Significance of Sudan III Staining in Macrophages

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Peritoneal macrophages of mice, 48 hr after intraperitoneal administration of albumin (egg white), were studied. Almost all granules in the cytoplasm of macrophages stained by the supravital staining with neutral red were revealed to be colored brick red by the staining with Sudan III in the smear preparation. Because of lack of lipids in the inoculum, it is difficult to explain that these granules stained with Sudan III were lipids phagocytosed. The observation led the suggestion that the Sudan III stained granules in macrophages were out of all relation to lipids phagocytosed and that the granules indicated some functional phase of macrophages.

- macrophages; Sudan III stained granules; atherosclerosis

In general, the granules of macrophages stained with Sudan III have been considered to be lipids phagocytosed. In a series of the cytological study of lepromatous leprosy, Usubuchi and Arakawa (1956) and Arakawa (1958) reported that the Sudan III stained granules in lepra cells were also stained by the supravital staining with neutral red. From this finding they raised a question about the prevalent view that the granules stained with Sudan III in lepra cells were derived, by phagocytosis, from lipids of Mycobacterium leprae (Harada 1955).

The question of the same kind about Sudan III stained granules of macrophages was brought out in various diseases besides leprosy.

We report the evidence that almost all granules of macrophages appearing in the peritoneal cavity in a short time after administration of albumin were stained with Sudan III.

MATERIALS AND METHODS

Twenty male dd mice weighing 20 to 30 g were used. Before the treatment, smear preparations of cells in the ascites of all mice were microscopically observed by Giemsa staining. Animals were divided into two groups each consisting of 10 mice. In the first group, 0.2 ml of 1% albumin (egg white, Sigma Chem. Co.) dissolved in the physiological saline solution was administered into the peritoneal cavity. In the second, 0.2 ml of 10% India ink (Kuretake Sumi Honpo Co., Tokyo) dissolved in the physiological saline solution was injected into the peritoneal cavity.

Forty-eight hr after administration, the observation of ascites cells was done by Giemsa staining of the smear preparation and by the supravital staining with neutral red. The Sudan III-hematoxylin staining of the smear preparation was also carried out.

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A part of specimens of both groups were embedded in epoxy resin and sectioned for electron microscopy. Thin sections stained with uranyl acetate and lead citrate were examined with a JEM-TS electron microscope.

**RESULTS**

*Experiments of the administration of albumin*

The findings in 10 mice injected with albumin were nearly the same. Compared with those before administration, the findings of macrophages 48 hr after administration were as follows:

*Giemsa staining.* There were observed a large number of vacuoles varying in size in the cytoplasm of macrophages (Fig. 1).

*Supravital staining with neutral red.* A large number of granules stained with neutral red were observed in the cytoplasm of macrophages (Fig. 2).

*Sudan III-hematoxylin staining.* A large number of granules stained with Sudan III were recognized in the cytoplasm of macrophages (Fig. 3). The size varied greatly. The findings by the Sudan III staining were much the same as those of the supravital staining with neutral red.

*Electron microscopy.* Phagolysosomes of varying sizes were observed in the cytoplasm (Fig. 4).

*Experiments of the administration of India ink*

The findings in 10 mice injected with India ink were nearly the same. Compared with those before administration, the findings of macrophages 48 hr after administration were as follows:

*Giemsa staining.* There were observed a large number of India ink granules in the cytoplasm of macrophages (Fig. 5).

*Supravital staining with neutral red.* A large number of granules of India ink were seen in the cytoplasm of macrophages and, besides those, granules stained with neutral red were also seen though various in number. Furthermore, it was observed that the central part of some of the large granules was stained with neutral red and the periphery of the same granules with India ink (Fig. 6).

*Sudan III-hematoxylin staining.* Many granules of India ink were seen in the cytoplasm of almost all macrophages. Granules stained with Sudan III coexisted with those granules. Moreover, in some large granules, the central part was stained with Sudan III and the periphery with India ink (Fig. 7).

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Fig. 1. Vacuoles of various sizes in the cytoplasm of peritoneal macrophages 48 hr after intraperitoneal administration of albumin. Giemsa staining. × 800

Fig. 2. Many neutral red stained granules in the cytoplasm of peritoneal macrophages 48 hr after intraperitoneal administration of albumin. Supravital staining with neutral red. × 800

Fig. 3. Many Sudan III stained granules in the cytoplasm of peritoneal macrophages 48 hr after intraperitoneal administration of albumin. Sudan III-hematoxylin staining. × 800
Sudan III Staining in Macrophages

Fig. 1

Fig. 2

Fig. 3
Electron microscopy. In the cytoplasm of macrophages, there were observed phagolysosomes full of minute particles of India ink (Fig. 8).

**DISCUSSION**

The present experimental results from mouse peritoneal macrophages 48 hr after intraperitoneal administration of albumin showed that a large number of granules stained by supravital staining with neutral red were almost in accordance with granules stained with Sudan III. Furthermore, these granules seemed to accord with granules taking up India ink. Electron microscopically, there were observed vacuoles corresponding to phagolysosomes in cases of the administration of albumin and were seen many phagolysosomes full of India ink particles in cases of the administration of India ink.

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**Fig. 4.** Vacuoles of various sizes in the cytoplasm of peritoneal macrophages 48 hr after intraperitoneal administration of albumin. Electron microscopy. $\times 9720$

**Fig. 5.** Many India ink granules in the cytoplasm of peritoneal macrophages 48 hr after intraperitoneal administration of India ink. Giemsa staining. $\times 800$

**Fig. 6.** Many India ink granules mingled with a small number of neutral red stained granules in the cytoplasm of peritoneal macrophages 48 hr after intraperitoneal administration of India ink. Supravital staining with neutral red. $\times 800$

**Fig. 7.** Many India ink granules mingled with a small number of Sudan III stained granules in the cytoplasm of peritoneal macrophages 48 hr after intraperitoneal administration of India ink. Sudan III-hematoxylin staining. $\times 800$
Fig. 5

Fig. 6

Fig. 7
It is difficult to understand that a large amount of lipids were produced in the cytoplasm of macrophages 48 hr after administration of albumin. It would be rather suggested that the Sudan III stained granules in the cytoplasm of macrophages may not be lipids phagocytosed, but that they may be the substance which was secreted into the lysosome from the cytoplasm so as to treat the exogenous egg-albumin transported into the phagolysosomes. This substance may be stained with Sudan III and also stained with neutral red by supravital staining. Moreover, after the administration of India ink, the phagolysosomes of macrophages were full of minute India ink particles as a result of phagocytosis as shown by electron microscopy. Mitamura and Nito (1929) described the close relation between the granules stained with neutral red and those full of India ink.

Considering that the Sudan III stained granules may not be exogenous lipids but may be the secretion into phagolysosomes of macrophages, the significance of the present view “lipids phagocytosis” of macrophages in various diseases must be re-examined. Generally, the sudan III stained granules in lepra cells have been thought to be lipids which were taken up into macrophages by a process of phagocytosis of the destroyed *Mycobacterium leprae* (Harada 1955). However, Usubuchi and Arakawa (1956) and Arakawa (1958) proposed the opinion that the Sudan III stained granules were not lipids derived from the broken bacterium and
that the granules expressed merely some functional phase of those macrophages, because the Sudan III stained granules in lepra cells were also stained by the supravital staining with neutral red. Considering the present results obtained from the experiments in peritoneal macrophages of mice, we can hardly refrain from entertaining serious doubts as to the ordinary explanation of the findings of the Sudan III stained granules in macrophages in other various diseases. The most important disease in relation with "lipids phagocytosis" is atherosclerosis. The prevalent theory on the pathogenesis of atherosclerosis is Anitschkow’s "cholesterol theory" (1915). However, there have been doubts from various points of view (Büchner 1956). According to our opinion described above, the Sudan III stained substance in atherosclerosis may not be due to the absorption of cholesterol from the blood, but may be due to the activated macrophages appearing in the intima of arteries in order to digest the degenerative material caused by various diseases, especially hypertension.

References