Hemagglutination by Viruses—Its Biological Implications

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THE host-parasite relationship in most virus infections is understood poorly because the physical and chemical processes governing the initial virus-host cell interaction have not been accessible to analysis. An understanding of these processes seems of the greatest importance if any advance is to be made in the systematic and not haphazard study of drugs with possible chemotherapeutic action in virus diseases.

The recent literature indicates that progress of great potential significance has been made, permitting a better understanding of the mechanics of some infectious diseases. Research workers investigating the dynamics of hemagglutination by viruses have reported findings which permit a glimpse into the mysteries of virus-host cell relationships. This review presents briefly some of the more important steps in the investigations which led from the discovery of an apparently simple phenomenon, hemagglutination, to the experimental proof that certain chemical substances may interfere with virus multiplication through the blocking of virus enzymes.

The simultaneous discovery by Hirst[14] and McClelland and Hare[21] of the phenomenon of red cell agglutination by influenza viruses was in itself a development of great significance. It provided an exceedingly simple and efficient method of determining the virus content of materials in the test tube, whereas all previous methods were tedious and expensive, using large numbers of experimental animals for virus titrations.

In the hemagglutination test, serial dilutions of virus-containing material are mixed with red blood cells from human, chicken, or guinea pig and the pattern of the settling red cells is observed in 45 to 90 minutes. In the presence of influenza viruses the cells clump in a characteristic fashion. This reaction is specifically neutralized by the serum of immune animals and man. The hemagglutination-inhibition test has formed the basis for the extensive studies on the epidemiology of influenza and the efficacy of vaccines to produce specific antibodies. The methods for this test have been well standardized[23,29] and provide a useful diagnostic tool for the clinical laboratory.

It was believed at first that only viruses of the influenza group possessed this hemagglutinating capacity. Soon it was demonstrated, however,[1,20] that the agents causing two diseases of chickens, Newcastle disease and fowl plague, also agglutinated red cells; and in 1945 mumps virus, grown for the first time conveniently in embryonated hen's eggs,[13] was added to the list.[18]

The mechanism of red cell agglutination appeared similar to that of the clumping of bacteria by specific antisera: The bridges between red cells were virus particles instead of antibody molecules, and the clumps of erythrocytes settling to the bottom of the tube carried with them most of the active virus. A significant difference from ordinary antigen-antibody reactions became evident when it was demonstrated[15] that red cells could be freed from the absorbed virus by incubating them at 37° C., and if resuspended, these same cells could not be agglutinated again by adding fresh virus. The virus eluted at 37° C. from the cells had lost nothing of its infective power or its hemagglutinating ability for other erythrocytes. In fact this virus material could be used over and over again to hemagglutinate and "stabilize" considerable quantities of red cells, suggesting that its activity was that of an enzymatic destruction of the receptor on the cell to which it attached. Subsequently[16] it was shown that similar processes occurred between influenza virus and its receptor cells in ferret lungs. Hirst[16] concluded from this work that the first step in virus-host cell interaction—that is, infection—was an adsorption of the virus to the susceptible cell and subsequent enzymatic destruction of a specific component of the cell surface to which the virus had attached. The nature of this important surface constituent remained unknown for the time being.

Unexpected relationships among hemagglutinating viruses became apparent when Burnet formulated the concept of the "receptor gradient."[21] The observation had been made that red cells treated with one virus and resuspended became inagglutinable by the same virus but retained agglutinability by other agents. This suggested the possibility that each virus might have a more or less specific point of attachment to the cell surface. Once this "receptor" had been destroyed by the action of one virus, only heterologous agents could attach to the cell's remaining receptors. Hemagglutinating viruses could be arranged in a series, indicating their relative affinity for surface receptors: mumps, Newcastle disease, influenza A, influenza B, fowl plague, swine influenza. Red cells resuspended after agglutination by any virus in this "gradient" could still be clumped by any virus following, but had become inagglutinable by viruses preceding it in the series. After agglutination with swine influenza virus, the resuspended cells often clumped spontaneously or when mixed with normal sera. This non-specific red cell agglutination resembled the phenomenon observed by Thomson[27] and Friedenreich[8] in blood specimens

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accidentally contaminated with bacteria, especially diphtheroids and vibrios. Substances produced by such bacteria could be adsorbed to, and eluted from, red blood cells, just as with influenza virus, and they similarly changed the character of erythrocytes. Such enzyme-like materials of high potency were obtained from anaerobic bacilli[22] and cholera vibrios,[4] but many other organisms produce red cell agglutination[12] and may form similar products.

The Australian workers[24,3] studied filtrates of cholera vibrio cultures in great detail. It was found that these preparations made erythrocytes inagglutinable by influenza viruses, that they were inactivated by heating, and could be neutralized by specific antisera. They were designated "receptor destroying enzyme"[3] because their enzyme-like action resulted in removal of surface receptors for virus particles and in a change of the surface pattern of red cells: Treated Rh positive cells could be agglutinated by "blocking" as well as by complete antibody, and could be hemolysed by fresh normal guinea pig serum.[24]

In view of the fact that this "receptor destroying enzyme" interfered with the attachment of virus to red cell it was of interest to determine its other effects on virus-host cell relationships. Such studies were performed in animal lung tissue and in the allantoic cavity of chick embryos.[5] It was demonstrated that this enzyme promptly liberated viruses of the influenza group which had been adsorbed to host cells, and that it prevented such adsorption if administered prior to the virus.

Much evidence suggests that this "receptor destroying enzyme" is a mucinase, an enzyme that attacks protein-polysaccharide complexes such as mucin. Several mucins are known to be present on the surface of red cells; and the substance carrying O, A, or B blood group specificity, for example, is a mucin. It was suggested therefore that blood group substances might actually be receptors for virus attachment. Soon it became clear, however,[9,6] that purified O and A group substances did not inhibit hemagglutination and thus apparently did not enter into reaction with the virus. On the other hand several investigators reported a variety of polysaccharides which interfere with hemagglutination or virus multiplication.[11,26]

It appears justified, therefore, to postulate that for the group of influenza-like viruses at least, a polysaccharide, or mucin, on the cell surface may be the point of attachment for the virus. Since adsorption of the virus to the cell surface is probably a prerequisite for cellular infection, the presence of such a polysaccharide may determine whether a cell is potentially susceptible to infection. Support for the hypothesis that polysaccharides are of great significance in virus-cell relationships is provided by the following facts:

1. Hemagglutination by viruses of the mumps-influenza group is inhibited by many more or less complex carbohydrates, from apple pectin to red blood cell extracts.[11,6]

2. The hemagglutination-inhibition effect of various sera[7] is abolished by the "receptor destroying enzyme" and by agents which specifically attack proteins (trypsin, for example) and carbohydrates (potassium periodate). This suggests that the inhibitor in serum may be a polysaccharide-protein complex.[5]

3. Saline extracts of various human and animal tissues inhibit hemagglutination by viruses of the mumps-influenza group.[9] Purified preparations from human red cells and lung tissue which strongly inhibited hemagglutination, proved to be at least 50 per cent polysaccharide by chemical analysis.[6] Filtrates and autolysates of pneumococci (containing much polysaccharide) inhibited red cell agglutination by influenza A virus.[25]

4. Takahashi[26] reported that the multiplication of tobacco mosaic virus could be inhibited by a polysaccharide obtained from yeast.

Most of this evidence is admittedly of an indirect nature, dealing chiefly with the phenomenon of hemagglutination as indicator of the participation of polysaccharides in the early adsorption of virus to tissue cells. Since such chemical compounds are known to occur on cellular surfaces it seems important, however, not to neglect their potential significance.

In studies of bacteriophage-host cell relationships it has been demonstrated clearly with the aid of the electron microscope[19] that adsorption of the virus particle to the surface is the first step in infection of the cell. Such adsorption is chiefly determined by the pattern of surface structures, responsible for the high specificity of these bacterial viruses. That similar specific surface reactions may be important in hemagglutination by viruses is indicated by the following findings: Influenza virus A (PR8 strain) agglutinates red cells of humans and chickens but not of sheep, while mumps virus clumps all three types. Extracts of human cells inhibit all these hemagglutinations, but extracts of sheep cells inhibit only agglutination of sheep cells by mumps virus and not that of human or chicken cells by influenza virus.[6] Thus it appears that the specific inhibiting substance present in the extract (principally of polysaccharide nature) is present on the surface of the cell and may well be responsible for initial virus adsorption.

A similar specific relationship has been demonstrated in a different virus-host system.[28] The susceptibility of animal species to infection with pneumonia virus of mice is proportional to the concentration of a lung tissue component which specifically combines with the virus. Little is known about the chemical nature of this tissue component, but it is interesting to note that multiplication of this virus in susceptible lung is inhibited by several polysaccharides.[17]

The facts presented suggest the possibility that in other animal and human virus infections similar conditions might prevail. It has been proposed[5] that the cellular tropism of viruses may be partly based on the presence of specific chemical sub-

stances on the surface of potentially susceptible cells. The mere presence of this surface structure would, of course, not make the cell susceptible, as evidenced by the presence of specific polysaccharides on red cells which are not invaded by viruses of the influenza group. Viruses possessing enzyme systems specific for such compounds could attach to such cells, whereas cells devoid of these surface structures would be susceptible to virus action.

If it is postulated that a virus has to attach to a specific chemical compound on the cellular surface prior to invasion of the cell, then a corollary postulation might be the possibility of blocking this adsorption by similar chemicals. Materials related to the original enzyme substrate could be administered to the host in order to compete with tissue cells for virus enzymes. Such blockage would offer an excellent method of chemoprophylaxis and chemotherapy of virus infections. Recent reports indicate that this possibility is not altogether idle speculation. Green and Woolley[11] found that several polysaccharides inhibited influenza A virus multiplication in the allantoic fluid of fertile eggs. Horsfall and McCarthy[17] showed that polysaccharide preparations lessened the severity of infection with pneumonia virus of mice and inhibited virus multiplication. Ginsberg, Goebel and Horsfall[10] were able to demonstrate that the multiplication of mumps virus in chick embryos could be inhibited by a polysaccharide from Friedlander's bacillus. Except for the large viruses of the lymphogranuloma-psittacosis group which respond to a number of drugs, this is the first evidence of a useful avenue of approach to the chemotherapy of virus infections. A great volume of work will probably be done in this direction in the immediate future. Even if it should be shown that the effect of polysaccharides is one of competition for virus enzymes not limited to the cellular surface, this would detract little from the value of the stimulation stemming from interest in the mechanism of hemagglutination.

SUMMARY

Some of the more important steps in the investigations on the mechanism of hemagglutination by viruses have been traced. Like pieces of a jig-saw puzzle, apparently unrelated experimental results begin to fit into a pattern which may open the way for better understanding of the dynamics of virus infection of cells and possible approaches to the chemotherapy of virus diseases. The following advances have been most notable:

1. Respiratory viruses of the mumps-influenza group are able to clump red cells by attaching to a specific substance on the cell surface.

2. The point of attachment, or "receptor," is destroyed by enzymatic action of the virus, as well as by enzymes from a number of bacteria and other sources.

3. Enzymes destroying the "receptor" of host cells for this group of viruses, are specific for more or less complex polysaccharides.

4. It is concluded therefore that the virus "receptor" is of polysaccharide nature, on the cell surface. This is supported by the fact that polysaccharides from a variety of sources inhibit adsorption of the viruses to host cells.

5. This primary adsorption is a preliminary step to the infection of the cell. If it can be interfered with, cellular infection can perhaps be prevented.

6. The early interaction of virus and host cell seems to be based on a virus enzyme acting on a specific chemical substrate of the cell. In the group of mumps-influenza viruses this substrate appears to be a polysaccharide.

7. It may be possible to interfere with this enzyme-substrate reaction, thereby preventing early virus action on the cell. This interference may be possible by introducing material similar to the cellular substrate of virus enzymes. Such material, competing for virus enzyme with tissue cells, may provide for effective chemoprophylaxis and chemotherapy.

8. In a few virus infections it has actually been shown that polysaccharides from a variety of sources have definite chemotherapeutic action under experimental conditions.

ADDENDUM

Since this review was submitted for publication much evidence has appeared supporting the affinity of mucoproteins and viruses of the mumps-influenza group. Such mucoproteins were found in egg white and in normal allantoic fluid suggesting a source of error in laboratory tests using hemagglutination techniques. The work on substances blocking the attachment of viruses to host cells has been extended but there has been no further and more definite indication of the chemotherapeutic significance of this mechanism.

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