

ON THE QUESTION OF THE PECULIARITIES IN THE
MORPHOLOGY OF THE VACCINAL TULAREMIA INFECTION
IN ACUTE RADIATION SICKNESS

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In connection with the wide usage of ionizing radiation the question of the peculiarities in the morphology of the infectious process acquires actual importance. Indications are present in the literature that in the presence of radiation sickness an unusual course in the inflammatory process is observed, manifesting itself in the pronounced depression of the proliferative component of inflammation and predominance of certain hemorrhagic changes [1-3].

However, these investigations concerned themselves principally with the local inflammatory process. At the same time a study of the morphological course of inflammatory changes in generalized infections under conditions of radiation injury would present considerable interest, especially in the presence of infections characterized by definite specific morphological changes, particularly granule formation.

As a result of these considerations, we assigned ourselves the task of studying the peculiarities of the morphology of vaccinal tularemia infection in acute radiation sickness. As is well known [4-5], in vaccinal tularemia infection the same specificity of morphological changes is fundamentally retained as is seen with the administration of virulent tularemia bacteria.

METHOD

The experiments were carried out on 50 guinea pigs, whose average weights ran from 400 to 500 g. Part of the animals were subjected to a single total roentgen-radiation dose of 290 r DL 20 - 30/30 in the RUM-3 radiotherapy apparatus. Conditions of the radiation: 180 kv, 10 ma, 0.5 mm Cu and 1 mm Al filters, surface area 20 × 20 cm, focus distance 40 cm, dose energy 32 r/min. Twenty-four hours after the radiation, twenty pigs were injected intracutaneously, in the region of the right hip, with 1 billion microbial cells of a 48-hour culture of *B. tularensis*, prepared from dried tularemia vaccine obtained from the N. F. Gamaleya Institute of Epidemiology and Microbiology of the AMN SSSR. Two

control experiments were set up: inoculation of an analogous dose of the vaccinal strain of bacteria without radiation (20 pigs) and radiation without inoculation (10 pigs); the irradiated control pigs were injected intracutaneously with physiologic saline. In addition, three normal pigs of the same weight, taken from the same group of animals as the experimental pigs, served as controls. Five pigs were sacrificed after 1, 3, 5, 7, 10, 14, 21, and 31 days post inoculation (two experimental, two inoculated controls, and one irradiated control). The investigated tissues were fixed in 10% formalin. The histologic preparations were stained with hematoxylin and eosin, and in isolated instances, by van Hieson's method. Blood was taken from each pig before it was sacrificed and determinations made of the number of leukocytes and the phagocytic index in relation to staphylococcus. In addition, beginning from the 14th day after inoculation, the titers of specific agglutinins were determined.

RESULTS

The dose of radiation used in the experiment caused death in 4 of the irradiated inoculated pigs and 2 of the irradiated control pigs. No deaths were observed among the inoculated control pigs.

Leukopenia developed in the irradiated pigs beginning from the fourth day after irradiation (3000 to 5000 per mm³); the number of leukocytes decreased still further on the sixth day (to 2000 per mm³), was maintained at this low level over the course of the eighth to eleventh day, rose on the fifteenth day to 5000 to 7000 and was restored to the normal magnitude by the 22nd day after radiation exposure. The degree of leukopenia was manifested approximately equally by the experimental animals (inoculated after irradiation) and the irradiated control animals which did not undergo inoculation. In the inoculated control animals on the 14th and 21st day after injection of the bacteria a minimally manifested increase in the number of leukocytes was noted.

In the experimental pigs and the irradiated control pigs on the eighth day after irradiation, the phagocytic index decreased to 7 -15 against 18 - 23 in the inoculated control animals. Beginning from the eleventh day after radiation exposure and later it was not possible to evidence a distinct lowering of the phagocytic index in the experimental animals. As a rule, the titer of specific agglutinins did not change under the influence of radiation exposure. A decrease in the titer of antibodies in the irradiated inoculated pigs compared with the inoculated controls was seen only on the 21st day after irradiation.

We present the data of the morphological investigation.

Skin at the Site of Injection. A seropurulent inflammation is noted in the experimental animals in the dermis and subcutaneous cell layers 24 hours after inoculation. In the center of the focus of infection the walls of the small blood vessels are impregnated with plasma, this not being seen in the inoculated control animals. In the experimental animals, after 3 days, distinct necrotic changes are noted in the center of the nidus with a simultaneous productive reaction about the periphery in the form of reproduction of a considerable number of histiocytes; no essential differences are shown at this stage in comparison with the changes in the control pigs.

On the 5th day after inoculation, the necrotic changes in the zone of inflammation increase. The epidermis is necrotized. Massive foci of necrosis with nuclear disintegration are found in the dermis, as well as the subcutaneous tissue down to the muscle layer. At the periphery of the necrotic foci are observed numerous hemorrhages, edema of the blood vessel walls, and a productive-destructive panvasculitis. There is also noted a small productive reaction in the form of histiocyte reproduction. In the zone of the productive reaction around the blood vessels appear tularemia granulomas, consisting of epithelioid and plasma cells, fibroblasts, neutrophilic leukocytes and isolated lymphocytes. In contradistinction to the inoculated control animals, in the experimental animals at these time intervals the alterative-necrotic changes predominate over the exudative-productive ones.

On the 7th and 10th day after inoculation, in the experimental animals, unlike the controls, there is still rather considerable necrosis with nuclear disintegration and hemorrhages along with an increase in the productive reaction and the formation of tularemia granulomas. By the 14th and 21st days after injection of the bacteria, in the skin and subcutaneous layers in between the separated elements of connective tissue, specific granulomas are still encountered, consisting of epithelioid cells, histiocytes, a small number of neutrophilic leukocytes, and multinucleated giant cells. The vaccinal tularemia skin changes are still not completed by the 31st day after inoculation. Here one can still encounter remnants of in-

completely healed granulomas and even small leukocytic infiltrates. The impression is created that at a specific stage (from the 21st day after inoculation) the processes of healing at the site of injection of the vaccinal strain is inhibited somewhat in the experimental pigs as compared with the healing processes in the inoculated control animals.

Thus, in the skin of the vaccinated animals under the influence of irradiation, as opposed to the inoculated control pigs, beginning with the third day and up to the tenth day after inoculation more apparent necrotic-destructive changes are observed, involving all the elements of the skin including the walls of vessels. After the 10th day these differences level off and fall into the boundaries of individual variation in the reactions of animals. Furthermore, beginning with the 21st day the impression is created of a certain inhibition of the reparative processes in the experimental animals; in addition the morphological specificity of the vaccinal process is preserved in the experimental pigs (see figure).

In the irradiated control pigs 24 hours after the injection of physiological saline, edema and fine hemorrhages were seen in the dermis. In the following days a weakly expressed histiocytic reaction was observed, and after 14 days, a small scar.

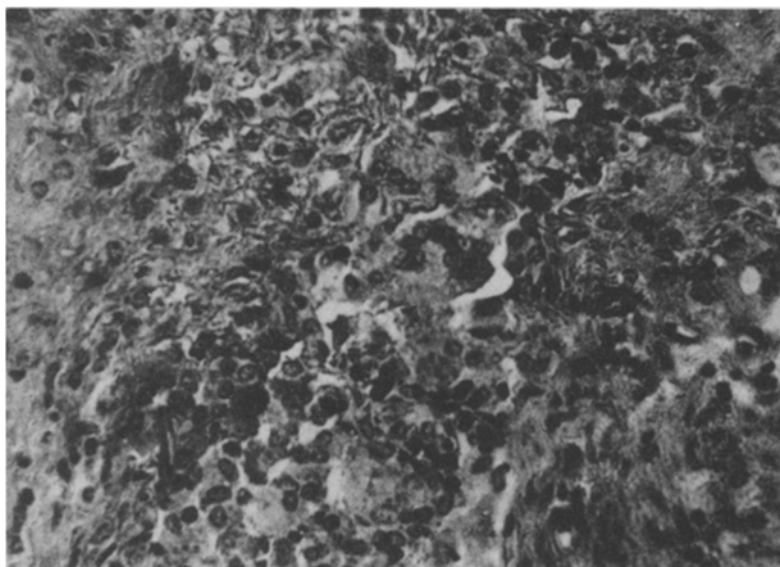
Regional Lymph Nodes. No essential differences were noted in the experimental animals in comparison with the nonirradiated pigs after 24 hours. In both cases the appearance of a seropurulent lymphadenitis took place. In the experimental animals after 3 days a necrotic process developed in the lymphatic follicles; the nuclei of the lymphocytes underwent rupture and fusion. In the cortical layer under the capsule single miliary granulomas were encountered, consisting of reticular and epithelioid cells, neutrophilic leukocytes and single lymphocytes.

After 5 to 7 days, in the experimental pigs, along with the presence of miliary polymorphocellular granulomas in the cortical layer, were observed radiation changes manifested by a depression of lymphoid hematopoiesis and hemorrhages in the sinuses. In the inoculated control animals only polymorphocellular granuloma were noted.

After 10 to 14 days, numerous tularemia granulomas of different degrees of maturity were seen in the regional lymph nodes. In the experimental pigs were encountered coarse granulomas with widespread necrosis and nuclear disintegration in the center and a distinct specific productive reaction about the periphery in the form of growth of the epithelioid, reticular cells and fibroblasts. Other granulomas consisted basically of reticular, epithelioid cells and leukocytes. Dissimilar changes were noted in the lymphatic follicles; in some there was depression of lymphopoiesis; in others, on the contrary, there was development of reparative processes, characterized by the appearance in the follicles of small clear centers, made up of young lymphocytes.

After 21 to 31 days in the experimental pigs we also observed in one case wider necrotic changes in the tularemia granulomas in comparison with the control

cases. The vaccinal changes were seen in a background of completely distinctive reparative-hyperplastic processes in the follicles.



Tularemia granuloma with giant cells in the skin of experimental guinea pigs 21 days after the injection of the vaccine. (Hematoxylin-eosin stain. Magnification: ocular, 7 x, objective 40 x).

Thus, in the regional lymph nodes of the experimental pigs the more frequently encountered alternative-necrotic changes can be noted in the tularemia granulomas in comparison with the inoculated control animals, with concomitant radiation changes and their sequelae.

The dynamics of radiation changes in the experimental pigs were not distinguishable morphologically from the changes in the irradiated control pigs.

The Spleen. In the experimental animals, as well as in the inoculated controls, in not a single case were specific tulareminal changes encountered in the spleen. Here the morphological differences were manifested in the state of the lymphoid apparatus. Just as in the regional lymph nodes, 3 days after inoculation a depression of lymphopoiesis in the follicles was noted in the experimental pigs. In them the clear propagating centers were not seen; in the central parts of the follicles there was the appearance of karyolysis and karyorrhexis in the lymphocytes. Subsequently, up to the 14th day progression of these processes was observed. A substantial hemosiderosis appears. Beginning with the 21st day, along with the destruction of the lymphoid tissue in numerous follicles and partial replacement of the latter with connective tissue, the signs of restoration of lymphopoiesis are noted in the preserved follicles. This

is expressed in the formation of light centers in the follicles, consisting of young lymphocytes, and in the restoration of normal follicle dimensions.

Comparing the appearance revealed with the changes in the irradiated control pigs, it is possible to conclude that all the described morphological changes in the spleen of the experimental animals were related to irradiation.

In the inoculated control animals, as opposed to the experimental pigs, there was observed a typical non-specific infection-type of hyperplasia of the splenic pulp.

Liver and Kidneys. Under the influence of ionizing radiation there early develops a granular dystrophy of the parenchymatous elements in the liver and kidneys.

The data obtained allows arriving at the conclusion that massive roentgen radiation of guinea pigs, causing acute radiation sickness with a mortality outcome of 20 to 30% of all cases, produced previous to injection into the skin of massive doses of the vaccinal strain of tularemia bacteria, does not change the morphological specificity of the vaccinal process. However, in addition, all this causes certain morphological peculiarities, demonstrated in the fortification of the alternative component of inflammation in the skin and regional lymph nodes. In the cases described by us, these peculiarities

were not as markedly manifested as those observed by other authors [1-3] with the action of ionizing radiation on a nonspecific nidus of inflammation. Despite the in-

dedicated morphological differences, the clinical course of the vaccinal process in both the experimental and the control animals did not essentially differ.

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