MECHANISMS OF INFECTION AND IMMUNITY IN VIRUS DISEASES OF MAN[1]

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The biologist concerned with the microbic world has numerous approaches with which to gain pertinent information of the agents with which he is concerned. In many instances attention is centered upon morphologic and basic composition of the species studied, but in a great proportion the detection of specific properties and their evaluation are based on measurements of the behavior of the agent upon specific substrates. When a living organism is the major site of recognized activity of the parasite it becomes the substrate; and if the action of the parasite creates injury and active response on the part of the host the reaction constitutes disease. Theoretically, it should be possible to measure the specific components of host and parasite which are involved in a primary injury but the instances in which it has been accomplished are extremely few. And even where special activities of the parasite have been found to be exerted upon definite tissues the manner in which the effects are brought about is in most instances obscure—limited largely to a description of the morphologic changes in cells which the agent attacks or to the type of inflammatory response which follows. The fundamental interactions which determine selective localization and pathologic injury are not known. Conversely, the phenomena which differentiate naturally susceptible tissues and species from the resistant are essentially unknown.

Nevertheless, the ability of the susceptible host to modify his behavior after proper experience with a given agent has been seen to be associated with the acquisition or orientation of mechanisms not commonly encountered in the naturally resistant host. It has been increasingly obvious that regardless of the biologic level of the parasite, multicellular, bacterial or viral, the responses of the animal host are much the same, due in part perhaps to limitations in experimental methods of measuring them. In accordance with this trend a number of features formerly said to differentiate reactions to viruses from those to other infectious agents have fallen away or have required sharp limitations. They include the concepts that permanent immunity is uniform; that antibodies can be elicited only in a fully susceptible animal; that serological reactions such as precipitation, complement-fixation and agglutination are essentially directed against components of infected tissues of the host rather than to the virus moiety; that immunity can be obtained only by active infection.

An effort to bring the reactions of immunity to virus infections into conformity with those of other disease-producing parasites may appear on the one hand to be

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ultra-conservative as seeking to limit philosophic exploration; or progressive in seeking to apply a general law to presumably dissimilar behaviors. Many of the ideas currently debated in the virus field are direct transfers from those of bacterial and other parasitic infections: cellular immunity independent of antibodies; immunity and antibodies derived only by persistence of the parasite; the significance of strain variation; the advantage of infection over inactivated organisms in producing immunity; the meaning of antibodies in terms of antigenic structure; the inefficacy of specific serum therapy. In the latter connection it is important to recall how few are the instances, to date, of effective serum treatment of bacterial diseases or of effective prophylactic immunization apart from those involving circulating exotoxins. One can here point out as well that as procedures for studying the various diseases have improved the role of independent tissue immunity has declined, leaving it a prominent factor in only those diseases for which serological procedures are undeveloped; and that an understanding of the participation of antibodies in terms of bacterial constitution has scarcely been broached.

The patterns followed by viruses in creating disease are as different and complex, or more so, as those observed with other classes of parasites, although the viruses represent a more homogeneous biological group in that they are considered obligate intracellular parasites. Nevertheless, the routes of infection, the tissues attacked, and the character of injury induced vary so widely it seems impossible to consider the mechanisms of immunity except in terms of the mechanisms of infection, i.e., the pathogenesis of the different diseases. Despite the inadequacies in precise data there are many clinical and epidemiological observations of the virus diseases of man which, combined with immunological studies, afford opportunities to visualize the mode of action of viruses as reflected in the responses of the host. Conversely, an understanding of the mechanisms of infection at play offers a much better opportunity to clarify the natural history of a disease and the direction of specific control measures. The discussion will deal primarily with virus diseases of man and the significance of protective antibodies when considered in terms of the disease process, the portal of entry, the type of tissue injury, and the fate of the virus. But the opportunity will be taken to comment on other characteristics of viruses suggested by the study of the disease processes. Discussion will be largely limited to virus diseases of man. Those of plants will be carefully avoided for while they offer many interesting aspects, the reported absence of antibodies, the transformation of a high proportion of the plant proteins to virus, the limited conditions under which infection can be instituted, and other features suggest at present red herrings rather than aids in interpreting diseases of man.

Similarities in disease pattern, pathogenesis, and the agents involved tend readily to separate certain of the virus diseases of man into groups which illustrate the sharp differences in immunity exhibited, and also invite explanations of the common and discrepant features of the constituent groups.

Four sets of conditions suggest themselves: *1*, virus diseases in which infection persists but immunity does not; *2*, virus diseases in which immunity persists but

infection does not; *3*, virus diseases in which neither infection nor immunity persists; *4*, virus diseases in which both virus infection and immunity persist. In order to fit some of the examples into the groups suggested, a certain amount of freedom has been taken with established concepts and the classifications may have been stretched a bit, but with the hope of provocation rather than of pure distortion.

Group 1. Virus Diseases in which Virus Infection Persists but Immunity does not. The agents of the Psittacosis-Lymphogranuloma venereum group clearly belong in this category. In their natural hosts, especially, they may be initially introduced and maintained as resident or carrier infections with little general evidence other than serological reaction to indicate that the virus has been present. Under these conditions the virus is contained by the host in a well adjusted stage of parasitism. But exposed to physiologic insult which may disturb the equilibrium, the host factor is depressed and the virus may assume the offensive, reappearing in organs and their excreta so as to make the animal an active distributor.

In man the details are less clearly established. Nevertheless, with lymphogranuloma venereum there are: the recorded evidence of prostitutes transmitting the disease without significant signs of its presence, the known reservoir among the colored population especially in warm climates, and the continued chronic infection which can occur after chemotherapy. In psittacosis *of man* several instances have been recorded which Meyer has suggested may be actual relapses rather than newly acquired second attacks; and certain individuals have given strong evidence of being carriers.

The fact that other agents of the group follow so distinct a pattern in their native hosts is strong indication to support the available data in man. The viruses of pneumonitis in mice, hamsters, cats, and ferrets have all been encountered in stock being subjected to inoculations attempting to establish other viruses, thus clearly demonstrating the activation of a carrier state.

One of the theses proposed in explanation of continued immunity ascribes that fact to the persistence of virus in the host's tissues. The same thesis also attributes the persistence of protective antibodies for a prolonged period after infection to the continuing stimulus of virus remaining in the tissues. Diseases associated with the group of viruses mentioned are characterized by persistence of virus which tends to maintain itself through the proliferative and exudative response in lymphatic tissue and serous membranes. Rather than exhibiting a permanent immunity, however, they are typically subject to relapse. They represent persistent, unstable but balanced infections instead of high grade immunities. The failure to respond to inoculation of the same agent with a chain of reactions similar to that exhibited by the uninfected host is commonly called infection immunity. It is scarcely immunity but a stage of tolerance to amounts of infectious agent which appear insignificant in terms of the amount available in the tissues of the infected host.

The diseases of this group strikingly exemplify the equilibrium between infection and resistance, between host and parasite. Moreover, if these be invasions

by the host species' indigenous parasites they illustrate how troublesome a problem infection with one's own saprophytes may be. Viruses of the group have also been found to be susceptible to sulfonamides and penicillin, leading to the suggestion that an extra-cellular phase of metabolism is an essential stage in their cycle. It has also been suggested that these agents do not represent the true viruses, but it is difficult to see why they should be separated from a class of agents so inhomogeneous and poorly defined. Certainly, they belong as much as the pox viruses do, and one can speculate that because of their similarities the poxes may be the next ones to respond to drugs and antibiotics.

Infection with the virus of lymphocytic choriomeningitis in mice, dogs, monkeys, and probably man displays similar unstable continued infection even when antibodies are present in good amounts.

Special care has been taken to avoid the problem of the cold sore, herpes febrilis. The evidence at present available is generally interpreted to indicate that the initial experience with that virus produces a diffuse infection and a general immunity with circulating antibodies. There is little information of the persistence of virus deep in the tissues, but several reports describe the residence of virus in the mouth and its appendages. The current concept is, then, that herpes virus persists in superficial tissues and erupts when insult to the area occurs. Furthermore, the individuals in which virus recurs possess good levels of circulating antibody. The picture strongly suggests an accelerated local reaction. From the point of view of pathogenetic pattern, I would be much happier if the recurrent attacks of fever blisters could be shown to be newly introduced infections, for in other ways this virus has close similarities to those assigned to the third group and I shall ask the privilege of taking up certain points at that time even if it be a little out of keeping with usual interpretations.

Group 2. Virus Diseases in which Immunity Persists and Evidence Indicates that Virus does not. A. Infections limited to man. The rash-producing infections, the exanthemata, of virus etiology appear historically and epidemiologically to be diseases which in their evolution have become so thoroughly adapted to man as to have eliminated other hosts from their infection cycle. Smallpox, measles, chickenpox, are characterized by high transmissibility by the respiratory route, an incubation period of ten to twenty days, a period of fever, catarrhal symptoms, and blood invasion followed by florid cutaneous localization. Second attacks of the full blown disease do not occur so that immunity appears to be a permanent result of the primary attack. Relapses are not noted. Nor do segregated populations which include a nucleus of previous cases of disease appear to derive infection or immunity from association with those individuals who should be the carriers of virus. Infection is introduced by the fresh case. Here, then, are instances of virus disease which are maintained apparently by the human species alone, which elicit prolonged immunity, which result in the production of antibodies for many years, but in which evidence of continued residence of the virus has never been presented. Interpretation of the pathogenetic mechanisms offer, however, a reasonable explanation of why this group of diseases yield lasting immunity.

The portal of entry and the primary site of localization of virus appear to be in the upper respiratory tract from which the virus progressively extends. With the onset of acute illness invasion of the blood stream takes place and, along with lesions of the mucous membranes, a secondary localization of the virus occurs in the skin producing the characteristic rash upon which clinical identification largely depends. After recovery, a rich supply of circulating antibodies is provided and if virus were again to be introduced it might be able to establish itself superficially in the mucous membranes, but the stage of extension through the blood to produce the diagnostic eruption would be eliminated by the circulating antibodies which the virus would encounter. Under these conditions it is not unreasonable to believe that repeated upper respiratory infections with measles, smallpox or chickenpox viruses can occur and that the presumed permanent immunity is, in fact, immunity only against the generalized disease.

That measles can actually occur a second time is seen under two conditions. Rake, Stokes, Shaffer and their associates, cultivated in eggs what appeared to be measles virus as demonstrated by its ability to elicit a mild disease with Koplik spots and rash. Among infants tested for immunity to unmodified measles within a few months after the modified disease, clinical measles again occurred, indicating that infection with an active virus had not resulted in uniform immunity.

It is extremely interesting in terms of pathogenesis of the disease to observe the gradations from absence of disease to but slight modification of measles which can be attained by use of antibody after exposure to infection. The fact that complete protection is readily gained within three days after exposure suggests that either the virus has not entered vulnerable cells by that time or that the number of cells parasitized is too small to yield a significant amount of virus for transportation beyond the portal of entry. The modification of disease which takes place when serum is not given until the sixth day suggests that virus has been sufficiently distributed so that great amounts of antibody are required to catch all that has been produced. The modified disease is, however, measles infection and pediatricians assure me that children having had the modified disease can contract measles again after a later exposure, demonstrating that mild, active disease has not given permanent immunity.

Moreover, vaccine virus may be attenuated to such a degree that while a primary lesion results from its inoculation it does not give more than a brief protection to the more virulent parent lymph-strain. Even with a potent vaccine strain, immunity is not permanent although vaccination represents frank infection with active virus. The modified strains might well be considered to represent stages of parasitism which could or should become resident and persist, but do not. These observations also indicate that induced variation and attenuation must be of a limited nature so as to retain the proper immunogenic components in order to function protectively.

One wonders whether these diseases, with their fixed patterns and prolonged incubation periods in man, have not come to depend upon their presence in the

human host to complete a definite phase in their life cycles, thus accounting for their uniform, predictable behavior, and whether the persistent immunity is related to a genetic conditioning of the human cell—acquired through long experience with the same agent—which results in ready production of immune substances. Certainly, there is no reason to believe that secondary exposures and minor infections would not participate as secondary stimuli of the flush mechanism or booster effect.

B. Infections transmitted by biting insects. This group of diseases is derived from other hosts and transmitted to man by insect vectors which introduce the virus into the blood as feeding occurs. The site of essential injury is a distant organ to which the agent is transported by way of the blood stream and there localized to multiply and produce the characteristic disease. Yellow fever is a splendid example as is the group of viruses associated with acute encephalitis which appear to be variants of one basic agent. In one instance, the liver serves as the major site of injury; in the other, the brain. Mosquitoes are the most frequent vectors and apparently bring the virus to the human individual in a vegetative stage readily adaptable to human tissues, since prolonged incubation periods are not required. Immunity develops after infection and is persistent; that from yellow fever is essentially permanent. It is easy to see why. Antibodies are present in the blood so that when virus is again inoculated by the blood sucking insect, the virus introduced into the blood is immediately met by antibody and neutralized. There is little evidence of persistence of virus in man (the virus of St. Louis encephalitis persists for a time in experimental animals), relapses of infection are not described, and artificial immunization can be effectively done. The conditions are ideal for circulating antibodies to be most efficient. Other diseases presenting similar mechanisms of infection should behave in a similar fashion. Three years ago I ventured to predict, because of the disease pattern, that if dengue did not result in permanent immunity it probably indicated the existence of antigenically different strains, a fact since demonstrated by the beautiful studies of Sabin.

It is of further interest to point out that inactive virus can induce immunity to Japanese B encephalitis and equine encephalomyelitis, whereas the antibody titer resulting from vaccination with active yellow fever virus gradually declines although it represents the response to an active infection.

Group 3. Virus Diseases in which neither Virus Infection nor Immunity Persists. In contrast to those virus diseases of man in which evidence points to an effective, prolonged immunity there are certain others in which evidence of repeated infection is clear. Among them are influenza, common cold, foot-and-mouth disease, possibly poliomyelitis, and herpes, maybe. Although they appear at first glance to have little in common, they are characterized by short incubation periods and by the fact that the primary injury is to a superficial tissue.

Regardless of whether herpes virus is aroused from the tissues or freshly introduced, the recurrent infection is limited to a local lesion in the superficial squamous epithelium. The virus can become established and institute injury in

surface cells not readily reached by antibodies in the circulating blood which, nevertheless, can and *do* limit extension beyond the local site. Recently Evans, Slavin, and Berry have demonstrated that induced passive immunity can, in the experimental animal, similarly restrict infection with herpes virus to a local lesion; results duplicating those obtained with foot-and-mouth disease by earlier investigators. In fact, the pathogenesis of recurrent local infection in foot-and-mouth disease bears close resemblance to that presented for herpes.

Influenza virus selectively destroys the ciliated epithelium of the respiratory tract. In this instance, again, the susceptible cells to which the virus is readily introduced through inspired air, are superficial and essentially extravascular. Repeated infection with the same virus can occur in man or animal even though antibodies are present in the blood, and the epithelium is more than once a site of injury. But the damage is limited to a local lesion and the extension of injury to characteristic pulmonary disease is prevented. It has been clearly shown in influenza that the secretions of the respiratory tract may contain antibody which is enhanced in amount after infection or vaccination, paralleling at a lower level the content of antibodies in the blood; and it seems probable that the antibody exuding into the local secretions are the significant factors in determining whether virus can become established while the circulating antibody itself bountifully supplies and protects the lungs. The protective effect of vaccination by para-respiratory route can be attributed to its influence upon the antibody content of the respiratory secretions. On the other hand, repeated infections may, in addition, result in functional modifications of the epithelium which make it less likely to virus injury. Persistent virus in man has not been demonstrated. In swine, Shope has presented evidence that virus in the form of masked marvels can be maintained in worm parasites in the hog's lung,—*but* it does not give rise to immunity, and disturbance of that equilibrium incites disease.

Although influenza virus behaves as a perfectly good member of that class of agents, certain features are intriguing enough to stimulate the question as to whether it is of necessity an obligate intracellular parasite. The speed of its action is such that clinical disease occurs and cell destruction takes place in as little as twenty-four hours. In vaccinated animals the process may even be accelerated but virus is difficult to detect, thus suggesting its superficial neutralization. The manner in which influenza virus attaches to red blood cells and elutes is a striking example of virus attachment without apparent penetration. Moreover, Hirst has shown that the same phenomenon occurs when the virus is brought in contact with respiratory tissues of the ferret or mouse. Virus adsorbed to red cells is apparently still neutralizable by antibody. The comparative inefficacy of repeated dosage of virus in heightening antibody titer suggests that a surface antigen is of primary significance. If virus be given to mice by the intraperitoneal or intravenous route, it ordinarily does not cause typical pulmonary lesions although plenty of virus can be found in suspensions of lung tissue. One might anticipate that the virus would be able to penetrate the desired cells from the other side of the membrane, but lesions seem to occur

only when very large amounts are given. In that case the virus appears to overflow from the blood into the upper respiratory tract where it takes up its pathogenic action on the surface of the epithelium. Supporting this concept, experiments, as yet unpublished, have shown that a small amount of serum given into the nose protects against pulmonary disease from virus administered intraperitoneally. It might be expected that a virus which produces its effect so rapidly would penetrate the cell rapidly, as other viruses appear to do. Further evidence that it may not is seen in the observations of numerous workers that several hours after the intranasal administration of virus, immune serum given by the same route may prevent significant infection in mice. Moreover, Magill and I were able to show that influenza virus which had been exposed to susceptible cells in tissue culture for an hour, at least, could still be neutralized by immune serum. On the presumption that antibody does not penetrate the cell these various facts point to a continued existence at the surface. It may be that the surface stage is the period of essential damage and that cytolysis is a secondary autolytic phenomenon unrelated to intracellular virus and that the virus may obtain at the surface the materials required for multiplication.

To return from this digression, how does poliomyelitis fit into the group pathogenetically? The idea that the initial infection of poliomyelitis virus is of the alimentary tract has been accumulating support. The presence of virus in the mucosa of the pharynx and intestines strongly suggests a superficial localization although the higher incidence of paralytic disease in younger age groups may be an indication that first experiences are frequently more penetrating.

Accepting the idea that antibodies in the blood represent previous experience with a virus of poliomyelitis, the fact that more than one infection can take place is seen in the observations that antibodies may be present in the blood at the time clinical disease is recognized. Virus in considerable amounts may be demonstrable in the stools of apparently well individuals with circulating antibodies, indicating that propagation of the virus is going on in tissues unaffected by antibody of the blood. Moreover, in monkeys second infections can be frequently instituted under a variety of conditions. In general, while the data indicate that recovery or growth does reduce the probability of severe disease, repeated infections of the superficial alimentary tissues may be experienced.

Rabies is not a superficial infection and does not belong in this group. Nevertheless, the virus is ordinarily introduced by bite directly into the nervous tissue where it may escape circulating antibody. On the other hand, proper vaccination with inactive virus appears to have a protective effect, although it is not clear how much of the effect of active virus may be related to an interference rather than to specific immunity. Immune serum given into the area of injection of virus has been shown to be effective in protection, again by countering virus at the initial site.

There is one additional factor in this group of virus infections which adds to the difficulty of maintaining permanent immunity. That is the existence of antigenically variant strains. The significance of certain minor variations is not at all times clear but the broader type differences are unquestionably clear.

And so the superficial type of localization which appears to limit the efficacy of antibody unless available at that site and the existence of multiple strains of virus combine to give temporary immunities.

Group 4. Virus Diseases in which both Virus Infection and Immunity Persist Independently. In those instances of tumors definitely incited by viruses the circumstances differ from any of the other groups discussed—although resembling some features of Group 1—in the continued stimulation which they apply to cell growth. It is commonly accepted and the serological data support the idea, that virus may be transferred through numerous generations of cell division while antibodies in the surrounding fluid are helpless except when cell destruction takes place, a state which Rous has termed a clandestine relationship. Nevertheless, the antibodies are capable of limiting extension of the process as is shown by the fact that with Shope papilloma the tumors do not readily migrate to the adjacent areas of the skin. Moreover, there is seen at times a sudden regression of growth indicating that immunity has gained ascendancy. Green has recently suggested that in the tumors the virus actually hybridizes the cell so as to alter its specificity and make the virus antigen the dominant characteristic. In the mouse mammary cancer, immune serum added beforehand inactivates the presumed virus which resides within the cell transplants. Perhaps to the present discussion the most pertinent point is, however, that among the virus tumors persistence of virus is associated with active evidence of infection circumscribed by a demonstrable, persistent immunity and that the limited effectiveness of antibody is related to the mechanism of virus action. The manner in which the two agencies, virus and antibody, exhibit their respective influences almost independent of one another further indicates that complete immunity is not derived from active infection and persistence of virus.

COMMENT

In general, it appears that immunity in virus diseases is, as with infections by other agents, related to the interplay of antibodies and the cells of the body, varying according to the mechanisms of infection and the characteristics of the agent. In the instances when virus becomes entrenched in, and protected by cells, antibodies can serve only to prevent the free dissemination of virus by meeting it when it emerges from the cells and by gradually reducing the focus to the point of obliteration. In those circumstances, antibodies to be effective must be maintained at a high level and, if a disturbance in the host's functions is initiated by physiologic insults, the agent may gain the ascendancy through a reduction either in antibody production or in the capacity of the disposal mechanisms to remove the sensitized agent, or both. In most of these instances the important item is not merely whether antibody is present in the blood alone but its concentration and its availability to the tissues where the virus is encountered. This fact is shown by the relative efficiency of immunity according to the route employed for its testing in experimental animals. When unphysiological routes, such as direct inoculation into susceptible brain cells, are employed the degree of immunity is low since virus may be enabled to avoid interstitial antibody.

When routes are employed which give acquired antibody an opportunity to come into action its effectiveness is enhanced and greater protection results. Morgan, Schlesinger and Olitzky, in a series of studies, have shown the variation in the quantitative relationships between the amount of antibody required for protection and the method of testing against the virus of Western equine encephalomyelitis. In order to get detectable antibody in the spinal fluid of rabbits antibody in the blood had to be pushed to high levels, and in these circumstances protection against intracerebral inoculation was achieved. This is similar to the probable behavior of antibodies in furnishing protection to the respiratory epithelium in influenza.

The efficacy of vaccination with inactive viruses is also more reasonably estimated when challenge is made by normal physiologic approaches. In this respect, from consideration of the mechanisms of infection one might predict that diseases in which a blood stream phase is essential to full development are those in which immunity could be most readily induced. The results of passive immunization with measles are a clear invitation to active immunization. The fact that gamma globulin is effective in prophylaxis of infectious hepatitis indicates that the blood stage is an essential part of its pathogenesis and that vaccination should be effective. The encephalitides, already mentioned, dengue and others of that group are similarly inviting. In other conditions the effect will depend upon the amount of antibody that can be made available locally at the portal of entry. Protection of the local area where the virus makes its entry may protect the body as a whole.

It is not intended to imply that continued infection may not influence antibody levels or that cells may not be altered in their receptivity to virus or in their capacity to dispose of virus. Cells may be rendered refractory to infection by virtue of their being infected, as with interference, or by alterations in physiological reactivity which create a non-specific impermeability as is seen to some extent after physical, chemical or developmental stimuli. There seems to be, however, no more need to call upon the virus to persist in order to have continued production of antibodies than in the case of soluble toxins or egg albumin. After the type is set the printing press continues to print.

To summarize, then, antibodies are effective in virus immunity according to the invasive mechanism involved, the type of parasitism and the availability of antibody at the portal at which virus makes its entrance to the body. The behavior of different viruses and their diseases can be interpreted according to these factors, and the behavior of immunity in others can be predicted when certain of the mechanisms are disclosed. The need for physiologic conditions of testing for proper evaluation of immune mechanisms is becoming increasingly apparent, emphasizing that it is not sufficient that antibody be present but that there be enough in the right place at the right time.