

THE ACTION OF ELECTRONEGATIVE COLLOIDAL PARTICLES ON THE INFLAMMATORY REACTION INDUCED BY **MYCOBACTERIUM LEPRÆ** AND **M. LEPRAEMURIUM** IN RATS, GUINEA PIGS AND RABBITS

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Electronegative colloidal particles injected into animal tissues produce an inflammatory reaction, with predominance of macrophages (5, 3, 2). The macrophages proliferate and display intense atrophic activity. Under the action of the colloidal particles the same elements which proliferate when *M. lepraemurium* is experimentally inoculated are stimulated (16, 19, 13, I, 20, 15, 17).

The injection of colloidal particles into animals inoculated with mycobacteria influences the evolution of experimental murine leprosy (17). Small doses of colloidal particles enhance the evolution of the disease, whereas high doses have an inhibitory effect. Histological differences between the lesions produced by the mycobacterium alone or by the mycobacterium plus the colloidal particles are not observed. The colloidal suspension in this instance should act by influencing the phagocytic activity of the macrophage against the *M. lepraemurium* (17).

In the guinea pig and the rabbit the action of the colloidal suspensions has been studied only with regard to the evolution of the experimental tuberculosis (21, 18, 22, 4). Although discrepancies have been observed (21, 18, 22, 4), it is generally agreed that the presence of electronegative particles in the tissues does not inhibit the development, and does not produce any modification in the histological structure of the lesions induced by the *M. tuberculosis* (14).

The electronegative colloidal particles, in all referred instances, were administered parenterally. In the present study an attempt is made to investigate the influence of these particles on the histological structure of the lesions produced by *M. leprae* and *M. lepraemurium*, when both the particles and the mycobacteria are topically inoculated together. The experiments were carried out on rats, guinea pigs and rabbits. These animal species were chosen because they react differently when inoculated with these mycobacteria (6).

MATERIAL AND METHODS

Seventy Wistar rats of both sexes, weighing 120 — 160g, 40 guinea pigs weighing 200 — 240 g and 10 rabbits weighing 2.000 — 2.500 g were inoculated with *M. leprae* or *M. lepraemurium* suspensions, previously heated at 120°C for 1 hour. Previous to the inoculation electronegative particles were added to the suspensions of bacilli. The experimental animals were divided into 5 groups:

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- a) 10 rats and 10 guinea pigs. inoculated by intraperitoneal route with approximately 5 mg of *M. lepraemurium*, with 1 ml. of 1% Prussian blue "solution" added.
- b) 10 rats and 10 guinea pigs inoculated intracutaneously with approximately 0.1 mg. of *M lepraemurium*, with 0.1 and of 1% Prussian blue "solution" added.
- c) 20 rats inoculated by intraperitoneal route with approximately 5 mg of *M. lepraemurium* plus 1 ml of 1% Trypan blue "solution".
- d) 10 rats and 5 rabbits inoculated by intraperitoneal route with approximately 5 mg of *M. lepraemurium* plus 1 ml of 1:2 diluted Higgins ink.
- e) 20 rats, 20 guinea pigs and 5 rabbits inoculated with mycobacteria suspensions without electronegative particles (controls). These animals were injected with approximately 5 mg of *M. lepraemurium* by intraperitoneal route and with 0.1 mg of *M. leprae* by intracutaneous route.

The rats and guinea pigs were sacrificed by ether inhalation at the 1st, 2nd, 3th, 6th, 9th, 12th, 15th, 20th, 30th, and 40th day after the inoculation. The rabbits were sacrificed at the 1st, 3th, 9th, 15th, and 30th day.

Pieces of omentum, lymph nodes, spleen, liver and skin, were fixed in Bouin's fluid, embedded in paraffin, and stained by hemalum-eosine, Masson's trichromic stain and Ziehl-Neelsen stain. Other pieces of these same organs were placed in a 15% formalin solution with 3% of calcium chloride added; the macrophages were stained by silver impregnation in frozen sections (11) ; the lipids were stained by Sudan Black B.

RESULTS

There are no morphological difference between the lesions produced by the mycobacterial species used. The effect on the lesions is also similar for the different types of colloidal particles (Prussian blue, Trypan blue and charcoal). This effect is however different in the guinea pig and rabbit on one hand and in the rat on the other hand. A small difference is generally observed in the rate at which the particles disappear within the macrophages. It is faster for the Prussian blue, intermediate for the Trypan blue and slower for the charcoal.

In the rat, the addition of electronegative particles to the mycobacterial suspensions has little effect on the intensity of the early inflammatory reaction. In both controls and animals which have received colloidal particles, the lesions initially have an acute aspect, with a central suppurative area. Around this area a chronic inflammatory reaction develops; the resting conjunctive cells are activated and soon undergo transformation into macrophages. The macrophages are argyrophilic and their cytoplasm contains bacilli and a few sudanophylic droplets. In the animals injected with bacilli plus colloidal particles, stained granules are also present within this cell.

There is an inverse relationship between the number of bacilli and of colloidal particles within a single macrophage. It does not appear however, that the macrophages act discriminating the intake of the two elements; the predominance of particles or bacilli is mainly controlled by the opportunity of contact between these and the macrophages. According to the local conditions the chance favours the uptake of one element more than the other one.

During the evolution of the lesion the macrophage undergoes transformation into the rat lepra cells, which stores bacilli and electronegative granules in their cytoplasm. The rat lepra cell is slightly argyrophilic. The inverse relationship between the number of bacilli and of electronegative granules presented by the macrophage is preserved in the lepra cell.

During the chronic phase of the evolution of the lesions some morphological differences develop between the animals inoculated with bacilli plus, electronegative particles, and the animals inoculated only with bacilli. In the former group the lesions are larger and show little tendency to spreading; when the inoculation has been performed by intraperitoneal route the lesions arising in the regional lymph nodes are scanty and show a small number of bacilli.

During the further evolution of the lesions these differences become more conspicuous. In the lesions of the control animals there is some proportionality between the number of inflammatory cells and bacilli, and the macrophages generally store numerous bacilli. In the lesions of the animals which have received bacilli plus electronegative particles, there is not such proportionality, since the bacilli are relatively less numerous than the inflammatory cells. This fact suggests that the electronegative particles alone induce an inflammatory reaction characterized by macrophage proliferation.

With the progress of the evolution of the lesion the granules situated within the macrophages become smaller and less numerous. This occurrence is observed throughout the evolution of the lesion and is found either in the animals injected with Prussian blue or in those inoculated with Trypan blue, or in a lesser degree in the cases where charcoal was used. The granules fade progressively a fact that suggests a process of digestion by the macrophage. This finding is not observed in all the macrophages, but only in those where the quantity of the granular material is neither very high nor very low. Some correlation appears to exist between the quantity of granules and their digestion by the macrophage.

When the macrophages contain a great number of granules or of bacilli they assume the characteristics of the rat lepra cell. They are globous, with granules and bacilli scattered within the cytoplasm and they present no evidence of digesting the intracellular granules.

When the macrophages are digesting the electronegative granules the mycobacteria that happen to be present in these macrophages show morphologic alterations (fragmentations, alcohol-acid resistant granules) and become less numerous. The study of the successive phases of the evolution of the lesions suggests that the macrophages which digest the electronegative granules are also able to lyse the bacilli contained within their cytoplasm. When the bacterial lysis is accomplished the macrophage still contains some slightly stained minute granules. In this instance the macrophage undergoes transformation into a globous cell which does not contain lipid droplets, is not argyrophylic and is free of bacilli. This cell has some morphological characteristics of the guinea pig epithelioid cell. Some electronegative particles which remain in its cytoplasm, are located in a limited area of the cell, not showing the irregular distribution seen in the macrophages and in the rat lepra cells.

It appears that the electronegative granules act on the rat macrophages in such a way that they develop the ability to lyse the phagocytised mycobacteria. This occurs only in some limited areas of the lesion; these areas assume the arrangement of the tuberculoid lesions and do not contain bacilli.

After the 15th day following the inoculation, the rats injected with mycobacteria plus electronegative particles present lesions of three different types: e) lepromatous lesions, (which are the predominant ones) constituted by nodules of rat lepra cells containing great number of bacilli and of electronegative granules; b) tuberculoid-like lesions constituted by epithelioid-like cells containing some electronegative granules but no bacilli; c) mixed type lesions; the bacilli, although still abundant are much less numerous than in the control and show only little morphologic alterations.

In the animals injected with bacilli alone no other type than the lepromatous lesion has been observed.

The modifications of the histological structure of the lesions found in rats inoculated with mycobacteria plus electronegative particles seem to depend upon the action exercised by these particles. In the liver in which the particles are generally absent, the structure of the lesions is always of the lepromatous type, like in the control animals.

In the guinea pig and in the rabbit the addition of electronegative particles apparently does not alter the intensity of the inflammatory reaction induced by the mycobacteria. However, in these lesions, the macrophages contain more numerous bacilli and show less tendency to necrosis than in the control animals. It appears that the presence of particles reduces the intensity of the macrophagebacilli interaction. This idea is supported by the fact that when the particles are present bacilli remain morphologically normal for a longer period within the macrophages a fact that suggests slower lysis of the bacilli. In the lesions containing electronegative-granules, bacilli are still numerous on the 12th —15th day of the lesion evolution. In the controls animals the lesions have only a few bacilli at this time. In animals that have received bacilli plus electronegative particles the evolution of the lesions is slower than in the controls; the difference although small may be observed until the end of the experiment (40th day).

These facts suggest that the electronegative particles in guinea pig and rabbit lesions decrease the rate of the evolution of the inflammatory reaction, by acting upon the lysis of the mycobacteria. However the macrophage does not appear to lose the ability of lysing the bacilli, since they can be transformed into epithelioid cells free of bacilli.

This transformation, however occurs later in the animals receiving colloidal particles (12th — 15th day) than in control ones (9th — 12th day). Structures of lepromatous type containing numerous bacilli are not observed even in the lesions developed near the point of the colloidal suspension injection; these facts support the idea that the lysis of the bacilli is not abolished.

There is some evidence that in guinea pig and rabbit lesions lysis of the bacilli and digestion of the stained granules occur simultaneously within the macrophages. The granules become smaller, and more poorly stained. They accumulate in a small area of the cell, probably the Golgi area and disappear only at the end phases of the evolution of the lesions.

DISCUSSION

In the rat, the transformation of the macrophage into the rat lepra cell is related to the absence of lysis of the phagocytised bacilli (6, 11). The previous inoculation of *M. lepraemurium* or of BCG by intraperitoneal or muscular route, is not able to modify the general course of the inflammatory reaction produced by the *M. leprae* or the *M. lepraemurium* (9, 12). In this case hypersensitivity develops; the inflammatory reaction is intense and necrotic areas appear, but there are no evidence of lysis of the bacilli by the macrophage.

In the guinea pig and in the rabbit the macrophages have the property of lysing the phagocytised *M. leprae* or *M. lepraemurium*. After completing the lysis they undergo transformation into epithelioid cells, devoid of bacilli (6). In these animals, the lytic property of the macrophage exists normally, and it is independent of specific or non specific experimentally induced hypersensitivity (6, 7, 8, 10, 11) However, the development of hypersensitivity in the guinea pig seems to accelerate the mycobacterial lysis by the macrophage (8, 10).

The different behaviour of the macrophage in the rat and in the guinea-pig when mycobacteria are inoculated appears to be a character inherent to

these animal species (6) Probably by this reason the tissue reaction in these two species is constant. The alteration of the degree of hypersensitivity induce only quantitative changes in the tissues reaction. However, the concomitant inoculation of electronegative particles proved to be able to modify in the rat, the structural type of the lesions induced by mycobacteria. This modification occurs only in limited areas of the lesions; it is characterized by greater intensity of the chronic inflammatory reaction, probably due to stronger interaction between macrophages and bacilli. In these areas, the macrophages develop the ability to lyse the bacteria. After completing the lysis of the bacilli, the macrophages undergo transformation into epithelioid-like cells, as it occurs in the guinea pig. The lysis of the bacilli within the macrophages proceed in parallel with alteration of the electronegative granules, suggesting its digestion by the macrophages.

In the guinea pig and in the rabbit, on the contrary, the electronegative particles seem to reduce the interaction macrophage-bacilli. This fact is probably responsible for the slower lysis of the phagocytised mycobacteria and for the slower evolutive rate of the lesions, compared with the controls.

These facts suggest that in the rat, in which the *M. leprae* and the *M. lepraemurium* actuate as a weak irritant, the concomitant administration of electronegative particles enhances the functional activity of the macrophages. It is probable that some enzymatic systems are activated. These enzymatic systems should be effective in lysing the bacilli and should be indirectly responsible for the appearance of cells having the character of the epithelioid cell. In such conditions the rat macrophage also acquires the ability of digesting electronegative particles. Therefore it seems that the enzymatic activation is not specific for mycobacteria.

This point of view is supported by the absence of bacterial lysis a) in lesions of animals previously vaccinated either by BCG or by *M. lepraemurium* (12), and b) in lesions not containing electronegative particles, even in animals inoculated with mycobacteria plus colloidal suspensions.

In the guinea pig and rabbit the mycobacteria alone have a strong injurious action upon the tissues. In such conditions the electronegative particles seem to act in an opposite way, namely inhibiting the functional activity of the macrophage. Then, it appears that the particles actuate differently according to the intensity of the injurious action of the mycobacteria upon the animal tissues.

The modifications of the histological structure of the rat lesions induced by the electronegative particles attain only limited areas of the lesions. Therefore, its practical value appears to be very restricted. Nevertheless it seems worthwhile to show that with the aid of experimental methods the rat macrophage (which behaves like the human macrophage of the lepromatous leprosy lesions) can acquire the property of lysing the *M. leprae* and *M. lepraemurium*.

SUMMARY

The action of electronegative particles on the histological structure of lesions produced by mycobacteria was studied in rats, guinea pigs and rabbits. *M. leprae* or *M. lepraemurium* suspensions were inoculated together with colloidal suspensions (Prussian blue, Trypan blue, charcoal) in these three animal species. The mixture were injected by peritoneal or intracutaneous route.

The lesions developed on the site of inoculation and in some organs were studied histologically during the lesions evolution.

In the rat the late lesions produced by mycobacterial plus suspensions of electronegative particles are stronger, more localized and present larger number of macrophages, than the lesions of control animals, which received mycobacteria alone. The macrophages phagocytise the bacilli and atrophy the particles in some limited areas of the lesion there is indication of bacterial lysis and electronegative particles digestion by the macrophages. In these areas, the macrophages after lysing the bacilli undergo transformation into epithelioid-like cells, and

the structural type of the lesions, is modified: tuberculoid dike areas develop within a lepromatous lesions. In the guinea pig and rabbit electronegative particles slow down the rate of mycobacterial lysis by the macrophage, resulting in a slower evolution of the lesions. The histological structure of the lesion, however, is not modified.

The alteration of the histological structure in the rat inoculated with mycobacteria plus electronegative particles is probably related to the enhanced stimulation by the bacilli and particles acting together; in this way lytic enzymatic systems of the rat macrophage are activated.

RESUMO

Estudou-se em ratos, cobaias e coelhos, a influência de partículas coloidais sobre a estrutura das lesões produzidas por micobactérias. Suspensões de *M. leprae*, ou de *M. lepraemurium*, foram inoculadas concomitantemente com suspensões coloidais (azul da Prússia, azul de Tripán, ou carvão), nessas três espécies animais, por via intraperitoneal ou intradérmica.

As lesões formadas no local da inoculação e em vários órgãos foram estudadas histologicamente durante a evolução da reação inflamatória.

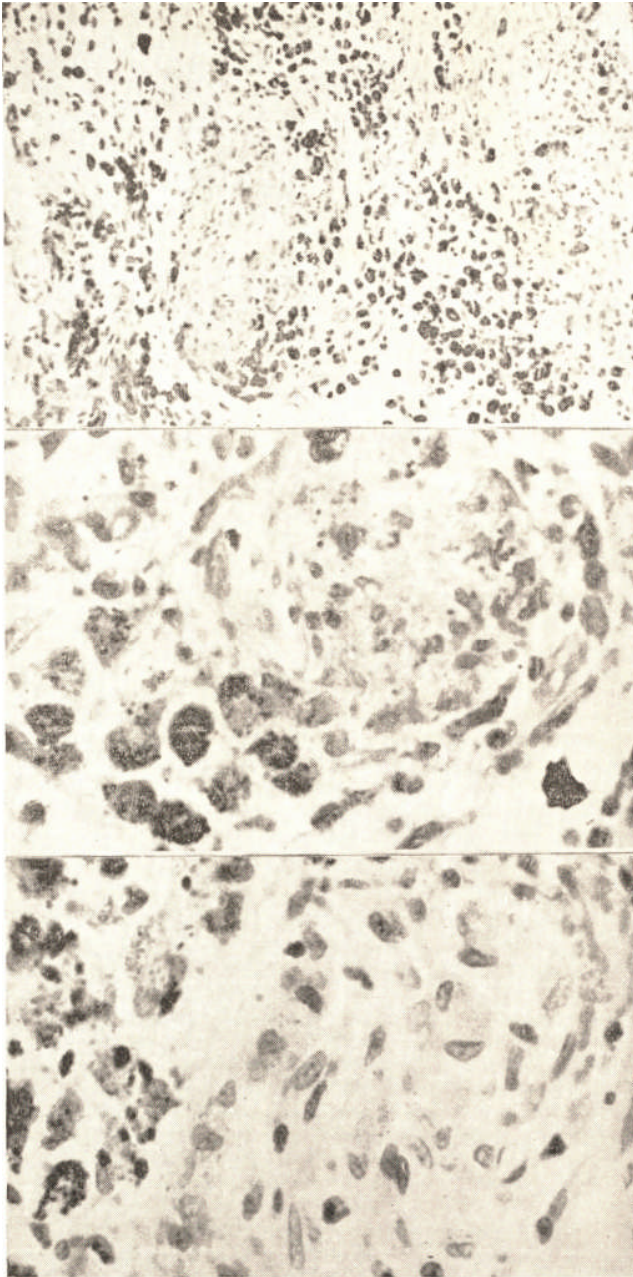
No rato, as lesões provocadas pela mistura de micobactérias e partículas eletro-negativas são mais intensas, mais localizadas e apresentam maior número de macrófago, comparadas com as lesões dos animais controles, que receberam apenas micobactérias. Os macrófagos fagocitam os bacilos e atrofizam as partículas injetadas. Em algumas áreas das lesões há indícios de lise dos bacilos e digestão das partículas, pelos macrófagos, o que não ocorre nos animais controles. Nessas áreas, que são limitadas, os macrófagos após usarem os bacilos se transformam em células de tipo epitelioide. Nestas circunstâncias, há modificação do tipo de estrutura das lesões, porquanto aparecem áreas de aspecto tuberculóide no interior de lesões lepromatosas.

No cobaio e no coelho, as partículas coloidais atuam em sentido oposto, tornando mais lenta a lise dos bacilos pelos macrófagos; como consequência a evolução das lesões é mais lenta. A estrutura histológica das lesões, no entanto, não é alterada.

A modificação da estrutura das lesões, verificadas no rato inoculado com micobactérias em mistura com partículas eletronegativas, é provavelmente devida à ação estimulante desses dois elementos agindo conjuntamente; nestas circunstâncias, haveria ativação de sistemas enzimáticos dos macrófagos, os quais seriam responsáveis pela lise das micobactérias.

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Rat lesions produced by the inoculation of **M. lepraemurium** plus Prussian blue. There are areas which cells contain numerous colored granules; in these areas bacilli are numerous: leprous cells. In other areas the cells do not contain granules and bacilli, taking a nodular arrangement: epithelioid-like cells.