A HISTOCHEMICAL STUDY OF CERTAIN PERIODIC ACID-SCHIFF-POSITIVE SUBSTANCES IN THE MACROPHAGES

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(Received July 25, 1958. Presented by Active Member AMN SSSR V. N. Chernigovskii)

The existence of a phagocytic function in macrophages is evidently due to specific biochemical processes occurring in them. For this reason, the results of histochemical examination might effectively supplement the morphological interpretation of the tissue changes which, appearing in response to different stimuli, including those of bacterial origin, are revealed in the form of a macrophage reaction.

It would be of interest to explain which of the histochemical characteristics are specific for certain forms of macrophage and which are common to the macrophages of all the tissues. Of particular interest,here,is the study of the polysaccharides (PAS-positive substances) found in the cytoplasm of the macrophages. The problem of the nature and origin of these substances, and whether they are identical in macrophages of different origin and function, has not yet been solved. It has acquired particular urgency in connection with the fact that the polysaccharides, detected by the PAS reaction, may play an important part in the energy metabolism of the cell and may also act as carriers of antigenic specificity, for macrophages are of obvious importance in the processes of immunogenesis [5].

In the present work,our objects were as follows: 1) to discover whether, in fact, the PAS-positive substances of the macrophages (other then glycogen) are substances of a polysaccharide nature, and how they are connected with lipids which, under certain conditions, may also give a PAS reaction; and 2) to determine whether the PAS-positive substances in macrophages of different origin and function are identical,histochemically.

The material used in the investigation was: 1) macrophages of the "foreign body cell" type, appearing in response to the subcutaneous introduction (transplantation) of finely cut adipose tissue; 2) macrophages formed in the kidneys under experimental conditions (ligation of vessels) [5], and 3) the macrophages of the lymphoid follicles of the appendix [4, 5].

EXPERIMENTAL METHOD

The investigation was carried out on rabbits. The material was fixed by Shabadash's method. The main histochemical technique to be used was the Schiff periodic acid reaction for polysaccharides. The PAS reaction, however, is not strictly specific; in certain conditions,it may be given by certain substances other than polysaccharides, in particular by lipids [2]. A possible method of control,in cases when the substance tested could not be broken down by enzymes,was by the acetylation and deacetylation reaction [8]. This is based on the fact that by acetylation with acetic anhydride mixed with amidopyrine, blockage of 1-2 glycol groups takes place, thereby leading to loss of the power to be oxidized by periodic acid and, hence, to react with Schiff's reagent. Subsequent deacetylation with a 0.1 N solution of KOH restores this power to the plysaccharides but not to substances

of another chemical nature. Thus,substances which give a positive PAS reaction, lose this power after acetylation and reacquire it as a result of deacetylation are, in fact, polysaccharides. We also employed staining with Sudan black after embedding the tissue in paraffin wax, and a combination of staining with Sudan black and the PAS reaction,in order to show the relative situation of lipids and PAS-positive substances. Preparations were treated with salivary amylase and with hyaluronidase, and also were stained with a 0.1% aqueous solution of toluidine blue for metachromasia.

EXPERIMENTAL RESULTS

Transplantate macrophages. On the 12-15th day, the formation of a considerable number of polymorphic macrophages was observed in the transplantates, and among them were encountered mononuclear forms, cells with 2-3 nuclei and multinuclear symplasts. The cytoplasm of the mononuclear macrophages contained PAS-positive substances in the form of fine granules or foam, distributed diffusely throughout the cell (Fig. 1.a). In some cases,the PAS-positive material appeared as fine, but nevertheless readily distinguished, round granules. Some macrophages contained no more than the general basic amount of PAS-positive material.

Acetylation completely inhibited the PAS reaction, but saponification with 0.1 N KOH solution led to full restoration of the power of the protoplasmic elements to give a positive PAS reaction. Saliva and hyaluronidase did not destroy the PAS-positive material. Sudan black stained the individual granules and irregular clumps in the cytoplasm an intensive black color. The almost complete absence of sudanophilic components from the cytoplasm of the mononuclear macrophages was conspicuous. The relative arrangement of sudanophilic and PAS-positive components in the cytoplasm of the mononuclear macrophages differed in individual cells. Not all macrophages giving a PAS reaction contained sudanophilic material. If it was present, it was in the form of more or less irregular granules and droplets, partly merged into clumps, against a background of clots of PAS-positive material.

The multinuclear macrophages were symplasts with more than 5-8 nuclei. The nuclei were most often situated at the periphery, in the form of chains. Many macrophages contained vacuoles of various sizes. The PAS-positive material was arranged unevenly in the form of fine granules and formed clots, but more often was spread diffusely throughout the cytoplasm (Fig. 1, b). Acetylation completely inhibited the reaction, but deacetylation completely restored,to the cytoplasmic elements,their power of giving a positive PAS-reaction. Saliva and hyaluronidase did not destroy the PAS-positive material nor change its morphology. Staining with Sudan black revealed sudanophilic components in the form of granules andloose agglomerations in different parts of the cytoplasm (Fig. 1, c). The distribution of sudanophilic and PAS-positive material in the cytoplasm of the symplasts,as shown by combined staining with Sudan black and Schiff's periodic acid,differed in the different macrophages. Some cells poor in PAS-positive substances contained a fair amount of sudanophilic material, and vice versa. However, PAS-positive substances were invariably predominant. Some macrophages had both types of component at the same time. Under these circumstances,black granules were clearly distinguished against the background of clots of PAS-positive material or were distributed around the periphery (Fig. 1, d).

Thus,in the macrophages of the foreign body cell type,a considerable amount of polysaccharides was observed, being distributed diffusely throughout the cytoplasm in the form of small granules and in places forming more compact masses. Lipoid substances were encountered in far smaller amounts, in the form of droplets and granules against a background of clots of polysaccharide.

Macrophages in the kidney after ligation of vessels. On the 15-20th day after ligation of the renal vessels in a rabbit, intensive formation of macrophages was observed in the adipose tissue situated near the pelvis. These macrophages consisted of mononuclear cells and multinuclear symplasts. In the latter, the nuclei were often arranged in the form of a horseshoe around the periphery of the cytoplasm, in which there were vacuoles of various sizes. The cytoplasm was filled with small granules of PAS-positive material (Fig. 2, a). Large PAS-positive granules, unevenly distributed in the cytoplasm, were also found. The PAS-positive material was not destroyed by salivary amylase nor hyaluronidase. Acetylation removed and saponification with weak alkaline solution restored the power of the cytoplasmic elements of the macrophages to give a positive PAS reaction. Sudan black stained numerous,more or less,compact formations in the cytoplasm of the macrophages. In this way,reticular or thread-like structures, rings, bands (possibly a Golgi zone; Fig. 2, b) were revealed. On combined staining with Schiff's periodic acid and Sudan black, large and small granules and droplets of sudanophilic material were situated mainly in places where there were clots of PAS-positive substance in the cytoplasm. The

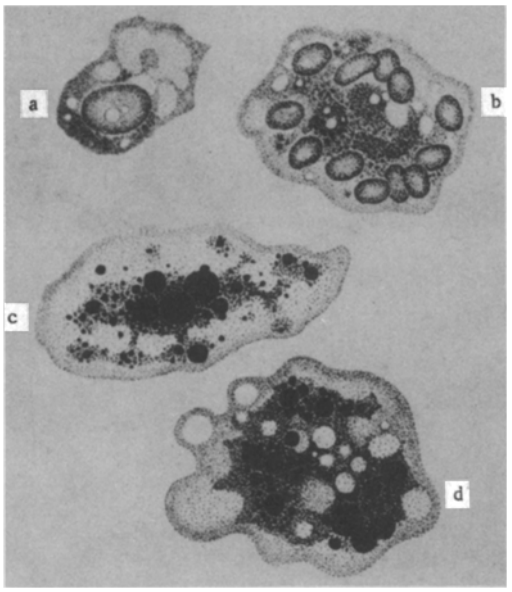

Fig. 1. Transplantation of finely cut adipose tissue (15 days). a) Mononuclear macrophage. PAS-positive substance in the form of granules. Schiff's periodic acid-hematoxylin; b) multinuclear macrophage. PAS-positive substance in the form of granules in the center of the symplast. Schiff's periodic acid-hematoxylin; c) multinuclear macrophage. Droplets of lipid. Sudan black; d) multinuclear macrophage. Droplets of lipid against a background of PAS-positive substance. Schiff's periodic acid-Sudan Black.

recticular and thread-like structures, which stained a deep black with Sudan black, at the same time were PAS-negative (Fig. 2, c).

The cytoplasm of these macrophages was thus seen to contain polysaccharide in the form of diffusely distributed small granules, and also as compact and coarse aggregations, giving an intensive PAS reaction. The lipids formed reticular structures not shown up by the polysaccharide stains; numerous sudanophilic droplets were, however, situated in those areas where there were also accumulations of PAS-positive substance.

Macrophages from the lymphoid follicles of the appendix. In the appendix of normal rabbits, macrophages containing PAS-positive material were distributed in the upper parts of the lymphoid follicles in the form of isolated cells, and in the lower parts as accumulations of cells, forming centers of proliferation.

In the upper parts of the follicles, the PAS reaction in the macrophages was dependent on the presence, in their cytoplasm, of a large number of phagocytosed microorganisms (Fig. 3, a), which continually penetrate the intestinal wall from its lumen and give a strongly positive reaction for polysaccharide. Various stages of phagocytosis were seen, with digestion of the microorganisms and liberation of polysaccharides (Fig. 3 a, b). Under these circumstances, polysaccharide, mainly formed as a result of intracellular digestion of microorganisms, was deposited in the reticular cells of the centers of proliferation in the lower parts of the follicles; isolated remnants of dead microorganisms could also be seen there.

In the centers of proliferation was also found a brown pigment, usually seen in lymphatic glands and bearing no relation to the products of phagocytosis of microorganisms. After acetylation, the PAS-positive substance of the microorganisms and of the accumulations in the centers of proliferation, like that of the remnants of dead microorganisms, lost its power of staining, to be restored after deacetylation. Amylase and hyaluronidase had no effect on staining with Schiff's periodic acid.

By staining for metachromasia, it was possible to show clearly the difference between the mucin of the mucosal cells of the intestinal epithelium, which gave metachromatic staining, and the polysaccharide of the microorganisms and of the centers of proliferation, which did not. Sudan black stained the large droplets in the individual macrophages of the centers of proliferation, which usually corresponded to the sites of distribution of the pigment (Fig. 3, c). The microorganisms and polysaccharides of bacterial origin did not stain with Sudan black. Staining with Sudan black at the same time as with Schiff's periodic acid confirmed this picture (Fig. 3, d).

Thus, in the appendix, the PAS-positive substances of microorganisms and the products of their phagocytosis, which were deposited in the macrophages of the centers of proliferation of the lymphoid follicles, were polysaccharides. These polysaccharides differed from the mucus of the goblet-cells and did not stain with Sudan black.

It may be generally stated that, in all the cases chosen, the bulk of the PAS-positive material found in the cytoplasm of the macrophages was polysaccharide. This was shown by the complete absence of staining in the experiment when the PAS reaction was followed by acetylation, and its restoration after gentle saponification

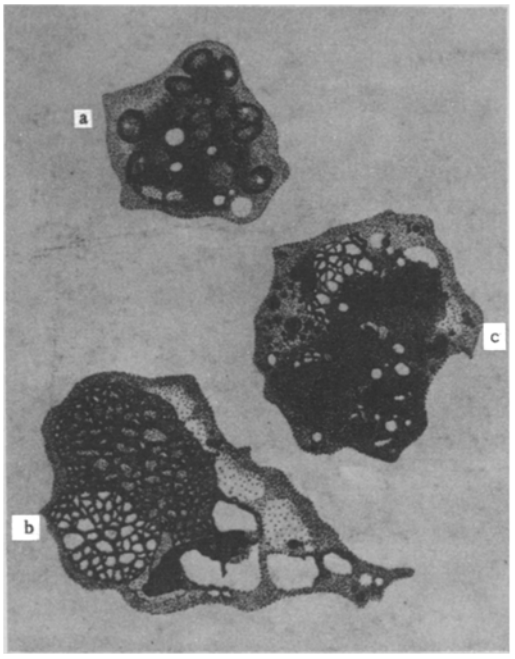

Fig. 2. Macrophages from the kidney (20 days after ligation of the vessels). a) Multinuclear macrophage. PAS-positive substance in the center of the symplast. Schiff's periodic acid - hematoyxylin; b) multinuclear macrophage. Lipids in the form of a reticular structure. Sudan black; c) multinuclear macrophage lipids against a background of PAS-positive substance. Schiff's periodic acid - Sudan black.

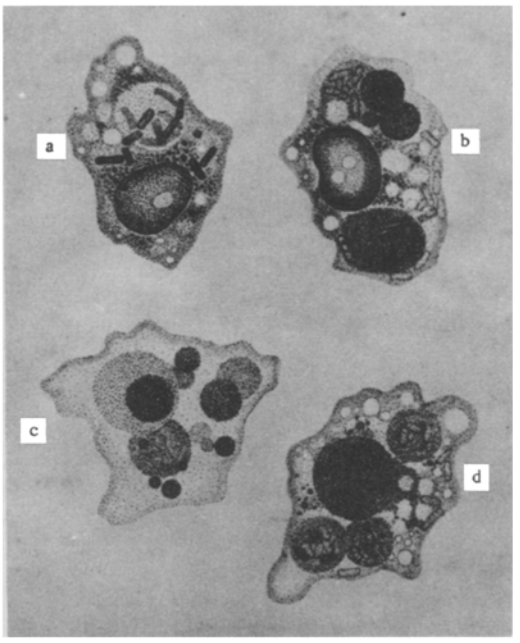

Fig. 3. Macrophages of the appendix of the normal rabbit. a,b) Successive stages of phagocytosis and digestion of microorganisms. Schiff's periodic acid - hematoxylin; c) lipids in the reticular cells of the centers of proliferation of the lymphoid follicles of the appendix of the normal rabbit. Sudan black; d) droplets of lipid among PAS-positive substance and remains of microorganisms in the reticular cells of the centers of proliferation in the appendix of the normal rabbit. Schiff's periodic acid - Sudan black.

with 0.1 N KOH. This polysaccharide was neither glycogen nor hyaluronic acid, as shown by the negative results of preliminary treatment with the corresponding enzymes. The macrophages may contain, at the same, substances of lipoid nature, whose relationship to the PAS-positive material may vary in different cases. Presumably macrophages may contain individually PAS-positive substances, i.e., polysaccharides, and individually sudanophilic components, i. e. lipids. In macrophages which differ from each other in respect of their origin and function, these substances may also differ in origin, and they must be identified histochemically in each particular case.

SUMMARY

The author conducted a comparative histochemical study of different types of macrophages appearing in normal and experimental conditions. Substances of polysaccharide and lipoidal origin formed in the macrophages as a result of the transformation of phagocytized material (including microbes) may be present in the macrophages, differing in genesis and function. These substances may be and are to be differentiated from each other and from the polysaccharides of the cytoplasm proper of the macrophages.

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