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Author: A. Eyquem, Institut Pasteur, 25, Rue du Docteur-Roux, F-75-Paris, France

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A living cell infected with a virus acquires resistance to penetration by another virus. This cellular phenomenon is independent of immunological reactions and is the result of a particular substance, "interferon".

Animal experiments and experience acquired in human pathology studies have shown that, in a similar manner, colonization by a certain species of bacteria is capable of protecting the animal against infection by another species. The mechanism of this interaction is not known, but knowledge of the existence of this phenomenon will henceforth explain certain epidemiological observations and opens therapeutic perspectives of great interest.

SUMMARY

For many years, the existence of nonspecific resistance had been known which was independent of the specific resistance factors against infectious agents composed partly of humoral factors (antibodies), partly on tissue factors, and especially on delayed hypersensitivity. The mechanism of the nonspecific resistance is discussed. It was found to be dependent on temperature, on the endocrine and nutritional state of the recepient, and occasionally on genetic factors. Certain species of mice are resistant to certain salmonellae as well as to Saint Louis encephalitis.

Certain aspects of the mechanisms involved can be understood from the results obtained by the study of viral interference of the stimulation of the reticuloendothelial system and bacterial interference. The experimental results obtained thus far have already permitted the proposal of new methods of human therapeutics.

VIRAL INTERFERENCE

Viral interference is defined as the inhibitory action exerted by one virus, alive or inactive, on the subsequent development of another virus which may or may not be related. The interference is independent of immunological mechanisms and phagocytosis. It is essentially based on the intervention of interferon

whose importance has been revealed by the investigations of Isaac.

Interference manifests itself under well-defined conditions which are dependent on the system under consideration. The interferring virus should be administered at a certain time before the virus to be inhibited which is called the experimental virus. However, in certain cases, the two virus can be inoculated simultaneously or the interferring virus can be even inoculated after the experimental virus. The phenomenon is dependent on the speed of virus multiplication as well as on the dose of the injected virus. Interference is localized in tissues and organs which are in contact with the interferring virus. Only cells which are in immddiate contact with the interferring virus are capable of resisting infection by the experimental virus. (4, 18). Many cases of viral interference are now known among which the following can be pointed out:

The influenza virus and the virus of Japanese encephalitis exert an inhibiting action on the multiplication of the equine encephalitis virus of the West and poliovirus 1 and 2. The virus of Rocky Mountain spotted fever interferes with the virus of Western encephalitis in chicken embryo tissue cultures.

Interference mediated by interferon can be observed with viruses which are biologically and biochemically quite different. For example, an RNA virus like the influenza virus is capable of inhibiting a DNA virus like the vaccinal virus.

A certain amount of reciprocity exists in the interference, in that the experimental virus can become interfering and prevent the development of the virus which is normally the interfering one. This reciprocity is not always found. For example, it does not exist between influenza virus and the West Nile virus.

On the other hand, there are examples of interference which are unexplainable at the present time; for example, Coxsackie A virus interferes with Rous sarcoma virus although the former does not reduce the production of interferon.

Interferon was detected by Isaacs (1957) after treating chicken embryo cells with influenza virus which had lost its ability to produce infection. It was obtained also from a number of other cells: monkey kidney, Hela cells, chorio-allantoic membrane exposed to a variety of viruses (influenza, Ourlien, Newcastle, polyoma, arbor...).

It is a protein with a molecular weight of 30,000 produced by animal cells of various species after treatment with different viruses.

The production of interferon is normally confined to the cell. Penetration of the virus into the cell initiates its synthesis. It is, therefore, a product formed by the cell in response to a viral infection and is capable of inhibiting the growth of viruses. The amount of interferon that is produced is partly dependent on the virulence of the virus. Optimum activity by interferon is achieved when it is administered during the time preceding viral infection. It suppresses viral synthesis most probably by inhibiting viral nucleic acid synthesis and by modifying the ability of the cells to adsorb viral particles.

Interferon has not only a protective role but also a curative one. a therapeutic role has been envisaged but it production is made difficult by the fact that there is quite limited cellular specificity and there is the adaptation by certain types of viruses. In addition, the inhibitory activity detected in vitro is not always accompanied by a therapeutic effect.

Other cellular inhibitors analogous to interferon have been discovered. For example, Chancy (3) observed an inhibitor in K.B. cell cultures infected with para-influenza 3 virus which was also active in kidney cells of monkeys. This substance seems to act by increasing the resistance of the cells to the cytopathogenic effect of the virus.

Other interference mechanisms have been mentioned. One of them involved interference with the cellular receptors formed on the membrane whose integrity is necessary for the fixation and subsequent penetration of the virus. For certain

viruses, the cells which have lost their receptors are resistant to infection.

Certain viruses, such as influenza virus inactivated by ultra-violet, can retain their enzymatic properties to "lyse" the cellular receptors and thus prevent the fixation of new viral particles. However, this hypothesis is also discussed.

The inhibition of multiplication is dependent on numerous other factors such as temperature, pH and metabolites (23). However, our knowledge of these aspects is still very fragmentary.

NONSPECIFIC RESISTANCE AND SEROLOGICAL AND CELLULAR FACTORS

There is actually a trend to minimize the bactericidal role of serum which is partially dependent on the different fractions of complement. Properdin, which is considered to have a fundamental role, could probably be considered to be a macroglobulin with high specificity, playing the role of opsonin. Lysozyme, which is bactericidal *in vitro*, does not play a well-defined role *in vivo*. The same holds true for beta-lysins, histones, globins, and protamines (26).

NONSPECIFIC RESISTANCE AND STIMULATION OF THE R.E.S.

The cellular factors are basically represented by phagocytosis and the activity of the reticuloendothelial system. However, these activities do not appear to be functional in the complete absence of antibodies of natural or immunological origins. The intracellular substances which are involved in resistance are not well defined: The action of lysozyme, phagocytin, histone, and lactic acid have been observed in polynucleocytes but not in the case of macrophages. It is known that the activity of the phagocytes can be stimulated by contact with particles of various types. This non-specific immunity can be elicited by the injection of various bacterial lipopolysaccharide extracts (31). The recent studies of Prévot, Halpern, and coworkers (19) showed that stimulating factor of the reticuloendothelial system could also be found to various degrees in the extracts of

anaerobic corynebacteria. This most active species in this regards was a strain of Corynebacterium anaerobium. This stimulation can be observed in mice after a single injection (intravenous) of dead microorganisms. It can be witnessed by the elimination of carbon particles which reaches a maximum in about eight days. These results compare favorably to those obtained previously using extracts of various salmonellae, particularly S. abortus which was studied by Westphal.

The exact mechanism of these modifications is not clear. Landy and co-workers showed that in mice injected with bacterial lipopolysaccharide, there was an increase in the glycolytic activity of the macrophages and, at the same, time, an increase in resistance to infections. The macrophages of animals that have received such injections have more phagocytic activity than macrophages from normal animals. The intracellular bactericidal activity, however, appears to be the same. This increase in phagocytosis can also be produced in vitro and can be observed 24 hours after the addition of small quantities of lipo-polysaccharides to macrophage cultures.

Fauve noted that the behavior of macrophages obtained from the peritoneum of germ-free mice was the same and that they possessed the same phagocytic capabilities as those obtained from normal mice. On the other hand, these macrophages did not prevent the multiplication of microorganisms that had been phagocytized. As a result, the organisms in macrophages from germ-free mice were more slowly destroyed than bacteria phagocytised by macrophages from conventional mice (12).

The role of the reticuloendothelial system and certain macrophages in the resistance of a particular strain of mice to the Arbor B virus was noted by Goodman and Koprowski (14). However, the mechanism responsible for this natural resistance is still unknown, although it is known that it is genetically determined. However, there is no difference between resistant mice and sensitive mice in the initial viremic period; in both cases, there is a viremia in

resistant mice, this process stops within 18 hours.

BACTERIAL INTERFERENCE

Studies carried out with germ-free animals have shown that the lymphoid tissues and reticuloendothelial systems of these animals are immature in comparison to those of normal animals. This immaturity can be detected histologically. The inhibition of the development of the digestive tract appears to explain, at least in part, the high sensitivity of these animals to bacterial infection. This is contradictory to the high resistance shown by germ-free animals to the action of endotoxins, radiation, or cortisone.

The sensitivity of pathogen-free mice to infections has been studied by Dubos and Schaedler. Pathogen-free means that these mice do not have any gram-negative bacteria in their intestinal tracts. These authors have shown that these mice were more sensitive than conventional mice to intravenous injections of Staphylococcus aureus and Klebsiella pneumoniae (7,8,8,10).

A decrease in resistance to infections can also be observed after modification of the intestinal flora and the action of neomycin or phtalyl-sulfathiazol (Dineen, 1961 (5)).

The results obtained by R. Dubos and co-workers have been recently recognized in other forms in four maternity hospitals. The latter observations confirm that the contamination of new-born infants with virulent staphylococci can be prevented if these infants have already accepted certain types of "resident" staphylococci (7, 11, 25). In this regards, contamination is especially severe in the case of children treated with antibiotics and then put into contact with the family environment. This particularly is true for staphylococci which are resistant to antibiotics, especially those which are lysed by phage types 52, 52A, 80 and 81.

An experiment (25) which was conducted in New York (Cornell Medical Center), Atlanta, Cincinatti, and in Louisiana (regions with different climates) on 524

nursing infants produced similar results. Staphylococcal strain No. 502A was chosen because it could be easily distinquished by its serological and bacteriophage characteristics.

The implantation of "resident" staphylococci belonging to this strain was carried out with 80 to 90 % success when it was introduced via the nasal fossae of 500 new born infants. The results have been obtained by the deposition of 55 microorganisms onto the umbilical stump. Protection persisted in 60 % of the children for 6 to 25 weeks. It was still apparent in 30 % of the children for 6 to 12 months. Among the children which were housed in the same rooms, 9 % became carriers of the strain of staphylococcus. In the course of implantation, the authors did not observe a single case of undesirable action of this prevention.

These results have been confirmed in a recent publication that reported on observations made on several thousand children as well as adults (11).

The interference phenomenon is not restricted to a single type of S. aureus. The resistance to colonisation by a second strain of S. aureus is dependent in part on the localised presence of a large number of resident S. aureus cells. The elimination of these by antibiotic treatment favors the artificial colonization by other strains

No hypothesis concerning the mechanism of this phenomenon has been suggested by the authors of these experiments. They assume that the use of the strain of S. aureus selected constituted the most effective means of stopping an epidemic of staphylococcal infections.

Henceforth, one will be able to consider the application of this principle to the control of other diseases.

The results obtained, especially those of the Rockefeller Institute and Cornell University, allow one to attribute a great deal of importance to the role of the normal intestinal flora in the behavior of animals (7, 8, 9, 10).

Dineen has shown that in mice treated with antibiotics, the increased sensitvity of these animals to infections can be modified by administering an implantation of gram-negative microorganisms into the digestive tract (5). This phenomenon has been shown for P. pyocyanus, P. vulgaris, and A. aerogenes.

Schaedler place mice treated with antibiotics and non-treated mice into the same cages and observed the composition of the intestinal flora. The flora of the treated mice came very close to that of the untreated mice. He also found that the density of lactobacilli increased faster in mice that were living in contact with untreated mice than in treated and isolated mice (22).

On the other hand, it is not possible to modify the intestinal flora of normal animals by trying to accustom them to the intestinal flora of treated animals. The normal animals seem to be able to defend themselves by preventing the invasion of their own flora by bacteria which are not part of their usual residents.

It is actually difficult to explain these various phenomena: It seems that one can assume the intervention of a constant stimulus from the microorganism in the form of bacterial products. It is logical to assume that different polyosidic antigens play a certain role and it is not necessary to discuss the importance of the penetration of bacteria present in the intestinal milieu through the intestinal coating in normal animals. This penetration and the passage into the ganglions and liver could be found in 20 % of the animals examined. It is also known that immunisation via the digestive tract is possible and that the digestive adsorption of antigens of E. coli is followed by the appearance of homologous antibodies. While it is possible to accept the intervention of intestinal flora, one is still ignorant of those species of microorganisms that play a major role. The German authors have placed particular emphasis on the importance of gram-negative bacteria, especially E. coli.

A primary role has also been attributed to the lactobacilli (Dubos).

The possibility exists that bacteriocins also play some role in these phenomena. As a matter of act, all enterobacteriaceae are capable of elaborating colicins, and vice-versa, the coliform bacteria elaborate the bacteriocins.

The bacteriocins elaborated by gram-negative microorganisms have a narrow spectrum of activity from a taxonomic standpoint. These bacteriocins act only on gram-negative bacteria belonging to the species producing them and th related species. The spectrum of activity can be altered: certain colicins act on a large number of strains while others act on only a small number. The bacteriocins exert their antibiotic action via intermediatary particles which attach themselves to a specific receptor located on the surface of the bacterial cell.

The action of bacteriocins, like that of the colicins, is slow and progressive. The gram-positive microorganisms, including S. aureus, are capable of elaborating bactericins with much faster activity. The exact chemical composition of these bacteriocins has not yet been completely elucidated. Certain one among them appear to be identical with the microbial toxins.

The activity of these bacteriocins in the intestinal milieu could be responsible for the dynamic equilibrium between different bacterial species, represented basically in the adult by the lactobacilli and E. coli, and to a lesser extent by the clostridia and the bacteriodes. In fact, the ability to elaborate colicins is present in about 25 % of the enterobacteriaceae. The evolution of the colicinogenic flora of the intestine has been followed in some special cases.(Robbins, 1957). The study of six subjects during a four month period showed that E. coli continued to elaborate the same spectrum of colicin during the entire period of observation in three of the six subjects. This examination confirmed the stability of a small number of resident strains and the instability of a great number of transitory coli strains (21).

The colicinogenic property appears to be very important in permitting the the implantation and persistence of certain strains of E. coli. The implanted strain very often possesses a colicin for the strains which it has replaced. However, it is possible that other factors can be involved also (Branch, 2). As a matter of fact, one can observe in the same subject the presence of colicinogenic factors and bacteria sensitive to these colicins.

The dynamic equilibrium between bacterial species in the intestinal milieu, which was the preoccupation of Metchnikoff at the beginning of this century, is based on a mechanism which is still unknown. One is ignorant of why the "resident" flora stays implanted during long periods of time in an individual and why there is symbiosis with the carrier as well with the other bacterial species. One can hypothesize that bacterial degradation products, which find their way into the reticuloendothel system, lead to a state of immunological paralysis in the carriers with regards to the homologous antigens and that this situation inhibits the production of antibodies with a specificity that would limit the implantation of bacteria which possess similar antigens.

There is still a great deal unknown about the intestinal flora of humans since it is practically impossible to study the flora at various levels of the intestinal tract. On the other hand, systematic examination of the flora of the digestive tract have been carried out with various animals. The recent studies of H.W. Smith on numerous animal species including the monkey, goat, horse, ox, sheep, pig, guinea pig, and rat have shown a very strong similarity in the intestinal flora but a large difference in the density of certain microorganisms in the intestinal tract. In a general way, the acid pH of the stomach cavity exerts some inhibition on proliferation. The rabbit is distinguished from the other species in that there is a much greater density of bacteriodes in the large intestine. The effect of different diets appears to the principle cause for differences in the floral distribution. The absorption of microorgan-

isms by the oral route appears to play a determinant role in certain species but not in the rabbit and chicken.

It is known that the substitution of a "resident" organism by another is difficult in a normal subject. On the other hand, it can occur easily after antibiotic treatment. In order to prevent the sterilizing effect of the gastric acidity, Lasserre, 1965 (16) attempted to introduce lactobacilli into the digestive tract by means of a special catheter used for pyxigraphy. In this manner, they could be deposited in the caecum and a rapid implantation could be brought about. This could shed some light on the conditions surrounding certain severe cases of colitis.

In the case of children, there is the natural implantation at birth of E. coli which is most likely of maternal origin, and especially of Bifidibacterium bifidum which develops in the presence of various growth factors of which the most important one is the bifidus 2 factor of Raymond. Recent studies have shown that a flora of B. bifidum play a natural protective role as well as a therapeutic role with regards to acute enterocolitis in nursing infants (29). A study by Vieu on new-born and nursing infants confirmed the existence of E. coli residents persisting even in the course of mixed nursing and of transitory E. coli colicins.

While it is difficult in human therapeutics to find evidence for an increase in resistance of the individual as a result of different intestinal flora and for improvements in intestinal disorders as a result of various bacterial populations, this is not the case in experimental animals. In this regards, one must make certain reconciliations between the stimulative activities of the reticuloendothelial system activity studied by Prévot and Halpern and the increase in resistance in animals having controlled bacterial populations.

CONCLUSIONS

A certain amount of analogy exists between the bacterial interference phenomenon and the viral interference phenomenon. There are, however, many points of difference. Viral interference is actually better understood and is attributable to activity of interferon. On the other hand, the actual mediator of bacterial interference is not known. One of the best means of defense against pathogenic microorganisms for which there are no standardized vaccines appears to be the establishment of viable microorganisms of the same species which are not virulent. The mechanism of antibacterial resistance acquired in this manner is still obscure, but the success achieved by implantation of bacterial populations has verified the prophylactic role of bacterial interference.

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