Experimental Studies on the Functional Condition of Reticuloendothelial System, Especially with Reference to the Various Manifestation Types of the Leprous and Tuberculous Foci

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INTRODUCTION

Long-continued studies in our department have revealed the fact that the functional condition of the reticuloendothelial system (RES) exerts significant influences upon the various manifestation types of the inflammatory foci. When its functional activity is promoted, the cellular proliferation and metamorphosis are rapidly and conspicuously provoked. On the contrary, its lowered functional activity results in the weakened cellular response corresponding to its stage and degree. It has been also revealed that the functional condition of the RES can be approximately shown by observing the cellular forms in the inflammatory foci.^{1)-5), 8)-11)}

The authors made the following experiments to elucidate the problem of how functional conditional differences of the RES changes the type of manifestation of such diseases, such as tuberculosis and leprosy which show the various manifestation types, based on the experimental records mentioned above.

Studies on the Leprous Foci of the Experimental Mice

(I) Experimental Materials and Methods

The authors adopted typhoid vaccine injection as a way of activating the functional condition of the RES. Marked effect of the vaccine on the function of RE cells was examined by Dr. Ohta of our department.¹⁴⁾ Beforehand, a small quantity of the vaccine (0.2 cc.) had been injected in the peritoneum of mice (0.2 cc./20 g., one injection). Evans blue injection was used as another way of lowering the functional condition of

the RES (0.5%/0.1 cc./20 g., every other day, totalling five injections). Cardiolipin, one of the constituents of the rat leprosy bacillus, is closely connected with antigen-antibody reaction mainly in leprosy of the lepromatous type¹³⁾ and was injected as the third pretreatment (0.5 mg./0.2 cc./20 g., every other day, totalling 15 injections). According to Dr. Ohta,¹⁴⁾ cardiolipin generally inhibits the function of the RES.

Two days after the three kinds of pretreatment mentioned above were injected, each dd/T mouse was injected with living rat leprosy bacilli subcutaneously (10^{-2} /0.2 cc., Hawaiian and Kumamoto strains). Observation of manifestation findings in subcutaneous foci of leprosy mice was continued until the thirteenth week from the cytohistopathological point of view, and a comparison was made with the control mice which have not received these pretreatments. Two to three mice were killed every week, and the sliced samples of their subcutaneous tissue, lymph nodes, liver, spleen, lung, kidney and genital organs were stained with H.E., PAS, PAP silver impregnation and Ziehl-Neelsen's stain. Furthermore, cytological observations by supravital staining with neutral red and Janus-green, May-Giemsa staining and under the phase contrast microscope were made.

(II) Records of the Experiments

Following are the results of lepromatous changes observed in both group of the control mice and in another group of mice which received pretreatments. Observation limited to the subcutaneous tissue of the mice, was carried on.

(1) Findings of the Group of Control Mice

Formation of rat leprosy tubercle was brought about by the monotonous proliferation of the so-called lepra cells. Lepra cells were filled with rat leprosy bacillus which kept their original shape.

In the foci of the leprosy rat, local histiocytes swell and become round. These histiocytes showed a tendency to become free. Formation of polynuclear histiocytes and that of neutral red rosette in their cytoplasm were hardly observable. Basophilism of cytoplasm meaning the proliferation of the cell that belongs to the RES was not so marked. The main findings of this case were that histiocytes were transformed into the large rounded cells.

(2) Findings of the Group of the Mice Injected with Typhoid Vaccine

Soon after the injection, proliferation of the spindle shaped cell and the polymorphous cell, medium in size, were seen in the foci of the leprosy mice.

These cells took the form of the lepra cell as they came nearer and nearer the center of the foci. Furthermore, in the center of the foci, necrosis and degeneration were noticed. The foci showed centrifugal

aspects, and infiltration of the small rounded cell was conspicuous.

In the eleventh week, the lepra cells were hardly seen, and the foci demonstrated the so-called tuberculoid type composed of the epithelioid cells and the spindle shaped cells or the round mononucleated cells. In the same part, the presence of the polynucleated giant cells was observed dispersely (Fig. 1). Bacillus of rat leprosy is hardly seen and the remnants of them become droplets. In this case, local response of the histiocytes was caused mainly by the basophile cell and the numbers of these cells and their basophilism were noticeable. Tendency for inversion of the fixed type histiocytes formed newly into lepra cells was weak (Fig. 2).

Fig. 1. Tuberculoid foci in the subcutaneous tissue of lepra mouse which received pretreatment of typhoid vaccine before 11 weeks. Epithelioid cells and giant cells are seen (H.E.).

Fig. 2. The same case. (Supravital staining with neutral red and Janus-green).

Lepra bacilli are hardly demonstrable and remain in those phagocytosed ruins as neutral red granules. (Phase contrast microscope).

(3) Findings of the Group of the Mice Injected with Evans blue
Local injuries of the tissue were remarkable from the beginning. Numbers of bacilli of rat leprosy were perceived in each cell body which appeared in the foci accompanying with the infiltration of leucocytes and diffuse necrosis or edema of the tissue. But these foci had a poor tendency towards formation of the lepra cell, and the medium-sized spindle-shaped rounded cells showed marked degenerative changes. Cytological observation made it apparent that in the cell body of the histiocytes shaped newly (their number was small), granules stained with Evans blue were accumulated. A number of vacuoles, large and small, was demonstrated. Nuclei of these cells atrophied (Fig. 3).

Fig. 3. Diffuse necrotic foci in the subcutaneous tissue of a lepra mouse which received pretreatment of Evans blue before 10 weeks (H.E.).

(4) Findings of the Group of Mice Injected with Cardiolipin
More typical and earlier formation of leprous foci similar to those of the control mice was noted (Fig. 4). These lepra cells contained vivid rat leprosy bacilli, and were poor in substance stainable with PAS. Tendency towards fibrous proliferation was very weak and capillaries were seen dispersed freely among these cell group. Basophilism of histiocytes was hardly recognizable, and their tendency for becoming free was not demonstrated (Fig. 5).

Studies on the Tuberculous Foci of the Experimental Mice

(I) Materials and Methods of the Experiments

(1) Animal Used: One hundred and ten healthy dd/N male mice. Their body weight was about 20 g., provided that the functional state of the reticuloendothelial system have had been ascertained to be within normal limits.

Fig. 4. Typical lepromatous foci in the subcutaneous tissue of a lepra mouse which received pretreatment of cardioliipin before 13 weeks. Uniform proliferation of lepra cells are seen (H.E.).

Fig. 5. The same case. (Supravital staining with neutral red and Janus-green).

Lepra cells are filled with lepra bacilli (Phase contrast microscope).

(2) Pretreatment: Same as those mentioned above.

(3) Methods of Experiments:

Two days after the first injection of pretreatment, $H_{37}R_v$ dried human type tubercle bacilli diluted with physiological saline ($10^{-3}mg/0.1$ cc.) were injected into the vein of tail of both groups of the mice.

Three to five mice were killed every week until the eighth week, and the sliced samples of their lung, liver, spleen, lymph nodes, intestinal tracts and kidney were stained with H.E., PAS, PAP silver impregnation and Ziehl-Neelsen's stain.

(II) Records of Experiments

Tuberculous change of both group were examined in the lung of both groups of mice.

(1) Findings of the Control Mice

Three weeks after the injection of tubercle bacillus stated above, the increase of cells in the alveolar wall was observed, and the formation of small tubercles composed of the middle sized rounded or oval shaped cell were found around small arteries. The foci characterized by the cellular proliferation increased their number gradually in subpleural part. The cells showed swelling and vacuole droplet degeneration.

Tuberculous foci, which appeared mainly around arteries during the sixth to the eighth week, were composed of the same kind of cells mentioned above. Appearance of the giant cell derived from these cells was not clear. Accompanying the vacuole degeneration of the epithelioid cell, leucocytic infiltration and exudative fluid were observed, but the formation of the caseous foci was not found. In the material stained with the Ziehl-Neelsen's method, the formation of the caseous foci was observed in bundle form parallel to the increase of the vacuole cell after the fourth week. The foci contained fairly large amounts of the droplet cell.

(2) Findings of the Group of the Mice Injected with Typhoid Vaccine

In contrast to the control group, marked formation of productive tubercle was noted. The boader of the tubercle after the fourth week was clear. Exudation and leucocytic infiltration were hardly seen. The kind of cells that constitute the foci were the medium-sized and the small rounded cell and epithelioid cell derived from them. Degeneration of those cells was not seen (Fig. 6). By means of PAS stain, PAS stain posi-

Fig. 6. Productive tubercles in the lung of a tuberculous mouse which received pretreatment of typhoid vaccine before 8 weeks.

Small and middle sized cells and epithelioid cells are seen in the tuberculous foci (H.E.).

tive granules were seen to be arranged in rosette shape. The bacillus was observed as droplets (Fig. 7). This meant that the functional state of the RES raised.

Fig. 7. The same case. (Ziehl-Neelsen's stain).
Tubercle bacilli are difficult to find in the foci.

(3) Findings of the Group of Mice Injected with Evans blue

Numbers of speckled tubercle composed of the medium-sized droplets cells appeared in the third week. Leucocytic infiltration and storage of exudate were fairly conspicuous. The fourth to the fifth week, the foci showed changes of diffuse caseous pneumonia. The sixth to the eighth week, degenerative changes of the confluent foci become conspicuous, and the central parts of it appeared to be in necrosis, and cavity formation was observed. Ziehl-Neelsen's stain made it clear that tubercle of the third week contained fairly amounts of the tubercle bacillus corresponding to the increased number of the vacuole cell. After the third week, the number and spread of the bacillus increased rapidly in the cavity mentioned above (Fig. 8). The presence of numbers of bacillus was confirmed, as if they had been cultured in a pure medium. But all through these processes cellular digestion of bacillus was very slight (Fig. 9).

(4) Findings of the Group of Mice Injected with Cardiolipin

Findings of this group were nearly the same as those of the control group. However, the formation of the productive foci was rather strong, that is to say, the foci showed a tendency to diffuse and spread; the droplets degeneration of the medium sized cell appearing around the arteries was conspicuous. Marked exudation and leucocytic infiltration were observed (Fig. 10).

SUMMARY AND CONSIDERATION

As indicated in the above statements, the authors tried to clarify the

Fig. 8. Exudative foci in the lung of a tuberculous mouse which received pretreatment of Evans blue before 8 weeks.

Large tuberculous cavity is formed (H.E.).

Fig. 9. The same case. (Ziehl-Neelsen's stain).

Tubercle bacilli are more numerous seen in this case than in the control.

role of the RES as the main factor of the living body that is concerned with the various histopathological metamorphosis and types of focus manifestation of such diseases such as leprosy and tuberculosis.³⁾⁽⁶⁾⁽¹⁶⁾⁽¹⁷⁾

Generally speaking, basophilism of the RE cell has a relationship with new cellular production, and also it is considered to be the necessary pre-condition of phagocytosis of the RES.¹⁾⁻⁵⁾⁽¹¹⁾⁽¹²⁾

The RE cells show both proliferation and various histological metamorphosis, and this system responds to the functional demands of the living body.⁷⁾⁻¹¹⁾ For example, the appearance of the small rounded free cell is the reflection of the increased functional state of the RES, and on the contrary, weak appearance of these cells means the lowered functional

Fig. 10. Diffuse productive and exudative foci in the lung of a tuberculous mouse which received pretreatment with cardiolipin before 8 weeks.

Many middle sized and degenerated cells are seen in the tuberculous foci (H.E.).

state of the RE cells.

Furthermore, neutral red granules and PAS positive substance are supposed to be associated with the digestive mechanism of the RE cells for the foreign bodies.

In this study, the authors used this fact as a standard for determining the functional state of the RES from the morphological point of view. Based on the experimental results, analyses of the phase of the tuberculous and leprosy foci of the group of mice whose RES was lowered or raised were made respectively. The leprosy foci which were produced in the subcutaneous tissue of the mice by the injection of the lepra bacillus were typical lepromatous type in the control. In the group of mice which received the cardiolipin, earlier and more marked appearance of the foci of lepromatous type than that of the control. In the group injected with typhoid vaccine, the appearance of the tuberculoid foci was noted.

The tubercle foci formation in the lungs of the mice infected with human type tubercle bacillus is somewhat affected by the functional state of the RES. The same results can be stated about the foci of the rat leprosy. The tuberculous foci formation corresponding to the pretreatments, is characteristic in each case, and the kind and number of the cells taking part in the foci formation vary. These facts and the staining tendency of the tubercle bacillus within the foci presents enough material to say that the RES exerts influence upon the foci formation.

In the control mice which did not received pretreatment, proliferative foci formation was aroused. This type of the focus formation was accentuated by the increased functional state of the RES; under the lowered

functional state of the RES, formation of rather worse proliferative foci was demonstrated. Moreover under the disturbed condition of the RES, formation of exudative foci is characteristic. Summarizing the results mentioned above, it can be deduced that the difference of the functional condition of the RES in the living body exert very significant influences upon the various manifestation types of such disease as leprosy and tuberculosis. Therefore the authors believe that the function of the RES plays an important role as a non-specific resistance of the living body.⁷⁾⁽¹²⁾⁽¹⁵⁾

CONCLUSION

Results of the experiment on mice leprosy are as follows: 1) Promotion of the functional condition of the RES resulted mainly in the formation of tuberculoid typed foci. 2) Disturbance of the functional condition of the RES resulted in the formation of diffuse necrotic foci in the early weeks. 3) Inhibition of the functional condition of the RES resulted in the early formation of typical leprosy foci with contrast to that of the controls.

Results of the experiment on mice tuberculosis are as follows: 1) Promotion of functional condition of the RES resulted in the formation of marked productive foci as compared with that of the controls. 2) Disturbance of the functional condition of the RES resulted mainly in the formation of the exudative foci. Caseous necrosis and cavity formation were also noticed. 3) Inhibition of the functional condition resulted in the formation of more degenerated foci in contrast to that of the controls.

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