REVIEW ARTICLE

HOST RESISTANCE TO INFECTION*

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THE application of chemotherapy to the control of infectious disease has had an unexpected result in revealing the contribution made by the resistance of the host to the desired result.

Most experience has been gained from a study of the pathogenesis and treatment of disease in the experimental animal. The reviewer has turned to infections with the tubercle bacillus as examples because he has had more experience with that parasite than with any other. But there is another reason; infections with the tubercle bacillus provide a model in which host resistance is not complicated by the presence of circulating protective antibodies.

Let us first saddle man with a definition. Thus a "host" must by definition supply the conditions favourable for reproduction of a bacterial "parasite." The use of the term "soil" by some tuberculosis workers refers to their belief that some unknown biochemical factors essential to the reproduction of tubercle bacilli may determine susceptibility, yet so far as can be traced only two factors have clearly been established, as growth conditions, to play a creative part in bacterial susceptibility. They are the availability of free oxygen and the temperature of the host.

The availability of free oxygen limits the pathogenic potentialities of all strictly aerobic bacteria. Tubercle bacilli are strictly aerobic. How different would be their pathogenic potentialities and how different the resultant patterns of disease were these parasites facultative rather than obligate aerobes. How much more dangerous to mammals would the tetanus bacillus be were it a facultative aerobe.

The reasons for the high pathogenicity of the bovine tubercle bacillus and the low pathogenicity of the human strain for the rabbit while both are pathogenic for the guinea-pig but have poor pathogenicity for dogs, rats, and mice, remain subjects for debate and speculation.

The presence of a genetically transmitted resistance to tuberculosis is recognised, notably in some strains of rabbits; and this knowledge may be subjected to a statistically significant experimental proof. A discussion of these experiments will be made later.

HOST RESISTANCE IN MAN

Those physicians who observe the tissue change seen with tuberculosis, recognise the presence of clinical disease as a manifestation of the absence of host resistance. Yet it was the advent of substances with the capacity to modify favourably the course of the disease which first enabled the

* Forming the basis of three lectures arranged by the Convention Culturelle Franco-Britannique, given in Angers, Tours and Rennes in May 1956.

significance of host resistance to be assessed. Those Gram-positive infections, a single attack (and recovery) of which conferred a solid lasting immunity, provided the first clue to the subsequent discovery of passive and active immunity. Diphtheria provides an excellent example.

It is difficult not to conclude that resistance of man to diphtheria is high, and unless swamped, or as the result of pharmacological damage with toxins, the body has little difficulty in digesting the parasite.

It is with the classical studies of Anderson with pneumococcal pneumonia that we are able to identify one facet of host resistance[1–4].

Epidemic uncomplicated (lobar) pneumonia in adults in Glasgow was caused by type I, II and IV organisms. Mortality before chemotherapy was about 30 per cent; with chemotherapy this was reduced to 8 per cent, the peak efficiency being reached with sulphathiazole; penicillin, aureomycin and other antibiotics did not modify this figure.

Endemic (broncho-) pneumonia was caused by types III, VII, XV and XXI, and, before chemotherapy, had a mortality of some 30 per cent; with the advent of chemotherapy the mortality was reduced only to two thirds of this figure. These organisms were recovered from the mouths of most adults[1–4].

Here then is evidence for a lack of host resistance in the endemic pneumonia group.

Host Resistance in Animals

We must now turn to work with experimental animals the better to see some possible sites of action of the mechanism of host resistance. By observing the incidence of infection in rabbits with high or intermediate native resistance to known numbers of tubercle bacilli Lurie and his colleagues[5–7] have been able to make a measure of host resistance.

With the bovine tubercle bacilli, each bacillus of maximum virulence which is ingested by an alveolar phagocyte gives rise to a tubercle, irrespective of the resistance of the rabbit. With the human type bacilli, 47 ± 10 organisms will generate a single tubercle in susceptible rabbits, whereas 684 ± 159 organisms of the same virulence are required to produce a single primary pulmonary focus in the genetically resistant rabbits, which are therefore about 16 times more resistant to disease caused by the human strain.

When pharmacological doses of cortisone are given to rabbits which inhale human type bacilli the number of primary tubercles is markedly increased. Also, the foci swarm with bacilli and there is little granulation tissue; in addition the draining lymph nodes contain few micro-organisms. The controls show typical tuberculous granulomata with well-developed caseation and marked lymphatic dissemination. Cortisone does not increase the number of foci generated in rabbits exposed to bovine bacilli of maximum virulence, for not more than one primary tubercle can arise from a single cell. The disturbed hormone balance produced by cortisone appears to deprive the phagocytes of their capacity to inhibit the growth of the bacilli within their cytoplasm though their phagocytosis is not impaired. In similar ways cortisone has been shown

to have a rapidly acting, deleterious effect on many bacterial, protozoal, viral and rickettsial infections in a variety of species[8]. The rapidity of the intervention by cortisone in acute infections suggests that its effect is not related to an interference with antibody mobilisation. Lurie's experiments also showed (1) there was a greater initial destruction of human type bacilli in the resistant animal; (2) the lag phase which precedes the logarithmic multiplication of inhaled human bacilli in the lungs of both resistant and susceptible animals is longer in the former; (3) the rate of multiplication during the growth phase is lower and (4) the acquired resistance developed is greater in the resistant than in the susceptible animal. This evidence was interpreted to mean that the inhibitory influence on the intracellular growth of the bacilli is of first importance in host resistance. The cortisone evidence was interpreted to mean that this property is under the influence of the hormone balance of the individual.

This knowledge of the part played by the phagocyte has received unexpected support by a chance discovery made by Hart and his colleagues[9]. It was observed that a commercial non-ionic surface-active agent Triton WR 1339 and similar large molecules synthesised for the purpose suppressed the development of experimental tuberculosis in the mouse. This observation introduced a new type of antituberculosis agent. Other workers[10] reported synergism with dihydrostreptomycin in the treatment of murine tuberculosis. Recently is was shown by Rees[11] that regression and healing of an established infection in the guinea-pig is possible.

Three series of these non-ionic macromolecules were synthesised differing in their polyoxyethylene chain length from 10 to 90 units. In these three homologous series, alterations in the lipophilic-hydrophilic balance in the individual products, brought about by varying the polyoxyethylene chain length, can influence the outcome of the tuberculosis infection. As the lipophilic to hydrophilic ratio decreases, activity passes from antituberculosis to inactive, and then to protuberculosis.

Yet none of the antituberculosis members of the different series inhibited the multiplication of tubercle bacilli *in vitro* even at high concentrations, while none of the protuberculosis enhanced growth. Moreover, no tuberculostatic substance was found in the blood or tissue fluids from animals heavily dosed with a therapeutically active agent. On this evidence, the authors presumed that the various effects were mediated more directly through the host. Mackaness[12] introduced tubercle bacilli *in vitro* into preparations of monocytes (macrophages) obtained from animals previously treated with Triton WR 1339. Although phagocytosis was not increased, the ingested bacilli were partially or completely destroyed. In the controls there was free intracellular multiplication.

Direct contact of the agent with monocytes was ineffective. It was recently shown that the surface-active agent entered monocytes. *in vivo* This was done using a dye, Victoria Blue B 150, which is made soluble by Triton. A suspension of the particulate dye (1μ in diameter) is rapidly phagocytosed by monocytes from normal and detergent treated animals.

Whereas the particles of dye ingested by monocytes from normal animals remain intact, those ingested by monocytes from the detergent-treated animals rapidly dissolved to form blue patches which then coloured the whole cell. From simple colour tests it was concluded that 0·1 per cent of detergent entered the cell and was uniformly and freely available within the cells.

The range from anti- to growth-promoting action seemed to mean that the physical properties of the detergents alter in some way the surface of the tubercle bacillus *in vivo* so that its suspectibility within the monocyte is increased or decreased.

Hart and Rees[13] have used to forward their argument the analogy which has been shown to exist between the behaviour to sudden cooling of human red cells previously exposed to these detergents, and the behaviour of tubercle bacilli exposed to detergents within the macrophages. Those agents that decrease the haemolysis of red cells in thermal shock are antituberculosis and those that increase it are protuberculosis; those that are inactive in one instance are inactive in the other.

Preliminary chemical analysis of the lipids of red cells after exposure to the detergents, was found by Lovelock[14] to be consistent with the fact that those detergents with few ethylene oxide units displaced cholesterol preferentially from the red cell membranes, while those with many ethylene oxide units displaced phospholipid preferentially. It is thought by Hart and Rees[13] that if the surface-active agents affect tubercle bacilli and red cells in a similar way then those with few ethylene oxide units per molecule would displace the more hydrophobic lipids of the tubercle bacillus and so make it more easily digested inside the phagocytes.

These model experiments of Hart and his colleagues have proved stimulating and no doubt direct observations of the effects of enzymes on both treated and untreated cells will follow. A stage nearer to the living animal would be reached should both red cells and tubercle bacilli previously treated with the "few ethylene oxide" detergent molecules prove to be more easily digested with pepsin than untreated similar cells.

There are, of course, other possible mechanisms by which the detergents could produce their effect, such as modification of enzymic activity of the monocyte or of the tubercle bacillus. However, whatever their precise mode of action, they demonstrably influence the operation of the cellular defences in tuberculosis infection. The immediate point of attack is apparently the same as that previously seen as a unique difference in genetically resistant or genetically susceptible rabbits by Lurie[7].

He showed that the host resistance could be overcome by virulent organisms—in this case bovine bacilli—and also observed from cortisone experiments that those tubercle bacilli which were not phagocytosed were multiplying vigorously—"swarming." Another factor known to influence this is cord factor or virulence factor.

Cord Factor

Virulent tubercle bacilli grow on or in artificial media in a characteristic manner; they form sheafs or "cords" consisting of bacilli in close parallel

arrangements. The phenomenon was described by Koch[15] and its significance reinvestigated by Middlebrook and others[16]. From these original observations and all that has been learned since, it seems that under the conditions in which tubercle bacilli are usually grown, this morphological pattern is characteristic of strains which multiply in susceptible hosts.

Let us see where these new observations may fit into the jigsaw puzzle. When strains which multiply in a host are cultured in an aqueous medium, cord factor, as Bloch[17,18] calls it, which is extremely water-insoluble, accumulates at the bacterial surface, and can be removed with organic solvents without killing the bacteria. It is produced only by cord-forming strains of the tubercle bacillus.

In a lipophilic environment, it is partly released into the surrounding medium. Bacteria which are specifically prevented from synthesising cord factor *in vitro*, or which are grown under conditions where they release the compound into the medium, are significantly less virulent than control suspensions of bacteria from the same strain consisting of equal numbers of viable tubercle bacilli[19].

Chemical Nature of Cord Factor

Noll, Asselineau and Lederer (quoted by Bloch and Noll[20]) have made a partial characterisation of cord factor. It is an ester of molecular weight 1580 containing α-mycolic acid. It is not identical with any of the known constitutents of the tubercle bacillus. On alkaline hydrolysis an ether-soluble part is split off which has been identified as α-mycolic acid. The water-soluble part is a non-reducing carbohydrate fraction containing one atom of nitrogen. Acid hydrolysis splits this fraction into a reducing sugar, indentified as glucose, and a nitrogen-fragment of unknown structure.

The well-documented relations between the presence of cord factor and bacterial virulence have been supplemented by experiments in which injections of cord factor were combined with controlled and standardised tuberculosis infections in animals of known susceptibility[20]. These results may be summarised.

1. A single injection of cord factor, by itself innocuous, aggravates the course of mild as well as severe tuberculosis infections when given between one and 72 hours before infection.

2. A similar injection given to mice with chronic but stationary tuberculosis causes a flare of rapidly progressive disease.

3. These effects are quantitative in nature and the amounts of cord factor required to produce the aggravation depends on the susceptibility of the animal. The minimum effective does for mice is 1 to 10 μg.

4. The enhancing effect is specific; staphylo-, pneumo- or streptococcal infections are not influenced. Likewise bacterial components related chemically to cord factor but lacking the characteristic toxicity of the compound show no influence on murine tuberculosis.

5. Cord factor interferes with the chemotherapeutic activity of isoniazid. Tuberculosis infections aggravated by the action of cord

factor do not respond to isoniazid as well as do comparable control infections. This effect is quantitative and observed only in the living animal.

When cord factor is injected intraperitoneally it is reabsorbed and deposited in the lungs and liver and some in the kidneys. The distribution varies with the solvent. Followed histologically[20], the main injury which is seen consists of small haemorrhages caused by increased permeability of capillary vessels. Necrosis appears after repeated injections. There is haemostasis in the capillaries of the area and transgression of cellular elements of the blood into the perivascular tissue. The nature of the tissue damage is unspecific, but it is of the kind which has long been observed in early tuberculosis lesions by Canetti[21] and more recently by Florey and his colleagues[22].

Bloch postulates that after penetrating an organ the tubercle bacilli release cord factor from their cellular surfaces into the surrounding tissue, thus causing the observed tissue reaction.

There are many obvious reasons why the bacilli would find the haemorrhagic, oedematous mentruum adequate for their multiplication and why phagocytosis would be less effective in such an environment. With fewer bacilli destroyed, multiplication would begin earlier. Thus cord factor would enable more bacteria to multiply in the host during the very early phase of infection and presumably in later stages whenever metastatic foci are formed and new tissues invaded. In this way cord factor is seen by Bloch to act as an "aggressin" (Keppie, Smith and Harris-Smith[23]), and so to play an important part in the virulence of the tubercle bacillus and consequently in the pathogenesis of tuberculosis.

Acquired Immunity

Antibody Responses

No discussion of host resistance, with tuberculosis as a model, is complete without reference to acquired immunity. It is usual to say that antibodies play no part in acquired resistance to tuberculosis. This view is supported by the inability to correlate antibody responses, however measured, and resistance in experimental animals; also the effects of serum or other tissue extracts from vaccinated subjects on the bacillus *in vitro* are variable, and again the transfer of passive protection from vaccinated donors to normal animals has failed.

Raffel[24] has reported a recent investigation which was unable to show any indication that antibodies or other humoral factors serve as defences in tuberculosis. The exercise was remarkable for the use of a variety of current serological methods, and the use of most of the major isolatable constituents of the bacillary cell as test antigens.

The serological tests included complement fixation, agglutination, agar diffusion, and haemagglutination and haemolysis[25]. The developing method[26] for detecting incomplete antibodies was also used. The antigens were cultured bacilli, freshly isolated bacilli from sputum or other fluids, synthetic medium filtrates from bacillary cultures, cytoplasm of bacilli, proteins, polysaccharide from wax[27], phosphatides, wax[28] and firmly bound lipid[29].

Sera were from guinea-pigs treated with B.C.G. or bacilli killed with heat, mechanically, cathode irradiation or ultra-violet irradiation; or with water soluble bacillary constituents, or tuberculoprotein, wax, wax fractions, polysaccharides and phosphatides alone and mixed with tuberculoprotein and with killed bacilli extracted with organic solvents.

Protection against tuberculosis follows vaccination with B.C.G., and in a degree the water-paraffin-dead organism emulsion[30]. In the above tests the titres of the sera obtained from the protected animals did not exceed the reactions of the sera from animals treated with the variety of components which did not protect.

Passive Serum Transfer

The conventional test by which acquired resistance is challenged is passive serum transfer. The fact that tuberculosis is a chronic disease is evidence of the inadequacy of acquired resistance[32] and forewarns of the special precautions needed to effect artificial transfer. Calmette[33] lists the early unsuccessful trials and Long[34] should be consulted for an up to date record. Raffel and Efford[24] made two exacting studies in which the effects of serum were investigated on the progress of a light and a severe infection in guinea-pigs. Serum was provided by a donor group of 235 guinea-pigs vaccinated during six months with B.C.G. and also by a second non-vaccinated group of equal numbers acting as controls. Careful design of the experiment, made it possible to inject subcutaneously freshly-drawn donor serum; the dose was adequate—equivalent to about 250 ml. daily for man. Raffel describes the results of both experiments to be unequivocal, the animals receiving immune serum deriving no benefit from the transfer. This failure should be contrasted with the successful demonstration in another chronic infection, malaria, in which Coggleshall and Kumm[35] demonstrated passive immunity with small amounts of low titre antibody.

Implanted Semipermeable Capsules

Immunity mechanisms have also been studied by implanting semi-permeable capsules in the body[36,37]. Usually a plastic capsule of about 1 ml. volume is used. A window at one end is sealed with a Gradacol membrane; an average pore diameter of 60 to 70 mμ does not pass tubercle bacilli or host cells yet is permeable to plasma proteins. Known numbers of tubercle bacilli are sealed in these capsules which are then placed in the peritoneal cavities of normal and immune guinea-pigs. At intervals up to three months capsules are removed from both kinds of animals and quantitatively sampled for growth of tubercle bacilli. No differences have been found[24].

Phagocytic Mechanisms of Resistance

The repeated failure to demonstrate the participation of humoral factors in acquired resistance directs attention to the action of phagocytes in vaccinated animals. Obervations in this field are complicated by difficulties introduced by the special methods used and by the life span of both

tubercle bacilli and phagocytes. Differing opinions which have been recently expressed will no doubt be resolved by the discovery of the common artifact.

Lurie[38,39] implanted phagocytes from vaccinated rabbits in the anterior ocular chamber of normal rabbits and observed them to inhibit bacillary growth. Suter[40] also found phagocytes from B.C.G. vaccinated guinea-pigs to inhibit growth of tubercle bacilli in tissue culture. But Mackaness[41,42] finds macrophages from vaccinated rabbits failing to inhibit the growth of virulent bacilli. Again Suter finds *normal* macrophages to inhibit avirulent tubercle bacilli but not attenuated strains like B.C.G. But Mackaness, although observing a prolonged lag in the growth of avirulent bacilli, finds an even greater inhibition of attenuated strains. Raffel[24] using macrophages from B.C.G. vaccinated guinea-pigs noted the same inhibitory effect observed by Suter and not seen by Mackaness. However, when Raffel used the avirulent strain H37 Ra with normal macrophages he found no bacillary inhibition. This disagrees with both Suter and Mackaness. With B.C.G. Raffel found growth with normal macrophages which agrees with Suter's work and disagrees with Mackaness.

At the present time the situation is confused and it may be as Raffel[24] suggests that the development of a method for the direct observation of bacilli within living macrophages may resolve some of the inconsistencies.

Acquired Immunity—Summary

The evidence up to date is that acquired immunity in tuberculosis is not activated by circulating antibodies or other elements of the body fluids. This observation is based on serological tests, passive transfer experiments, and the use of semipermeable containers seeded with tubercle bacilli and implanted in the peritoneal cavities of immune animals.

In short, the required proof, successful elsewhere, is absent. Whether a cellular immunity to *M. tuberculosis* exists is still a matter for research. Current studies of infected macrophages by the method of tissue culture and the intraperitoneally implanted semipermeable capsules have not yet supplied proof, but the advances are promising.

No antibacterial activity of plasma, splenic juice, or disrupted macrophage substance of immunised animals could be demonstrated.

Finally, although it seems probable that immunity to *M. tuberculosis* is not dependent upon an antibody-leucocyte relation, the nature of the mechanism is still unsolved.

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