SKIN LESIONS IN ALBINO RATS CAUSED BY THE ACTION OF FREUND-TYPE ADJUVANTS

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In addition to encephalomyelites, nephrites, tireoidites and orcheites, arising as a result of the introduction of adjuvants without accompanying tissue antigens, it is possible, under certain conditions, to reproduce certain clinico-morphological syndromes which do not fit into the framework of usual immunological reactions. Thus, when white rats were injected with a Freund-type adjuvant containing the fraction "wax D" of the tubercle bacillus, they developed arthritis, dermatitis, irridocyclitis or urethritis. This conditions resembled Reuter's syndrome in man [5, 7-10, 13].

The skin lesions at early stages of observation were characterized by papulous rash, and later they developed into a chronic dermatitis accompanied by loss of hair. Histological examination revealed hyper- and parakeratosis, acanthosis, and mainly a focal infiltration of the dermis with a predominance of lymphocytes and histiocytes (at early stages the infiltration was mainly perivascular). Sometimes the epidermis was invaded by cells and its basal layers were destroyed.

Pathological changes caused by adjuvants are regarded by the majority of authors as retarded allergic reactions [9, 12, 13]; this relates "adjuvant sickness" to certain forms of drug therapy disease [3].

The aim of this work was to study skin lesions in white rats produced by intraperitoneal injection of a Freund-type adjuvant.

METHODS

Seventy-two white rats of both sexes weighing 100-200 g were used in this work. The adjuvant used was a saline oil emulsion consisting of 1.5 ml of lanolin, 8.5 ml of petrolatum oil, 20 mg of the BCG vaccine (live or heat-killed) and 2 ml of normal saline or an equal volume of supernatant fluid obtained after centrifugation of a 50% homogenate of the rat kidney cortex in normal saline. The ingredients were thoroughly mixed and injected intraperitoneally into rats in 0.5 ml amounts with intervals of 9-10 days. Most animals received 4 injections, while the period of observation varied from 44 days to 8 months.

According to the composition of the adjuvant injected, the animals could be divided into 4 basic groups: 36 rats received the adjuvant with live BCG vaccine without the kidney tissue, 16 received the adjuvant with heat-killed BCG vaccine, without kidney tissue, 16 received adjuvant with live BCG vaccine and 5 received the adjuvant with heat-killed BCG vaccine and kidney tissue homogenate (with a view of inducing nephritis). The animals were killed by bleeding from femoral vessels. Pieces of skin were fixed in 10% neutral formalin and embedded in paraffin. Sections were stained with hematoxylin-eosin, with Massen's stain and by means of the PAS reaction.

RESULTS

Changes in the skin, observed in 49 rats, were characterized by a moderate or strong loss of hair, and by dermatitis sometimes accompanied by ulceration. In most animals these changes occurred not earlier than 20 days after

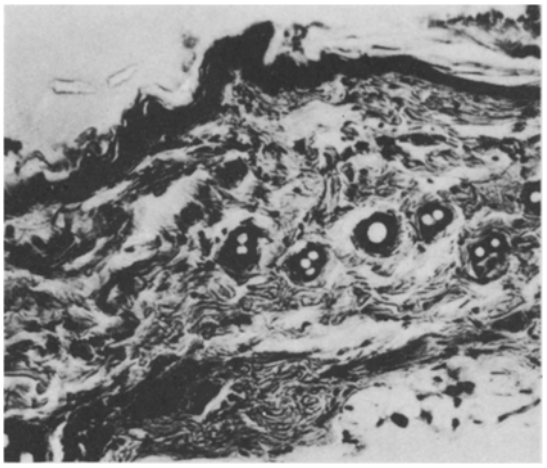

Fig. 1. Atrophy and sclerosis of dermis accompanied by thinning of epidermis and by hyperkeratosis. Hematoxylin-eosin. Magn. 170×.

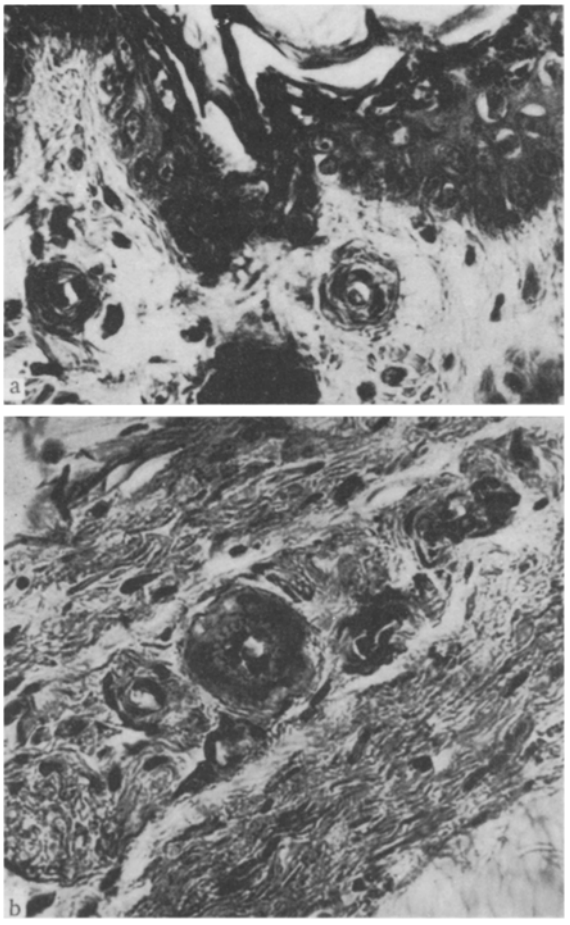

Fig. 2. Lesions in skin vessels. a) Sclerosis of arterioles in the subpapillary reticulum of skin. Hematoxylin-eosin. Magn. 437×; b) fibrinoid swelling of walls of arteries in deeper layers of dermis, and sclerosis of dermis. PAS-reaction and hematoxylin. Magn. 437×.

the first injection of the adjuvant, but the appearance of ulcerative dermatitis did not take place until the 30th day following the first injection. Skin lesions reached their maximum intensity usually 40-70 days after the beginning of the experiment, but subsequently in some animals the skin lesions became reduced, in that hair began to grow in some parts and the dermatitis either disappeared or became reduced. The intensity of skin lesions usually corresponded to the gravity of other clinical phenomena such as emaciation, accumulation of fluid in the abdominal cavity, an anemia, presence of different degrees of the lupoid-cellular phenomenon, etc. [1, 2]. Unlike the results of Pearson [9] arthritis was noted in 4 animals. Animals which received the adjuvant with the live BCG vaccine showed the most pronounced clinical symptoms and skin lesions.

Skin of 35 animals was studied histologically. The most significant changes were seen in those rats which received the adjuvant with live BCG vaccine but without the kidney tissue. Two types of changes could be noted: changes of the chronic type whose intensity increased with the duration of the experiment, and acute dystrophic and inflammatory changes. Changes in the 1st group at first became manifested as atrophy and sclerosis of the dermis accompanied by its reduction in thickness (Fig. 1). The sclerotic changes were most clearly defined in the papillary layer of the dermis and around hair follicles, where the soft connective tissue became replaced by dense collagen. In some cases there was sclerosis of hypodermal adipose tissue and of intermuscular connective tissue. Considerable sclerotic changes occurred also in skin arteries, especially in the subcapillary arteriole reticulum (Fig. 2a); when the sclerotic process was sufficiently pronounced this was accompanied by an emptying of the papillary capillaries and on the whole indicated a reduction of the vascular network of the skin. The sclerotic and atrophy changes in the dermis were accompanied by an atrophy of the epidermis, atrophy and reduction in number of the hair follicles, as well as by a pronounced atrophy of the smooth muscles. In addition to the above-mentioned atrophic and sclerotic changes there were also more or less pronounced acute reactive and inflammatory changes such as a diffuse histiocytic proliferation (especially strongly pronounced in the deeper layers of the dermis, where soft lymphocytic infiltrates were often found), fibrinoid swelling of the arteriole walls and focal swelling of dermis collagen accompanied by an intense PAS-positive reaction (Fig. 2b). The latter was most pronounced when the epidermis was ulcerated (Fig. 3). Some animals had productive phlebitis.

In those rats which received the adjuvant with live BCG vaccine and kidney tissue the changes resembled those in the preceding group but were less severe. At early stages of observation acute reactive and inflammatory changes predominated: fibrinoid swelling of the walls of small skin arteries, perivascular round-cell infiltrates, often with a

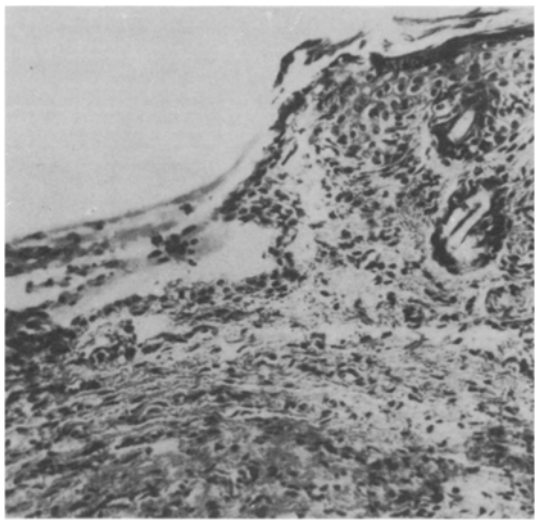

Fig. 3. Ulceration of skin, swelling and homogenization of dermis collagen with a proliferation of histiocytes. PAS-reaction and hematoxylin. Magn. 170×.

considerable admixture of lymphoid and plasma cells, and diffuse histocytic reaction. At later stages of observation old sclerotic changes predominated.

In the group of animals which received the adjuvant with heat-killed BCG vaccine, skin changes on the whole resembled those in the 2 preceding groups but there was a predominance of sclerotic and atrophic changes accompanied by ill-defined acute reaction and inflammatory processes in the dermis and in skin vessels.

Thus, in the animals belonging to the 3 groups there were skin lesions which differed only in their degree of intensity. These lesions were characterized by deep diffuse dystrophic processes in the connective tissue of the dermis accompanied by a fibrinoid change in the vessels and in dermis collagen along with a development of a diffuse histiocytic reaction and the formation of lymphoid and plasma cell infiltrates.

In addition to skin lesions there was noted the lypoid-cellular phenomenon, the blood serum contained antibodies to DNA in titers up to 1:160, there was vasculitis in a number of organs and the "wire loop" symptom in the kidneys [1, 2].

Thus, the peculiar syndrome which occurs in rats after repeated intraperitoneal injections of the adjuvant resembled systemic lupus of man according to a number of very important clinical and morphological manifestations.

It is of interest that skin lesions in animals, caused by the introduction of a Freund-type adjuvant are very similar to skin lesions in a similar disease of adult rats which occurs as a result of injection of immunologically active cells into resistant animals [11]. It must be stressed that according to a number of signs (arthritis, vasculitis, etc.) "the adjuvant diseases" [9] resemble closely homologous ones. As was already stated [11] the skin lesions noted by the authors were very similar to skin lesions in systemic lupus and in scleroderma of man.

It seems to us that the considerable resemblance of skin lesions in adjuvant and in homologous diseases (which are regarded as autoimmune diseases [11]) is not a chance one. It may be supposed that in hyperimmunization, processes of autoaggression assume a considerable significance, and that immunologically active cells play an important part in the development of these processes. The possibility of transfer of adjuvant encephalitis and nephritis to resistant animals indicates this likelihood [4, 6]. Possibly, as a result of hypersensitization there occur mutations of immunologically active cells and they become foreign to the host organism (in analogy with immunologically active cells of the donor, introduced into a resistant animal).

It is not excluded that a further study of adjuvant and of homologous diseases, which in some respects resemble collagenoses of man, will lead to an elucidation of the pathogenesis of the latter.

SUMMARY

Albino rats given repeated intraabdominal injections of a Freund-type adjuvant were found to have dermatitis and lost hair. Histological examination of skin lesions revealed deep diffuse dystrophic processes in the connective tissue of the derma with fibrinoid changes of vessels and collagen of the derma combined with the development of a diffuse histiocytic reaction and formation of lymphocyte-cell and plasma-cell infiltrates.

These changes closely resemble on the one hand skin lesions in systemic lupus erysematosus in man and on the other hand skin lesions in homological diseases. In view of the considerable similarity between skin lesions as well as other manifestations (lupus-cellular phenomenon, antibodies to DNA, "wireloop" syndrome in the kidneys, vasculitis, arthritis) described earlier with manifestations observed in homological diseases and systemic lupus, the question is raised of a possible similarity between certain pathogenetic mechanisms during these morbid processes.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
