MORPHOLOGICAL CHANGES IN THE NERVE CELLS OF THE SKIN ON IMMUNIZATION WITH LIVE TULAREMIA VACCINE

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Clarification of the role of the nervous system in the processes of infection and immunitys attracting the attention of microbiologists and immunologists at present. Morphological methods of investigation have not yet been applied sufficiently to the study of this problem. The morphological changes in the nerve cells with the administration of various bacterial preparations were studied by E. A. Kirilov [1]. He also investigated the morphological changes in the nerve cells when live smallpox vaccine is administered. A. S. Shevelev and M. N. Prudnikova showed (1954) that when a vaccine strain of tularemia bacteria is administered in different sections of the same tissue (skin, subcutaneous tissue), different immunizing effects are obtained. Thus, when the antigen was administered intracutaneously, the highest titer of specific agglutinins was obtained when the immunization was made in the skin of the nose, the lowest when in the skin of the abodmen.

The purpose of the present work consisted of the study of the morphological reaction of the nerve cells of the skin to immunization with live tularemia vaccine.

EXPERIMENTAL METHODS

Desiccated tularemia vaccine from the N. F. Gamaleya Institute of Experimental Medicine of the Academy of Medical Sciences of the USSR (series 63, control number 310) was administered to 16 guinea pigs intracutaneously—simultaneously into the abdominal skin and the skin of the tip of the nose, using 0.1 ml of vaccine each (6.75 of a human dose). Nine control guinea pigs were administered 0.1 ml each of a saccharose-gelatin solution in which tularemia vaccine is usually desiccated into the same areas of the skin. The animals were killed in 1 hour, 7 hours, 24 hours and also in 2, 5, 9, 16, 22, 30 and 46 days after vaccine administration. Pieces of skin were cut from each guinea pig in the area of the injection together with the underlying subcutaneous tissue and muscle and fixed with 20% neutral formalin. Staining was carried out according to Gross-Bielschowsky as modified by B. I. Lavrentyev with subsequent supplementary staining with carmine.

EXPERIMENTAL RESULTS

Clear changes were not observed on macroscopic investigation of areas of the skin where the vaccine was administered 1 hour and 7 hours after vaccination. In 24 hours, infiltration and redness 0.5 × 0.5 cm in diameter were observed at the place of administration into the abdominal skin; at the place of vaccine injection into the nasal skin, limited edema and compression were noted. In 48 hours approximately the same picture was observed. On the 5th day, the infiltration in the abdominal skin increased to a size of 1 × 1 cm, but hyperemia was no longer observed; at the place of injection into the nasal skin, the formation of a scab was noted, limited edema and compression of the tissue. On the 9th day, the intensity of the infiltration in both areas of skin was approximately the same as on the 5th day, a scab was evident on the nasal skin, and an area of necrosis appeared in the center of the infiltration in the abdominal skin. On the 16th day, a definite decrease in the local reaction was noted, a dot of defective tissue was visible in the center of a small infiltration. Beginning on the 22nd day after vaccination, clearly evident changes at the place of vaccine administration could not be found.

Histological changes in the local tissue elements were already visible one hour after vaccine administration. At this time, clear exudation and infiltration was observed, primarily around the blood vessels. These phenomena were most evident in the abdominal skin. The blood vessels were enlarged, hyperemic. The exudate consisted principally of polymorphonuclears and extended deep into the connective tissue and muscles (In guinea pigs, the layer of true skin is very thin on the tip of the nose and muscle fibers penetrate the skin, almost under the epidermis itself). At this stage, the emigration of leucocytes through the capillary walls could be seen. Later, the inflammatory reaction progressed and in 7 hours the exudative reaction increased, taking on a primarily diffuse nature. In places, the exodus of polymorphonuclears was so great that small abscesses, consisting primarily of polymorphonuclears, developed in some areas of the nasal skin. Along with the phenomena of exudation, diffuse reaction of the connective tissue was already observed at this stage, evidenced as a multiplication of the cells, primarily of the histocytes. The infiltration was more evident in the abdominal skin. In 24 hours the above phenomena increased. The diffuse reaction of the connective tissue increased. Definite edema of the tissues was observed. Degenerative changes in the epidermis appeared. The infiltration, which was located primarily in the papillary layer of the skin, even extended into the underlying tissues (subcutaneous tissue, muscles).

In 48 hours, the microscopical changes were approximately the same as after 24 hours. Powerful diffuse infiltration, extending into the underlying tissues, was under the epidermis. The infiltration consisted of polymorphonuclears with an admixture of plasma and lymphoid cells. In the vicinity, there was edema and a histocytic reaction. In 5 days, a powerful infiltration of approximately the same composition as after 2 days was found at the place the vaccine was administered, only the relative number of plasma and lymphoid cells increased somewhat. Diffuse leucocytic infiltration was visible under the necrotic epidermis. diffuse histocytic reaction increased with the distance from the epidermis.

On the 9th day, the vascular reaction and exudation became quiescent and the proliferative changes began to predominate, evidenced as the formation of specific granulation tissue, distributed either diffusely or in the form of nodes of epithelial cells primarily with an admixture of plasma and lumphoid cells, as well as polymorphonuclears. Among these cells, in places single giant cells of oval or disc shape with many nuclei, located primarily at the periphery of the cell, were found. At this stage a pustulous area was visible in one of the guinea pigs, which was surrounded by a strong wall of proliferating connective tissue cells. On the 16th day, a predominance of the productive reaction was observed. The number of leucocytes sharply decreased. Spindle-shaped connective tissue cells predominated in the infiltrate. Diffuse infiltration of epithelioid cells could be seen, node-like in nature in places, with an admixture of occasional multinuclear giant cells.

On the 22nd day, the young granulation tissue decreased sharply in volume. Edema of the tissues was still clearly evident. In places, granulomas were evident, in some of which the initial phenomena of fibrosis were observed. In 30-46 days after vaccination, the changes were approximately the same. In 46 days, only individual granulomas remained, around which evident fibrosis was observed.

The results we obtained confirmed the data of other investigators [3], according to whom the morphological reaction to immunization with live tularemia vaccine does not differ in essence from the reaction observed on administration of a virulent strain of tularemia bacteria.

We note that the intensity of the inflammatory reaction in the nasal skin was approximately the same as in the abdominal skin in most cases, but at earlier stages the inflammatory reaction in the abdominal skin was more evident.

Changes in the nerve cells of the skin were already observed 1 hour after vaccine administration: a clearly evident coarseness of the nerve fibers could be observed, their argentaffinity and also edema of the perineural investments of the large nerve fasciculi (Fig. 1). In 7 hours the phenomena of irritated cutaneous nerve cells increased. Nerve fibers with beaded thickenings were visible. At the ends of the nerve fibers, leakage of neuroplasma appeared in the form of bulbous extrusions. Enlarged Schwann nuclei, spherical in form, occurred in the sheaths of some of the nerve fibers. In 24 hours, along with the phenomena of the irritated nerve fibers (coarseness of contours, argentaffinity, uneven extrusions) degenerative changes in individual nerve cells first appeared, characterized by their fragmentation. These changes were only observed in the nerve fibers of the nasal skin. At this stage, it was possible to see the penetration of polymorphonuclear leucocytes into the sheaths of the nerve fascicles. A similar picture was observed in the period between the second and fifth days. Coarse irritated nerve fibers were observed in the abdominal skin as well as in the nasal skin. Degenerative changes of occasional nerve fibers were observed primarily under the epithelium. In the nerve preterminals and terminals, which end

at the muscles, only phenomena of irritation were observed (argentaffinity, coarse contours, leakage of neuroplams). Changes in the nerve fibers, as at the earlier stages, were noted, as a rule, not in the zone of infiltration, but at a certain distance from it.

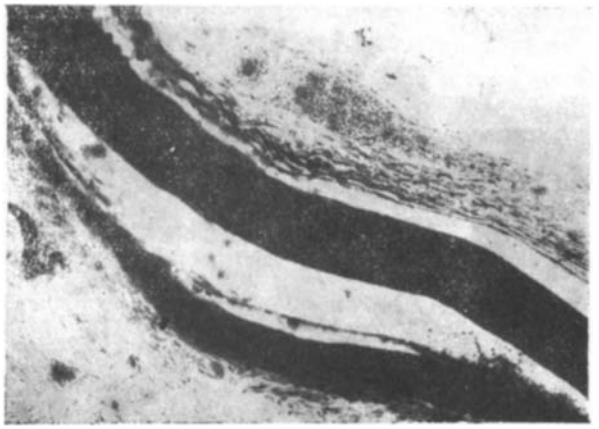

Fig. 1. Edema of the perineural sheath of a large nerve fascicle. The skin of the tip of the nose 1 hour after vaccine administration. Microphotograph: Magnification-ocular 8, objective 20.

By the 9th day, the number of irritated nerve cells increased somewhat; degenerative changes were still evident in an insignificant number of nerve fibers. On the 16th day, the intensity of these phenomena increased sharply. The percentage of affected nerve cells also increased. These changes were observed primarily at the place where the vaccine was injected into the nasal skin.

The degenerative changes in the nerve cells of the skin reached maximum development on the 22nd day after vaccination (Fig. 2). At this stage, the number of irritated preterminals and terminals in the muscles increased. The absence of degenerative changes in the motor nerve fibers in the muscles, while acute degenerative changes took place in the sensory nerve fibers located along the epidermis and hair follicles, and also in the neural apparatus of the sinuous hairs on the tip of the nose drew attention, as at the earlier stages. Some nerve fascicles and fibers were found to be completely degenerated, with granular degeneration. The degenerative changes were especially acute in the nervous apparatus of the sinuous hairs. The changes in the nerve cells were also most clear at this stage in the nasal skin.

Fig. 2. Fragmentation of nerve fibers of the root sheath of a hair. Skin of the tip of the nose on the 22nd day after vaccine administration. Microphotograph: Magnification-ocular 7, objective 20.

On the 30-46th day after vaccination, the degree of affection of the nerve cells decreased noticeably. Degenerative changes were found considerably less often and were primarily evident in the bulbs of the sinuous hairs.

Cutaneous pathological histological changes were considerably less evident among the control animals who were administered saccharose-gelatin solution than among the vaccinated guinea pigs. Diffuse leucocytic infiltration, accompanied by a weak connective tissue reaction, was noted 1-7 hours after injection. Later, however, the infiltration was considerably less evident than among the experimental animals, and on the 5-9th day and later, predominance of a weak histocytic reaction and edema of the tissues were observed. On the 30th day, weak fibrosis was noted. The formation of specific granulation tissue was not observed. Changes in the nerve cells were also considerably less evident than among the vaccinated guinea pigs and consisted primarily of irritation of the nerve fibers and their terminals. The most intensive changes in the nerve cells took place on the 9-16th day after injection. Consequently, the exudative-proliferative changes, as well as the changes in the nerve cells of the skin were considerably less evident in the control guinea pigs and reached their maximum development before the vaccinated animals.

Some peculiarities of the reaction of the cutaneous nerve cells on immunization of the guinea pigs with live tularemia vaccine drew attention. First of all, predominantly, affection of the sensory nerve fibers and their terminals was observed. Changes in the motor nerve fibers which ended in the muscles, were evident

primarily as phenomena of irritation, not degeneration. Another peculiarity consisted of the fact that changes in the nerve cells were usually found not in the mass of exudate (granulation tissue, scar), but at a certain distance from it. This was evident at various stages in the vaccination process, but was especially clearly evident during the first 5 days after vaccination.

The changes in the nerve cells were most evident in the nasal skin both at the earlier and later stages in the vaccination process. These changes were observed less frequently in the abdominal skin and were less clearly evident. In addition, the inflammatory reaction in the nasal skin was more intensive than in the abdominal skin. On the contrary, in a number of cases the inflammatory process proceeded more violently in the abdominal skin. Consequently, the more evident immunological effectiveness of immunizing guinea pigs in the nasal skin in comparison with their immunization in the abdominal skin can be connected with the more evident reaction of the nerve cells of the nasal skin to the administration of tularemia bacteria.

SUMMARY

We have described dynamics of changes in nerve cells of the skin and reaction of connective tissue in the case of injection of living tularia vaccine to guinea pigs into the skin of their nose tip and abdomen.

Better defined lesions in nervous elements have been observed not in the zone of infiltration but at a certain distance. For the most part sensory nervous fibers are affected. Changes in the nerve cells of the skin are seen more in the nose tip area than in the skin of abdomen, though the inflammation reaction in the first case is not more intensive than in the latter. Immunological aspects of the results obtained are being discussed.

LITERATURE CITED

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* In Russian.