KAWASAKI MED. J. VOL. 9 : NO. 2, 81-90 (1983)

Assessment of Macrophage-neutrophil Interaction in the Delayed Type Hypersensitivity Reaction of Guinea Pigs

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Accepted for Publication on March 8, 1983

ABSTRACT. Electron microscopic observations were made of exudate macrophages with phagocytosed autologous neutrophils in intraperitoneally implanted collagen sponges in sensitized and non-sensitized guinea pigs. In addition, the number of these macrophages per unit area of sponge was calculated. Of the mononuclear cells, 12.7% were such macrophages in the sensitized group and 4.9% in the non-sensitized group, 48 hours after implantation. Macrophages with ingested neutrophils or cell debris and ferritin as the antigen in cytoplasmic phagolysosomes often formed clusters with a lymphocyte series in the sensitized guinea pigs, but not in the non-sensitized guinea pigs.

Key words : macrophage — neutrophil-phagocytosis — cell-mediated immunity — electron microscopy — guinea pig

Neutrophil-phagocytosis by macrophages in vivo has hitherto aroused little interest since the macrophages may simply be phagocytosing whatever suitable foreign substances are present, including autologous degenerated cells. Occasionally, however, macrophages phagocytosing intact neutrophils has been observed in lesions of Behçet's syndrome[1-3] and Reiter's syndrome.[4] Various hypotheses about the etiology of this phenomenon have been advanced, but no convincing explanation has been given yet.

Although a considerable amount of information has accumulated on the morphology of interactions of macrophages with lymphocytes in relation to cell-mediated immunity,[5] there are few reports of interactions of macrophages with neutrophils.

Intraperitoneal implantation of a spongy biomaterial impregnated with an antigen induces a greater accumulation of peritoneal exudate cells in sensitized animals than in nonsensitized animals.[6] In the present study, electron microscopic observations of macrophage-neutrophil interactions using the sponge implantation method were made in addition to an analysis of the number of exudate cells per unit area of sponge.

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MATERIALS AND METHODS

Animals

Female English Hartley guinea pigs weighing 300–450 g were used throughout these investigations.

Immunization

Guinea pigs were sensitized by injecting into the foot pads 800 μg of cadmium-free ferritin (Nutritional Biochemicals Co.) emulsified in complete Freund's adjuvant (Difco Lab.). They were challenged 21 days later by implanting collagen sponges ($1 \times 1 \times 1.5$ cm, Koken Co.) containing 100 μg ferritin (ferritin sponge) into the peritoneal cavity. Guinea pigs which were not sensitized but received ferritin sponges served as controls.

Assessment of exudate cells into the implanted ferritin sponges

Thirty-six guinea pigs were investigated per group and 6 guinea pigs per subgroup. The guinea pigs were killed 1, 2, 3, 5, 10 or 18 days after the challenge. The ferritin sponges were immediately removed, fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.2 M cacodylate buffer (pH 7.4) at 4°C for 2.5–3 hours. Dehydration was perfomed with a graded ethanol series, and the specimens were embedded in Epon 812 (TAAB Lab.), acrytron E (Mitsubishi Rayon Co.) or paraffin. For electron microscopy, silver to pale gold ultrathin sections were cut and stained with uranyl acetate and lead citrate, and then examined under a HITACHI H-500 electron microscope. Some of the ultrathin sections were analyzed by an EDAX (model 711) energy dispersive X-ray spectrometer in combination with a HITACHI HU-12A electron microscope.

For light microscopy, thin sections were cut from specimens embedded in acrytron E or paraffin and stained with haematoxylin and eosin. The dimensions of five to ten thin sections per sponge were measured, and the cells were counted by means of a reticle fitted into the eyepiece of the microscope and calibrated at a magnification of 400 $\times$. The field outlined by the outside frame corresponded to an area of 0.0625 mm^2. In each section, the cells were counted in 16 or more fields selected from the periphery of the section. The cell populations were expressed as the average number of cells per 1 mm^2.

RESULTS

Electron microscopic observations of macrophage-neutrophil interactions

Ultrastructural examination of the neutrophils in the sponges removed 24 hours after intraperitoneal implantation revealed large ferritin-containing phagosomes in the cytoplasm (Fig. 1). Moreover, at 24 hours most cells containing ferritin were neutrophils in both the sensitized and non-sensitized guinea pigs.

Many macrophages containing phagocytic ferritin were also recognized in the sponges removed 24 hours after implantation, while only a few macrophages containing autologous neutrophils with phagocytosed ferritin were observed in either group.

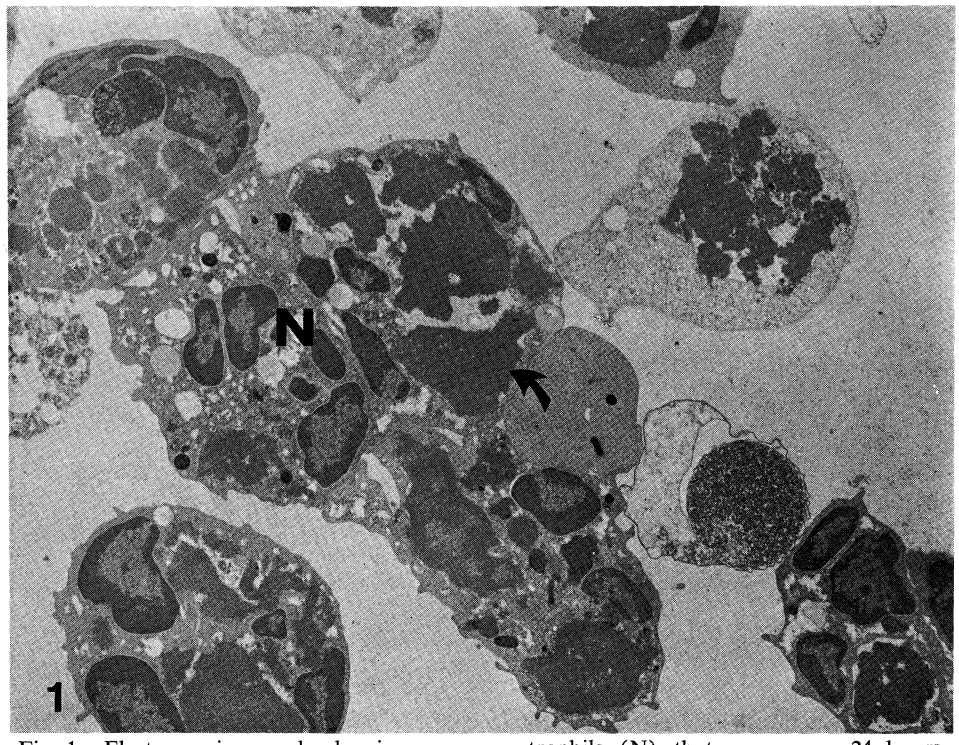

Fig. 1. Electron micrograph showing many neutrophils (N) that appear on 24 hours within an implanted ferritin-sponge in sensitized guinea pigs. Note that ferritin appear to be contained in the cytoplasmic phagosomes (arrow). (Epon 812 section, ×5500)

Neutrophils phagocytosed by macrophages were observed by light microscopy in the acrytron E sections for comparison. Those which appeared morphologically intact under the light microscope often showed necrotic signs such as a condensed degeneration in the entire cell, particularly the nucleus, but the ultrastructural characteristics were retained relatively well in electron microscopic observations in both groups (Fig. 2).

Out of consideration of the acrytron E resin embedding, specimens were not subjected to postfixation with osmium tetraoxide. As a result, electron microscopic observations revealed various artifacts in the ultrastructure of the cell. However, it was not difficult to determine the morphology of the cell, and a morphological comparison of phagocytosed neutrophils could be made without trouble between the two groups. No specific difference was observed in the morphology of macrophages containing intact neutrophils in either group, and ferritin was commonly observed in phagosomes and/or phagolysosomes of the cytoplasm of the macrophages.

With the lapse of 72 hours after implantation, the number of phagocytosed

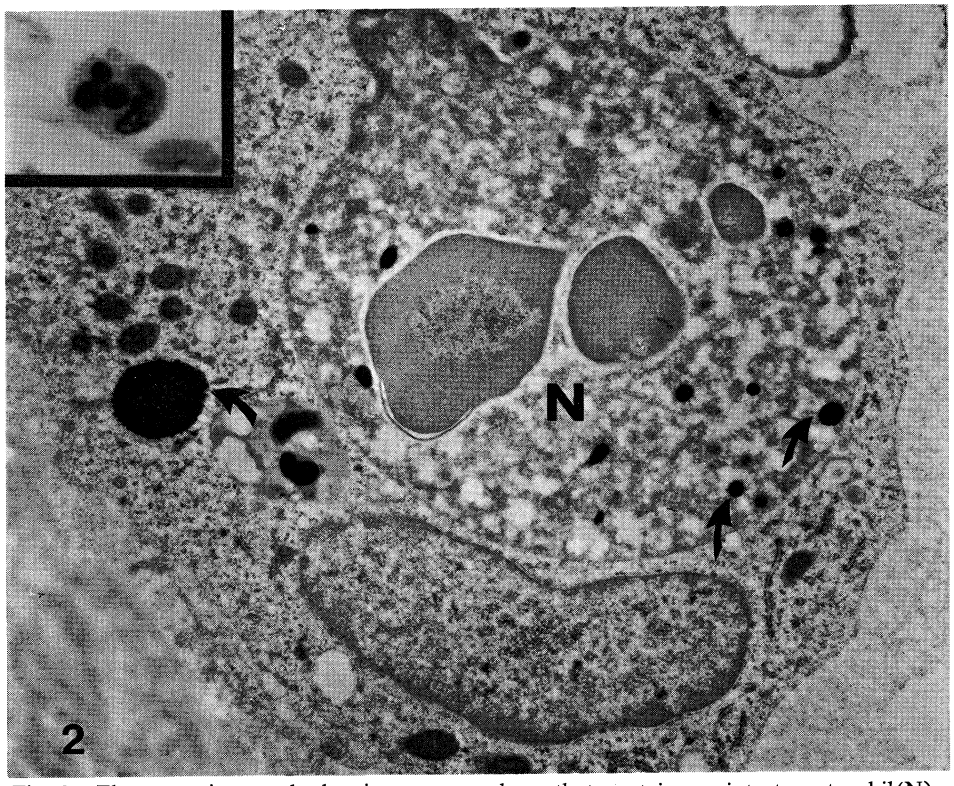

Fig. 2. Electron micrograph showing a macrophage that contains an intact neutrophil (N) in the cytoplasmic phagosome, and that appears on 48 hours within an implanted ferritin-sponge in non-sensitized guinea pigs. Note ferritin in the cytoplasmic phagosomes of the macrophage or the neutrophil (arrows). (Acrytron E section, ×9000) Inset : thick section of the macrophage with phagocytosed neutrophil same as the electron micrograph. (H & E, ×1250)

neutrophils in phagolysosomes which had lost their original form and were in various stages of digestion increased in both groups (Fig. 3). As a result, ferritin contained in cytoplasmic phagolysosomes of macrophages, derived from phagocytosis of macrophages or phagocytosis of neutrophils or both, accumulated in such a large quantity that it occasionally filled the cytoplasm (Fig. 4).

In the sensitized guinea pigs, macrophages containing neutrophil debris and ferritin in phagolysosomes in the cytoplasm often formed clusters with lymphocytes (Fig. 5). The lymphocytes ranging from those having few organelles in the cytoplasm to those with well-developed rough-surfaced endoplasmic reticulum were identified as being derived from T-cell and/or B-cell populations. Macrophages which formed clusters with lymphocytes often were degenerated along with being dilated. Clusters were also found in the granulomatous tissue that was formed in sponges on the 10th-18th day after

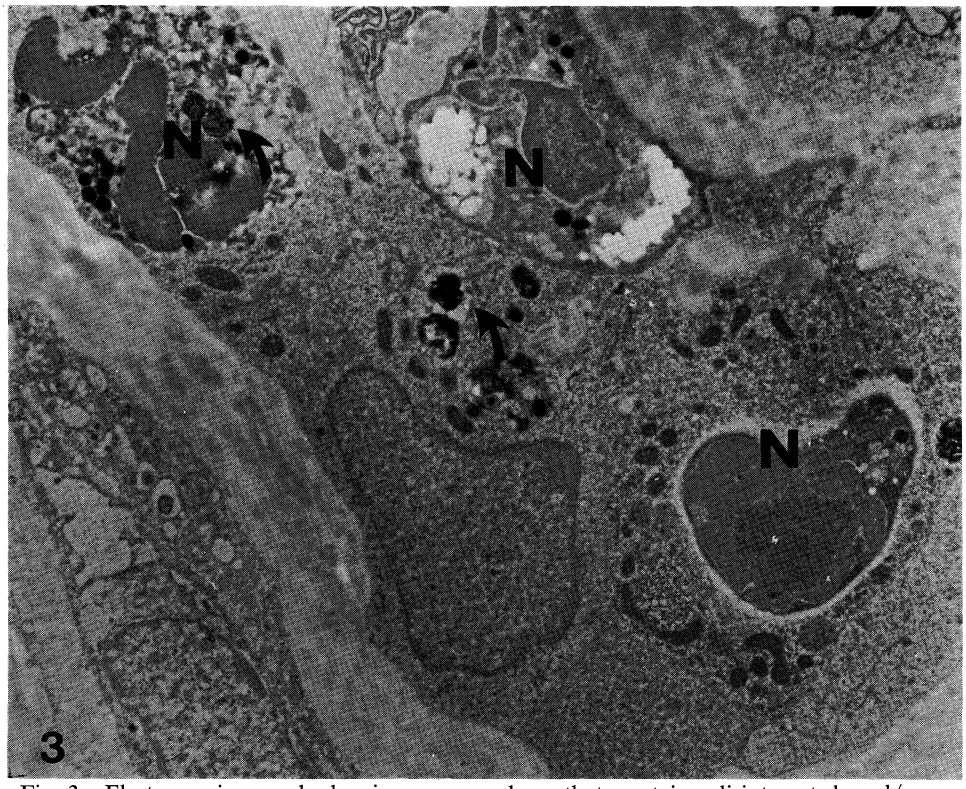

Fig. 3. Electron micrograph showing a macrophage that contains disintegrated and/or intact neutrophils (N) in the cytoplasmic phagosomes, and that appears on 72 hours within an implanted ferritin-sponge in sensitized guinea pigs. Note ferritin in the cytoplasmic phagosomes of the macrophage or the neutrophils (arrows). (Acrytron E section, ×5500)

sponge implantation. However, such cluster formation was not found in the non-sensitized guinea pigs.

When a large quantity of cell debris and ferritin accumulated in the cytoplasmic phagolysosomes, macrophages showed signs of degeneration and eventually collapsed. The remains of ruptured macrophages were phagocytosed anew by macrophages, resulting in ferritin being accumulated again in phagolysosomes in the cytoplasm of macrophages. Such a localization of ferritin in the cytoplasm was recognized as Fe by analytical electron microscopy (Fig. 6).

In granulomata which formed in the sponges on the 5th–18th day after sponge implantation, it was very rare to find macrophages with morphologically intact phagocytic neutrophils in either group.

Quantitative study on exudate cell populations

The method of counting exudate cells has been described previously.[6]

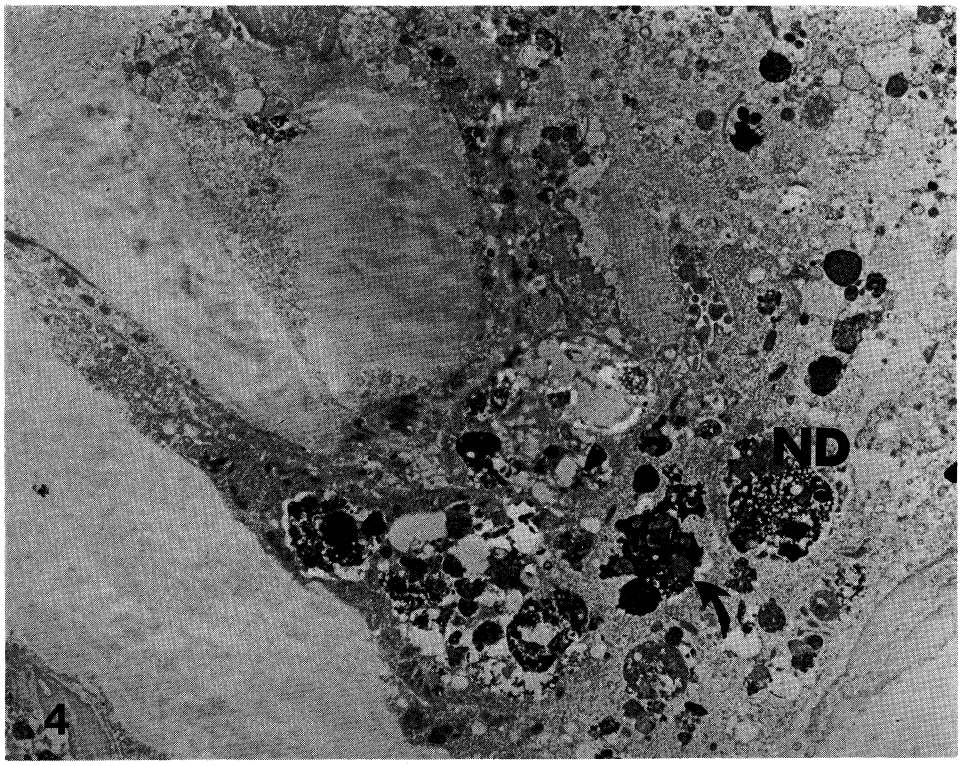

Fig. 4. Electron micrograph showing a macrophage that contains numerous neutrophil debris (ND) and ferritin in the cytoplasmic phagolysosomes (arrow), and that appears on 5 days within an implanted ferritin-sponge in sensitized guinea pigs. (Acrytron E section, ×4000)

Accumulation of exudate cells into ferritin sponges was significantly different quantitatively between the two groups of guinea pigs at 24 hours after sponge implantation. Neutrophils were predominant at 24 hours, but mononuclear cells became predominant in both groups 48–72 hours after sponge implantation.

The average number of macrophages with phagocytosed neutrophils per unit area of ferritin sponge was 5 times larger in the sensitized group than in the non-sensitized group 48 hours after implantation (Table 1). Of mononuclear cells, 12.7% were such macrophages in the sensitized group and 4.9% in the non-sensitized group, the ratio between the two groups being 5 : 2.

Seventy-two hours after sponge implantation, neutrophils observable in the cytoplasm of macrophages had little resemblance to the original form, and, thus, it was difficult to confirm that they were neutrophils. Polymorphonuclear cells which accumulated in the ferritin sponges showed necrotic signs such as blurred cell borders and pyknosis, and few remained intact morphologically.

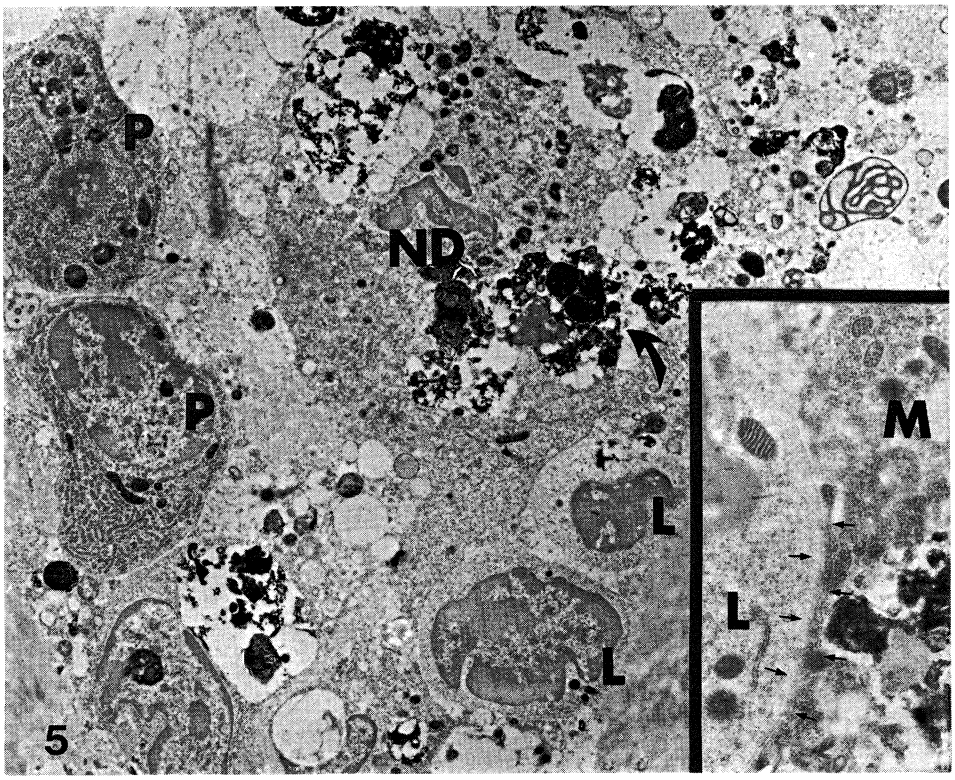

Fig. 5. Electron micrograph showing a macrophage-lymphocyte (L)-plasma cell (P) cluster that appears on 10 days within an implanted ferritin-sponge in sensitized guinea pigs. Note the degenerated macrophage containing neutrophil debris (ND) and ferritin in the cytoplasmic phagolysosomes (arrow). (Acrytron E section, ×4000) Inset : close contact between a macrophage (M) and lymphocyte (L). Note the area with narrow intercellular space (arrows). (Acrytron E section, ×12000)

Very few polymorphonuclear cells accumulated in ferritin sponges after 72 hours.

From approximately the 5th day after implantation, mature granuloma formation with multinucleated foreign body giant cells occurred, extending into ferritin sponges.

DISCUSSION

The phenomenon of intact neutrophils being phagocytosed by macrophages has attracted attention because neutrophils are so easily broken down by degeneration with the release of neutrophil granules.[7,8] Phagocytosis by macrophages predominantly involves foreign substances such as sponge matrix, ferritin or cell debris. Accordingly, the appearance of macrophages with phagocytosed morphologically-intact neutrophils can be said to be a rare phenomenon in terms

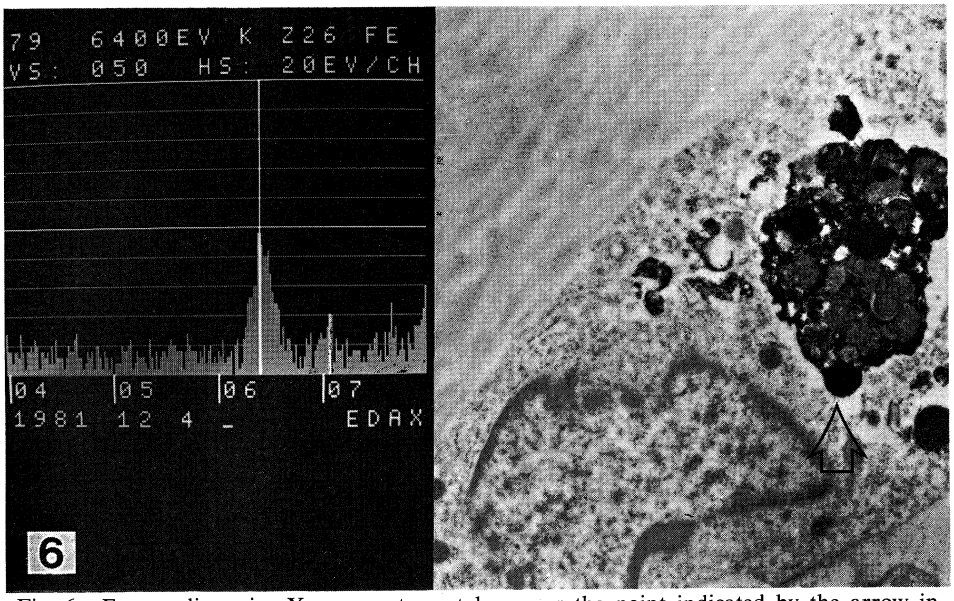

Fig. 6. Energy dispersive X-ray spectrum taken over the point indicated by the arrow in the cytoplasm of macrophage, that appears on 18 days within an-implanted ferritin-sponge in sensitized guinea pigs. Note the characterisitic peaks for iron. (Acrytron E section, ×9000)

TABLE 1. Number of macrophages with phagocytosed neutrophils in exudate cells into the intraperitoneally implanted collagen sponges

Group		Exudate cell count analysis		
		Time after implantation		
Sensitized Animals		24 hrs	48 hrs	72 hrs
Ferritin sponge implants	[a]Macr	36± 4	192± 14	161± 17
	[b]Mono	1042± 74	1515± 88	1531± 97
	[c]Poly	2577±169	1115± 93	704± 73
Non-sensitized Animals				
Ferritin sponge implants	Macr	19± 3	39± 6	21± 4
	Mono	440± 64	793± 82	843± 91
	Poly	1072±110	601± 75	442± 32

Each group consisted of 6 guinea pigs. [a]Macrophages with phagocytosed neutrophils were counted the number of macrophages containing polymorphonuclear cells. [b]Mononuclear cells were counted the total number of macrophages, monocyte and lymphocytes. [c]Polymorphonuclear cells were counted the number of dominant neutrophils. Results were expressed as mean counts per 1 (=0.0625×16) mm² of 30 sections from 6 sponges ± SE. All values are significantly different ($p<0.01$) from non-sensitized (Student's paired t-test).

of the time for appearance and the number. In this study, the presence of such macrophages in ferritin sponges was nil even after the 5th day of implantation. Consequently neutrophils are all but broken down by cell degeneration,

and there can be no new mobilization into the sponges.

There was no morphological difference in macrophages between the sensitized and non-sensitized guinea pigs. However, such macrophages tended to appear more frequently in the sensitized group, the ratio between the sensitized and non-sensitized groups being 5 : 2 in terms of the percentage of macrophages out of the total number of mononuclear cells.

Accumulation of mononuclear cells in the ferritin sponges reached the maximum level 48–72 hours after implantation in both groups though the accumulation of neutrophils and mononuclear cells was greater in the sensitized guinea pigs than in the non-sensitized guinea pigs. These results show that intraperitoneal implantation of the ferritin-impregnated, collagen sponges induced a delayed type hypersensitivity reaction in the ferritin sensitized guinea pigs.[6] Therefore, the quantitative differences of macrophages with phagocytosed autologous neutrophils seen between the sensitized and non-sensitized guinea pigs may reflect the increased number of exudate cells.

It is neither known, however, what role these macrophages play in the development of the delayed type hypersensitivity reaction nor whether or not they merely reflect the strength of phagocytic potential at the site of inflammation,[9] and have nothing to do with cell-mediated immunity.

To answer these questions, the fate of such macrophages needs to be followed in sensitized and non-sensitized guinea pigs.

Macrophages with the remains of ingested neutrophils and with ferritin in cytoplasmic phagolysosomes often formed clusters with a lymphocyte series in the sensitized guinea pigs but never in the non-sensitized guinea pigs.

The interaction of the macrophages with the neutrophils may be interpreted as one step in the cellular transmission of antigen information from neutrophils to macrophages involving cell-mediated immunity, and this interaction may induce an interaction between macrophages and lymphocytes.[10]

This work was supported in part by the project fund (grant 55–702) of Kawasaki Medical School.

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