TIME-DEPENDENT CHANGES OF MICROSCOPIC LOCALIZATION OF INTRAVENOUSLY ADMINISTERED COLLOIDAL CARBON PARTICLES IN MOUSE LYMPH NODES

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Abstract.....Time-dependent changes of microscopic localization of intravenously administered colloidal carbon particles were studied in mouse lymph nodes. Carbon particles were preferentially trapped by postcapillary venules (PCV) immediately after injection, and migrated easily outside of PCV either through intercellular space of the PCV endothelium or by phagocytic process during the 1 hr after injection. Particles were thereafter up-taken by pericytes and macrophages around PCV during the 24 hr, and consequently distributed throughout the cortex and medulla. Finally, they migrated to the medullary lymphatic sinuses, and phagocytosed by endothelial cells. Redistribution process of particles via the lymphatic sinuses from the regional lymph was observed during 30 minutes or 10 to 14 days after injection in different lymph nodes.

Key words: Colloidal carbon, lymph nodes, microscopic localization, time-dependent.

INTRODUCTION

Since particulate materials show variable distribution patterns in lymph nodes after their entry by different routes, their cytotoxic or immunogenic effects in the reticuloendothelial system (mononuclear phagocyte system) could be different [Yoffey and Courtice, 1970; Raviola, 1975]. We previously found colloidal carbon particles to be preferentially trapped by postcapillary venules (PCV) after intravenous administration in mice [Oghiso and Matsuoka, 1979]. The PCV is an important recruiting route of immunologically responsible lymphocytes in lymph nodes [Gowans and Knight, 1964;

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Marchesi and Gowans, 1964 ; Farr and De Bruyn, 1975] . Many of recent studies have also shown variable transfer patterns of particles through PCV, although the time-dependent changes of microscopic localization of particles are not fully clear [Mikata and Niki, 1971 ; Blau, 1978 ; Kotani et al., 1980] . It is important to obtain information about the time of particle transfer in lymph nodes for the assessment of particle translocation in the body. The present study was, therefore, performed to establish the time-dependent changes of microscopic localization of colloidal carbon particles (transfer patterns) through PCV or lymphatic sinuses in mouse lymph nodes after intravenous administration.

MATERIALS AND METHODS

Animals : Four-week-old female C3H mice, 16 to 20 g of body weight, which were kept under conventional conditions in our institute, were used.

Administration and Animal Sacrifice : Colloidal carbon (Pelikan ink, C11/1431, Günther Wagner, Hanover, West Germany) was used as the indicator particles. Total 6-8 animals of each group were sacrificed by exsanguination under anesthesia in ether atmosphere, and were autopsied at the following time points after intravenous injection of 0.1 ml (about 10 mg/animal) of carbon suspension ; 1, 3, 5, 10, 15 and 30 minutes, 1, 3, 6 and 12 hr, 1, 3, 5, 7, 10, 14, 21 and 28 days later.

Histology : Superficial, thoracic and abdominal lymph nodes of each animal were carefully collected and fixed with 5% glutaraldehyde in 0.2 M cacodylate buffer (pH 7.1) at 4°C for 2 hr, followed by postfixation with 1% OsO₄ in 0.2 M s-collidine buffer (pH 7.2) at 4°C for 1.5 hr. These samples were dehydrated with graded ethanol and acetone, and embedded in Epon 812. One μ m thick sections were prepared by glass knife-ultramicrotomy (Porter-Blum MT2B, Sorval Inc., USA) and stained with 0.5% toluidine blue in 0.5% borax.

Time-dependent changes of microscopic localization of colloidal carbon particles in lymph nodes were examined on light microscopy, and their deposition sites inside or outside of PCV were determined at scheduled time.

RESULTS

Figure 1 (A and B) reflects diagrammatically how many animals (percentage) of the group at each time point after injection had carbon particles at different deposition sites of lymph nodes, which were divided anatomically into the 8 parts.

(1). *Microscopic localization during the 24 hr after injection.*

It was observed that carbon particles were aggregated in the lumen of PCV and were occasionally engulfed by blood phagocytes 1 minutes after injection. Particles were also seen on the outline surface and intercellular space of the PCV endothelium 3 to 5 minutes after injection (Fig. 2). In some animals, particles were already seen at the basement membrane of PCV 5 to 10 minutes after injection (Fig. 3). From 15 minutes after injection, particles were observed to be deposited in endothelial cells of PCV (Fig. 4). Particles were seen at the basement membrane from 30 minutes to 1 hr after

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Deposition Sites	Postinjection Time																	
	Min.						Hr.				Day							
	1	3	5	10	15	30	1	3	6	12	1	3	5	7	10	14	21	28
PCV Lumen & Blood Phagocytes																		
PCV Outline Surface																		
PCV Intercellular Space																		
PCV Endothelium																		
PCV Basement Membrane																		
PCV Pericytes & Macrophages																		
Cortical & Medullary Macrophages																		
Sinusoidal Endothelium & Macrophages*																		

Fig. 1A Microscopic localization of carbon particles in mouse lymph nodes at each time point after intravenous injection.

Each checkers reflects how many animals (percentage) of the group at each time point had colloidal carbon particles at different deposition sites as the followings; 75 - 100% of animals, 30 - 75%, 0 - 30%, none

* The upper checkered column at the deposition site of sinusoidal endothelium and macrophages indicates the deposition in thoracic and pancreatic lymph nodes, and the lower column shows that in other lymph nodes.

Fig. 1B Diagram of anatomic locations in a lymph node unit.

1: peripheral lymphatic sinus, 2: lymphoid follicle, 3: postcapillary venule (PCV; 3a: lumen, 3b: outline surface of endothelium, 3c: intercellular space of endothelium, 3d: endothelium, 3e: basement membrane, 3f: pericyte, 3g: macrophage), 4: paracortex, 5: medullary cord, 6: medullary sinus

Fig. 2 Aggregated colloidal carbon particles in the lumen of the PCV and outline surface and intercellular space of the PCV endothelium. (3 minutes later, Bar=10 μ m)

Fig. 3 Carbon particles in the intercellular space of the PCV endothelium, and at the basement membrane (arrows). (10 minutes later, Bar=10 μ m)

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Fig. 4 Carbon particles in the PCV endothelium. (15 minutes later, Bar=10 μ m)

Fig. 5 Carbon particles in the pericytes (arrows). (1 hr later, Bar=10 μ m)

injection, and also distributed in surrounding pericytes from 1 to 3 hr after injection (Fig. 5). In most of animals, particles were distributed mainly at the endothelial cells of PCV, basement membrane and pericytes 3 to 12 hr after injection (Fig. 6). Paracortical macrophages outside of PCV were occasionally seen to phagocytose particles 12 to 24 hr after injection. Despite the reduction in amount of them, the aggregation of particles in the PCV lumen was occasionally noticed in lymph nodes of some animals during the observation period of 24 hr after injection.

In addition to the above findings, particles were also observed in the endothelial cells and macrophages of peripheral sinuses of thoracic and pancreatic lymph nodes from 5 to 30 minutes after injection (Fig. 7), and they were widely distributed from peripheral through medullary sinuses during the 24 hr after injection.

Fig. 6 Carbon particles in the PCV endothelium, basement membrane and pericytes (arrows). (12 hr later, Bar=10 μ m)

Fig. 7 Carbon particles in the peripheral sinus (arrows) of the pancreatic lymph node. (5 minutes later, Bar=10 μ m)

(2). *Microscopic localization 3 to 7 days after injection.*

Particles were mainly seen to be aggregated in pericytes of PCV, paracortical macrophages outside of PCV, and even in lymphoid follicles or medullary cords in most of animals at this observation period (Fig. 8).

More extensive distribution of particles was seen in cortical and medullary areas of thoracic and pancreatic lymph nodes than the other nodes at this period.

(3). *Microscopic localization 10 to 28 days after injection.*

Only a trace amount of particles was observed in PCV, and most of them were widely distributed throughout lymph node structures outside of PCV. Macrophages in medullary cords were seen to phagocytose particles, and endothelial cells lining medullary sinuses were occasionally seen to uptake particles (Fig. 9).

The deposition of particles in peripheral sinuses was firstly seen in cervical, axillary and mesenteric lymph nodes 10 to 14 days after injection, while it was already observed at the earlier time points (5 to 30 minutes) in thoracic and pancreatic lymph nodes.

Fig. 8 Carbon particles in the paracortical macrophages around the PCV (arrows).
(5 days later, Bar = 10 μ m)

Fig. 9 Carbon particles in the medullary cord and the medullary sinus (arrows). (28 days later, Bar=10 μ m)

DISCUSSION

We previously reported that intravenously administered colloidal carbon particles were preferentially trapped by PCV of lymph nodes, and distributed throughout lymph node structures 24 hr after injection [Oghiso and Matsuoka, 1979]. The present study was done to establish time-dependent changes in microscopic localization (transfer patterns) of particles either through PCV or through lymphatic sinuses.

Carbon particles migrated easily in lymph nodes through PCV from the blood. They were preferentially trapped by the PCV endothelium immediately after injection. Some particles were already observed to reach the basement membrane a few minutes later. Uptake of particles by endothelial cells of PCV started 5 to 10 minutes after injection, and phagocytic process of endothelial cells was noticed 30 minutes to 1 hr after injection. Particles were then seen at the basement membrane and pericytes in many animals 3 to 6 hr after injection. Macrophages of the paracortex and medulla were seen to engulf aggregated particles by 24 hr.

Such a prompt transfer of particles through PCV was considered to be due to the migration via intercellular space of the PCV endothelium [Blau, 1978]. Otherwise, particles could penetrate intercellular clefts associated with migrating lymphocytes or phagocytic macrophages [Marchesi and Gowans, 1964; Blau, 1978; Anderson and Anderson, 1975]. It is also possible that some particles could be transferred by the phagocytic process of PCV endothelial cells [Mikata and Niki, 1971].

Some particles were seen in the PCV lumen during the observation period of 24 hr, suggesting that they were continuously circulating in the vasculature of lymph nodes, and trapped by PCV during this period.

One day after injection, particles were seen outside of PCV and uptaken by macrophages of the paracortex, medulla and even lymphoid follicles, and only a trace

amount of particles was deposited in PCV 2 to 4 weeks after injection. During this later period, macrophages in the medullary cord and endothelial cells lining the medullary sinus were seen to uptake particles. Although it is unclear how phagocytosed particles migrated within lymph node structures, transfer or migration process of particles by macrophages might be suggested [Burke and Simon, 1970 ; Nopajaroonsri and Simon, 1971] .

As shown in the previous study, some particles were also redistributed in lymphatic sinuses of thoracic and pancreatic lymph nodes from the regional lymph 24 hr after intravenous injection [Oghiso and Matsuoka, 1979] . This redistribution process was observed 5 to 30 minutes after injection in these lymph nodes. Similar process was, however, seen later (10 to 14 days after injection) in other lymph nodes. It is supposed that time-difference in redistribution process in different lymph nodes might be due to the variable saturation of reticuloendothelial system or the differing active conditions of lymph nodes [Yoffey and Courtice, 1970] .

It is suggested in the present study that particles migrated through PCV were finally uptaken by medullary macrophages and endothelial cells of medullary sinuses. It is, however, conceivable that redistributed particles via the lymph were also uptaken by macrophages and endothelial cells of the medullary lymphatic sinus. These particles migrated to the medullary sinus either through PCV or via the lymph, thereafter, could be transferred to other tissues via the lymph.

We could exhibit, thus, microscopic localization process of intravenously administered colloidal carbon particles in lymph nodes as follows: (1). Particles are easily trapped by PCV in lymph nodes, and migrate outside of PCV either through intercellular space of the PCV endothelium or by phagocytic process within 1 hr.

(2). Particles are uptaken by pericytes and macrophages around PCV, and distributed widely in the paracortex, lymphoid follicles and medullary cords within 1 day. (3). Particles are uptaken finally by endothelial cells of the medullary sinus during the 1 month. (4). Redistribution of particles via the lymphatic sinus from the regional lymph occurs 5 to 30 minutes after injection in some lymph nodes or 10 to 14 days later in other lymph nodes.

These time-dependent changes of microscopic localization of particles in lymph nodes after intravenous injection could be significant both in transfer mechanisms and in effects of radioactive, cytotoxic or immunogenic particulate materials in the reticuloendothelial system.

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