virus may be during at least the first seven days following the exposure of a susceptible is not known, but at least the parenteral injection of homologous antibodies during this phase of its development will either attenuate or prevent the disease, depending upon the amount used and upon other well-known factors. From the time of exposure, the use of immune bodies has been considered by clinicians as prophylaxis until the onset of fever, after which their use has been considered as treatment. The lack of justification for such a division between prophylaxis and treatment has already been indicated. The fact that by means of immune bodies the rash may be limited after the fever has started to a few lesions on the face and neck, or even prevented from appearing, suggests that the virus is neutralized in transit from one group of cells to another and by a protective action of the circulating immune bodies surrounding the susceptible cells. It would thus appear that future success in the treatment of measles with immune bodies will depend to a great extent upon the development of methods for earlier diagnosis of the disease. In most instances the first appearance of Koplik spots will be a great aid in such treatment, but in certain instances, as indicated previously, their appearance is close to or almost co-incident with the rash. Obviously also, a history of exposure would assist in the diagnosis.

The fact that treatment in the children mentioned was more successful when the rash appeared two to three days after the appearance of Koplik spots may only indicate that the rash was delayed by gamma globulin. However, this does not seem probable inasmuch as immune bodies injected soon after exposure do not appear to lengthen the time between the appearance of the Koplik spots and of the rash, even though they attenuate the disease.

(b) In attempts to investigate the value of immune bodies in influenza by means of animal hosts, life or death must too frequently be used as the criterion of success or failure of a therapeutic measure. Particularly in small animals, fever and symptoms cannot be properly recorded and even in larger animals studies such as those required for measles or epidemic influenza are usually hampered by the lack of the secondarily invading bacteria so common and serious in the human host. Thus one would not be justified in the conclusion that homologous immune serum is of little value in the treatment of human influenza type A at the height of the disease merely because of the fact that infected mice cannot be prevented from dying if the immune serum is administered more than 48 hours from the time of intranasal injection of the virus. On the other hand, because the respiratory tract of the mouse is relatively small, it is possible that impracticable amounts of homologous immune sera would be required by inhalation in the human being for the same favorable result as is obtained in the case of the infected mouse. The respiratory tracts of the ferret or of the hog are more closely analogous to that of the human being and here also secondarily invading bacteria are more common, but unfortunately the practical problems of the use of immune sera in such animals have not proceeded jointly with the studies of the causative agents of epidemic influenza and their effects.

The adaptation of the intracellular parasite in influenza to its new animal host or to the chick embryo and the resulting antigenic changes in the parasite make it more difficult than is the case in bacterial pathogens to transfer the results of the use of immune bodies from these hosts to the human host. That such antigenic changes are greater in influenza virus type A than was at first supposed is suggested by the more recent work of Burnet concerning the O and D forms of virus.[2] The differences in the synergistic activity with bacteria of the human influenza virus type A and the swine influenza virus as studied by Bang[1] suggest also that if immune bodies are

to be used in treatment, their antigenic source must be properly analyzed.

Possibly in virus diseases other than influenza A, if a more accurate knowledge of the mode of attack were available, a more effective application of immune bodies could be obtained. The virus enters the respiratory tract usually by the airborne route and probably progresses along the epithelium from above downwards or outwards from its point of impingement, denuding the epithelium to the basement membrane. Resistance to the virus depends apparently not so much upon the epithelial cells of the respiratory tract as upon the antibody titer of the secretions or fluids which bathe them and which in turn reflect the antibody titer of the blood plasma.[3] Circulating antibodies may not, therefore, be so important as is the concentration of antibodies locally, and in this manner the use of concentrated antibody preparations such as immune globulin may be of great assistance. Also, the method of spread of the virus locally suggests that only certain epithelial cells are parasitized first and that the progress of the viruses from cell to cell may be checked or completely stopped by immune bodies locally or parenterally administered. A sufficient concentration of antibodies at the proper point and sufficiently early in the course of the disease in human beings may be difficult to obtain. Studies in which human volunteers are exposed to large amounts of virus by inhalation do not duplicate field conditions. The virus under field conditions may be more virulent but the exposure to it may be less intense than under experimental conditions. Nevertheless, the favorable results in animals justify further studies of the use of homologous immune bodies, particularly for treatment in the larger animals and in human beings under epidemic field conditions. Even moderate diminution in the severity of the disease would appear to justify such studies.

(c) In poliomyelitis the need for additional data is even more striking. In studies of passive immunization and particularly in the studies of treatment with immune bodies, the lack of the proper type and amount of immune bodies, the lack of suitable animals, the variability of incubation period, the differences in strains of virus, and the low incidence of human cases have all tended to prevent the development of sufficiently critical experiments.

In the studies on mice mentioned previously, evidences of illness and paralysis in almost all instances occurred within approximately 24 hours of death, because no satisfactory method has been

developed for determining early symptomatic or febrile evidence of poliomyelitis in these animals. It is possible that the mouse, which is protected by heterologous immune bodies when injected intraperitoneally 96 hours after the intracerebral injection of virus, is symptomatically ill from the virus at or near the time of the intraperitoneal injection. Many of the infected mice when immune bodies are not injected die within 4 to 8 days of the time of intracerebral inoculation. Intracerebral inoculation of virus may be considered also as a severe test of the effectiveness of immune bodies. In view of these considerations, further studies are indicated concerning the early recognition of signs and symptoms of the disease in the mouse as well as in the monkey and the development of a method of inoculation of virus in such animals which would permit a more constant incubation period and under conditions which would resemble more closely those of natural infection.

From a general consideration of these three infectious diseases as related to others caused by intracellular parasites, it is obvious that the value of immune bodies varies with each agent of disease, its site of attack, the incubation period, and a number of other factors. It appears probable that upon the concentration or availability of the immune bodies at the time the cells are selected for attack by the parasite depends the success of such therapy. In poliomyelitis, for example, the concentration of immune bodies at the site of attack may be far less and thus less effective than for influenza in which immune bodies may be concentrated in the secretions, the lymph, and upon the surface by inhalation. In neither of these diseases, however, are the limiting factors thoroughly understood.

In the rickettsial disease Rocky Mountain spotted fever, it has been demonstrated that death can be prevented in infected guinea-pigs if sufficient homologous antibodies are injected during the early febrile period of the disease. Similar observations in human beings appear to confirm these findings.[10] As in measles, the value of immune bodies is evident after the febrile onset of the disease has occurred, but only if they are administered shortly after fever has appeared.

From the foregoing findings, the conclusion may be drawn that for each intracellular parasite the appropriate time should be determined experimentally beyond which the parenteral injection of antibodies, even in large numbers, is no longer effective. It also may be concluded that if large amounts of antibodies are effective in

modifying the disease when they are injected in its early stages, they would be more effective in modifying or attenuating the disease if even earlier injection were made possible by earlier diagnosis.

Conclusions

The effective use of gamma globulin from large pools of normal adult plasma in the attenuation of measles even when injected after the onset of fever and the appearance of Koplik spots indicates the need for the development of earlier diagnostic criteria for this disease and possibly for other diseases caused by intracellular parasites. Data bearing on epidemic influenza type A in mice and ferrets and on poliomyelitis in mice (Lansing strain) also emphasize the need for additional studies in these diseases.

In view of the confusion in the terminology used by clinicians and the need for earlier diagnostic criteria and treatment, it is suggested that the term prophylaxis be limited to the use of immune bodies previous to the exposure of the susceptible individual and that their use following exposure and during incubation should be termed treatment, whether or not one can demonstrate the presence of the disease agent in the exposed susceptible at the time of treatment.

It is anticipated that with the development of more concentrated and effective homologous antibody preparations and earlier criteria of disease, additional intracellular parasites will be brought under more effective therapeutic control.

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