

RETICULOENDOTHELIAL RESPONSE IN MURINE LEPROSY¹

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Murine leprosy lesions occur most frequently in reticuloendothelial tissue, and bacilli proliferate electively in reticuloendothelial cells. A characteristic feature of both primary and metastatic lesions is that they invade mesodermal tissue and develop primarily around veins and capillaries. This clear relation to the small vessels is most relevant as metastatic lesions occur in the organs: in the liver around the central veins and capillaries, around the vessels of submucous connective tissue in the tubular organs. The same is valid for lesions in the lymph glands, spleen, bone marrow, or even in the connective-tissue cells around the vessels in the pia mater or around the vessels in the epineurium of the peripheral nerves. The interrelation of murine leprosy and the reticuloendothelial system as well as capillaries has been carefully studied by Tanimura and Nishimura (⁶).

The question then arises as to what is the reticuloendothelial and capillary endothelial response to this disease which is so closely related to these two types of cells, both representing the first defense barrier against injury. We have investigated this problem, and a summary of our observations is reported in this paper.

MATERIALS AND METHODS

Albino rats of the Wiersing strain, of both sexes, were used throughout the experiments. The rats weighed 50 gm. at the time of infection, and were kept on standard diet. Using a 3-month-old rat granuloma, the bacilli were separated by successive centrifugation, washed, and diluted with saline to 20 times the weight of the granuloma. No serum albumin was added. Eighty rats were inoculated with 0.5 cc. of the freshly prepared suspension of bacilli subcutaneously into the left scapular region. At the same time 80 other animals received 0.5 cc. of saline and served as controls. The capillary reaction as well as reticuloendothelial response was investigated: (1) 20 days after inoculation, when no palpable granulomata were present at the sites; (2) after 70 days, when palpable lesions had begun to develop; and (3) after 120 days, when all of the test animals presented huge granulomata, some of them with necrotic skin perforations, and showed loss of weight.

Upon each occasion, 20 infected and 20 control rats were chosen arbitrarily and were separated into four groups: (1) 10 controls; (2) 10 infected rats; (3) 10 serotonin

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(5-hydroxytryptamine (5-HT)) depleted controls; and (4) 10 5-HT-depleted infected animals.

The serotonin depletion was performed by intraperitoneal injection of 0.3 mgm/100 gm. of Reserpine 18 hours before the experiment. Each rat of each of the four groups received 30 mgm. of dextran intraperitoneally, after being weighed. Twenty minutes later, 5 rats in each group received 10 μ gm of histamine hydrochloride in 0.1 cc. saline into the abdominal skin, depilated the previous evening with an electric razor. Immediately after the histamine injection, 0.5 cc./100 gm. of India ink containing 3 per cent carbon and 3 per cent gelatin was injected intravenously. Eighty minutes after the dextran injection, the remaining 5 rats in each group received the same injection of histamine intradermally and India ink intravenously.

The carbon content in the blood was determined as by Heller *et al.* (³), photometrically at 5000A. To determine the rate of India-ink clearance, blood samples were taken from the tail vein of each rat every 10 minutes. Carbon content in the circulating blood was expressed as μ gm/cc.

India-ink accumulation was registered at the site where histamine was injected into the skin, in and around the granuloma, and in the extremities. Intensities of these reactions were assigned as negative to 4+.

Dextran-induced edema of the extremities and genitalia was registered 20, 30, 40, 80 and 110 minutes after the dextran injection. Intensity of edema was also noted as negative to 4+.

At the end of the experiments the animals were killed, the abdominal skin was removed, and India ink accumulation was read inside the removed skin. The livers and spleens were weighed. Autopsy was performed to reveal gross pathologic lesions.

EXPERIMENTAL RESULTS

The experimental set-up and a summary of the findings are presented in Table 1.

RESULTS 20 DAYS AFTER INOCULATION

At this time, when the inoculated animals were still virtually normal, no detectable granulomata were palpable through the skin; at autopsy an incipient lesion could be found in the subcutaneous tissue. There was no, or at the most weak, India-ink accumulation in or around the inoculation focus.

If India ink is injected before edema is induced by dextran, the dye accumulates at the site where histamine is injected and in the extremities, face and ears, where later the edema will develop. This India-ink accumulation is extremely intense in the infected rats as compared with the control animals. India ink clearance from the blood was at a normal rate in all of the rats.

A surprising difference was noted between the infected and uninfected groups when India ink was injected 80 minutes after dextran, at the time when dextran-induced edema had developed in the extremities. As previously described (²), in normal animals no dye accumulation can be produced with histamine, during the presence of dextran-induced edema, nor is there any dye accumulation in the extremities. However, a reversed capillary response was present in the infected rats, manifested by an intensified dye accumulation in the extremities and at the

TABLE 1.—*Capillary endothelial and reticuloendothelial response in rats with murine leprosy compared with uninfected controls; experiments performed in normal and serotonin-depleted rats 20, 70 and 120 days after inoculation.*

Serotonin status	Dextran edema	Murine leprosy or control	Time after inoculation											
			20 days				70 days				120 days			
			Hist. site ^a	Extremities	Granuloma	Dye clearance	Hist. site ^a	Extremities	Granuloma	Dye clearance	Hist. site ^a	Extremities	Granuloma	Dye clearance
Normal	Before	Infected	very intense	very intense	weak	normal	very intense	very intense	intense	normal	intense	intense	normal	
		Control	medium	medium		normal	medium	medium	intense	normal	intense	intense	normal	
	During	Infected	intense	intense	none or weak	delayed	intense	intense	intense	intense	intense	intense	delayed	
		Control	none	none		delayed	none	none	none	medium	none	none	delayed	
Depleted	Before	Infected	intense	intense	none	normal	medium	medium	normal	medium	none	normal	normal	
		Control	none	none or weak		normal	none	none	normal	none	none	normal	normal	
	During	Infected	intense	none	none	delayed	medium	medium	delayed	medium	none	delayed	delayed	
		Control	none	none		delayed	none	none	delayed	none	none	none	delayed	

^a Site of histamine injection.

No difference between infected and uninfected rats. No dye accumulation in and around granuloma.

site of histamine injection. During the presence of dextran-induced edema, as before, there was but weak dye accumulation in the incipient granulomata, and again no differences in India-ink clearance were observed.

In the serotonin-depleted, uninfected animals, if India ink was injected before the appearance of dextran-induced edema no dye accumulation occurred, as also described previously (²). The infected rats of the 5-HT-depleted group, however, presented a reverse capillary response: an intense dye accumulation occurred at the site of histamine injection, whether India ink was injected before or during the presence of dextran-induced edema, but there was no difference in dye accumulation in the extremities if India ink was injected during the presence of edema. No dye accumulation occurred at the site of infection. There were no differences between these two groups with respect to India-ink clearance.

RESULTS 70 DAYS AFTER INOCULATION

At this time, granulomata were palpable through the skin, and at autopsy there were well-developed but nonnecrotic lesions at the sites of infection. At this stage of the progressing disease, remarkable differences were found again between the capillary behavior in the infected and uninfected rats, characterized by an intensified capillary response to histamine injection before the occurrence of dextran-induced edema. During the edema, when no dye accumulation can be provoked by histamine in the control rats, there was an intense dye accumulation in the infected animals. At this stage of infection India ink particles accumulate intensely in the granuloma and the nearby connective tissue.

In animals depleted of 5-HT, no dye accumulated in the granulomata. In the depleted controls, there was no dye accumulation at the site of histamine injection or in the extremities, while again there was accumulation in these sites in the infected rats. The India-ink clearance rate was the same whether the animals are infected or not.

RESULTS 120 DAYS AFTER INOCULATION

At this time huge granulomata had developed in every infected rat which was tested. Some of the animals had necrotic skin perforation, and at autopsy all lesions presented necrotic centers.

At this advanced stage of infection, all tested reactions were the same in the infected and uninfected rats, whether normal or 5-HT-depleted. As usual, India-ink clearance was at the same rate. The remarkable thing is that there was no dye accumulation in the huge granulomata or in the nearby connective tissue.

DISCUSSION

The capillary wall is not only a selective barrier between the blood and the surrounding tissues, but it represents a rather special and unique functional entity. Every component of the semipermeable membrane undergoes profound alterations in different conditions and under different influences, and the capillary endothelial cells alter their physical and chemical characteristics accordingly. The transport of molecules and particulate matter through the capillary bed is particularly altered as mechanical, physical or chemical stimuli occur at or near the capillary bed. If stimuli or injuries occur, capillary endothelial cells acquire a peculiar adsorptive capacity. The inner surface of the cells becomes sticky, and particulate matter, like colloid dye circulating in the blood stream, adheres to the sticky cell surfaces at and near the site of the injury.

This capillary response is the most important feature of the first stage of the inflammatory process, and it is an important defense barrier permitting the localization of injurious agents. The acquired stickiness of the capillary endothelium is visualized by the India-ink accumulation at the site of lesions or stimuli, such as intradermal injection of histamine. The India ink test also demonstrates the "activated" functional capacity of capillary endothelium, a state in which the cells can perform the same function as any other cell of the reticuloendothelial system: an active engulfment of particulate matter. In this stage of endothelial "activation" there is no functional difference between "common" (reticuloendothelial) or "special" (capillary) endothelial cells. Accumulation of India ink or other colloid dyes, therefore, represents an activated state of the capillary endothelium.

In 5-HT-depleted rats, however, the capillary endothelium cannot be activated, and, as previously described (³), there is no accumulation of India ink or acid dye at the site of injury or stimulus. It cannot be concluded, however, that endothelial activation is mediated by 5-HT; it seems rather that 5-HT interferes indirectly with endothelial activation (⁵).

In the light of this knowledge, an interpretation of the results obtained can be attempted. There is a definite fluctuation in capillary response during the progression of murine leprosy, as shown by altered India-ink accumulation in the infected rats. In the early stage of the disease there is an increased alertness of the capillary endothelium. This stimulated response is demonstrable when 10 μ gm. of histamine is injected into the skin. While India ink accumulates with medium intensity at this site in control animals, an extremely intense dye accumulation occurs in the infected animals.

More characteristic are the findings if the dye is injected during the presence of dextran-induced edema, or into serotonin-depleted rats. In this latter case there is no dye accumulation at the site of injury or

stimulus, as we reported earlier and as is shown in the present experiments. Not so in the animals infected with murine leprosy. In the earlier stages of infection (20-70 days) the capillary endothelium of the infected rats is activated by minute amounts of histamine or by the anaphylactoid substance dextran, even during the presence of edema and even if animals are depleted of serotonin. This altered capillary response during the infection reveals a *stimulated readiness of capillary endothelial cells in the combat against injury*.

As the murine leprosy advances and huge necrotic lesions appear the stimulated capillary response is no longer present, since no difference was found in the capacity of the endothelial cells to accumulate colloid dyes in the control and infected groups, whether normal or serotonin-depleted. So long as there is an active defense against the invading parasite, the organism is mobilizing its armamentarium as reflected in the stimulated capillary response. This response is no longer present in the late stage of the infection, when the destiny of the host is determined and the disease advances to the inevitable fatal outcome. The stimulated capillary response disappears at the time of the appearance of metastatic visceral lesions, which were shown by Tanimura and Nishimura (6) to occur at this time.

Similarly instructive is the colloid-dye accumulation in and around the granuloma. In the incipient lesions and in those which are slowly progressing, the intravenously-injected dye is accumulated intensely. This capillary reaction shows an activated state in and around the lesions. Since India-ink accumulation is a visible sign of the acquired stickiness and adhesiveness of capillary endothelium and of its increased permeability, the dye accumulation in the early granuloma reflects a continual endothelial activation, a continual acquired adsorptive capacity of the endothelial surface. In the late stage (120 days), this defense mechanism of the capillary endothelium is no longer maintained. Although the same injury (granuloma) is present, no endothelial activation, no increased permeability, is evident: there is no dye accumulation in the late, huge necrotic granuloma. It is at this stage that the local defense barrier is broken through and metastatic lesions occur.

It is too early for speculation as to which factors mediate the increased capillary response during the early phase of the disease, which factors maintain it during the early progression, and then break it as the disease approaches the fatal outcome. It is tempting to investigate the participation of the activated "permeability factor" (4) in the stage of active defense, and the interference of the "inhibition permeability factor" in the stage of passive defense, to obtain a more accurate explanation of the present observations.

It was quite natural that, during the period of experimentation, the rate of India-ink clearance from the blood stream of infected rats did

not differ at any time from that of the control rats. These results reflect a normally-functioning reticuloendothelial system during this period of the disease. It is not excluded that in even more advanced stages, when the reticuloendothelial system becomes progressively invaded, the rate of clearance becomes delayed. These experiments were not attempted, because in aged rats, weighing more than 400 gm., the India-ink clearance cannot be evaluated because of great individual fluctuation. At the 120th day the average weight of the controls was 423 gm., against 285 gm. for the infected rats (without the weight of the granuloma).

The relative weight of livers and spleens of infected and control rats did not show any differences during the whole period up to the 120th days. Biozzi *et al.* (1) found that the weight of these organs increases progressively during infection with BCG as the immunity progresses. Whether the unaltered weight of these organs in murine leprosy reflects a complete absence of immunity remains an open question.

SUMMARY

The capillary-endothelial and reticuloendothelial responses in rats with murine leprosy were compared with responses in uninfected controls. Experiments were performed on normal and serotonin-depleted rats, 20, 70 and 120 days after inoculation. Capillary reactions were provoked with dextran and with histamine, then visualized with India ink. A definite fluctuation in capillary response is demonstrable during the progression of the disease. In the early stage of the infection there is a stimulated systemic capillary response, but in the advanced stage this capillary alertness is no longer present. Similar stimulated endothelial response is present in the incipient and slowly progressing lesions of inoculation, whereas in the late necrotic stage there is no endothelial activation in the granuloma. Apart from the altered capillary response there is no change in the phagocytic function of the reticuloendothelial system as shown by the India ink test made 20 and 70 days after inoculation.

RESUMEN

Las respuestas capiloendoteliales y retículoendoteliales en las ratas con lepra murina fueron comparadas con las observadas en los testigos indemnes. Los experimentos se ejecutaron en ratas normales y desprovistas de serotonina, a los 20, 70 y 120 días de la inoculación. Se provocaron las reacciones capilares con dextrán y con histamina, visualizándolas con tinta china. Durante la agravación de la enfermedad puede observarse una fluctuación bien definida de la respuesta capilar. En el período incipiente de la infección, se nota una excitación de la respuesta capilar constitucional, pero en el período avanzado deja de existir esa viveza capilar. Existe una excitación semejante de la res-

puesta endotelial en las lesiones incipientes y de avance lento producidas por la inoculación, mientras que no se observa activación endotelial del granuloma en el período necrótico tardío. Aparte de la alterada respuesta capilar, no existe modificación de la función fagocítica del aparato retículoendotelial según revela la prueba de la tinta china ejecutada a los 20 y 70 días de la inoculación.

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