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DIRECT TRANSDIAPHRAGMATIC TRAFFIC OF PERITONEAL MACROPHAGES TO THE LUNG

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INTRODUCTION

Intraperitoneal (i.p.) inoculation is a parenteral route of immunization which results in priming of the mucosal immune response. We hypothesized that this may be related to the processing of antigen either in the lymphoid tissue associated with the peritoneal cavity or in the sites that drain it. Particulates injected into the peritoneal cavity are removed principally by way of transdiaphragmatic lymphatic channels situated under the diaphragmatic mesothelium^{1,2}. These channels drain into larger intrathoracic lymphatics which in turn enter anterior mammary and parathymic lymph nodes before emptying into the bloodstream.

We have identified a novel pathway for the clearance of particulates from the peritoneal cavity which could be a significant source of lung interstitial macrophages as well as a pathway for immune priming of the lung and mucosa-associated lymphoid tissue (MALT). Some of the injected colloidal carbon is phagocytosed by peritoneal macrophages. These macrophages migrate across the diaphragm through the pleural space entering the lung at specific sites along the visceral pleural surface and then penetrate into the interstitium through short channels that serve as potential lymphatics.

MATERIALS AND METHODS

Adult female Swiss Webster mice were given a single i.p. injection of 0.1 ml of a 1:2 dilution of Pelikan special black ink in phosphate-buffered saline (PBS). This dose was empirically selected to prevent rapid overflow into the blood. Four animals per group were killed 5 minutes, 10 minutes, 1, 2, 3, 24 and 72 hours post i.p. injection. Three additional groups of

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animals Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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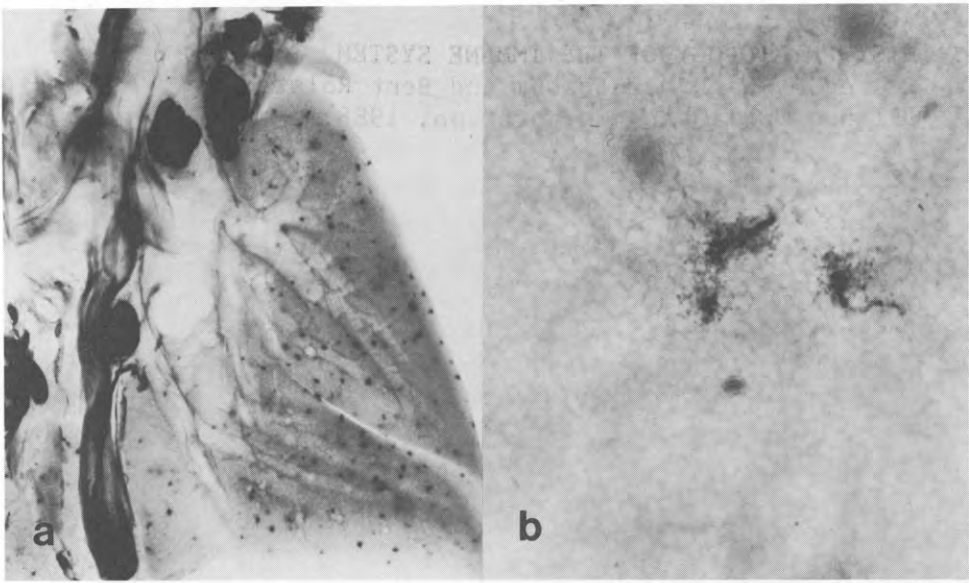


Figure 1. (a) Slice of a whole lung 18 hrs post i.p. inoculation of carbon showing accumulation in mediastinal and parathymic lymph nodes and in the short lymphatic channels leading from the visceral pleura. (b) Macroscopic view of the lymphatic channels showing dissemination into the alveolar septae.

mice were given an equivalent amount of carbon either intravenously, intranasally or intrapleurally and killed 30 minutes and 24 hours post injection.

Blood was collected in EDTA and the leucocytes isolated. Cells present in both the pleural cavity and lungs were harvested by washing with Ca^{++} - and Mg^{++} -free Jokliks medium. The resulting cell suspensions were centrifuged at 200 x g for 10 minutes at room temperature and resuspended in RPMI 1640 plus 10% foetal calf serum. The number of macrophages/monocytes present in the suspensions of blood leucocytes, pleural cavity cells and bronchial lavage was estimated using a haemocytometer. Cytocentrifuge preparations were made and the percentage of macrophages/monocytes containing carbon was determined using light microscopy.

Following bronchial lavage the lungs were inflated with either 10% formalin or 2% glutaraldehyde in PBS and fixed overnight at 4°C. Formalin-fixed lungs were processed for histology. Glutaraldehyde-fixed lungs were dehydrated in ethanol and cleared in methyl salicylate to study the carbon deposition in situ. The cleared lungs were cut into thick slices and embedded in plastic. The plastic blocks were oriented along the planes of carbon-laden channels and 1 μm sections of these channels were cut, mounted and studied by light microscopy. Also, the pathways of carbon movement across fixed and cleared diaphragms were studied. The diaphragms of untreated mice were examined by transmission electron microscopy for identification of the cell types present in diaphragmatic lymphatics.

RESULTS

Translocation of intraperitoneal colloidal carbon: Carbon was observed in the diaphragmatic, mediastinal and internal mammary lymphatics

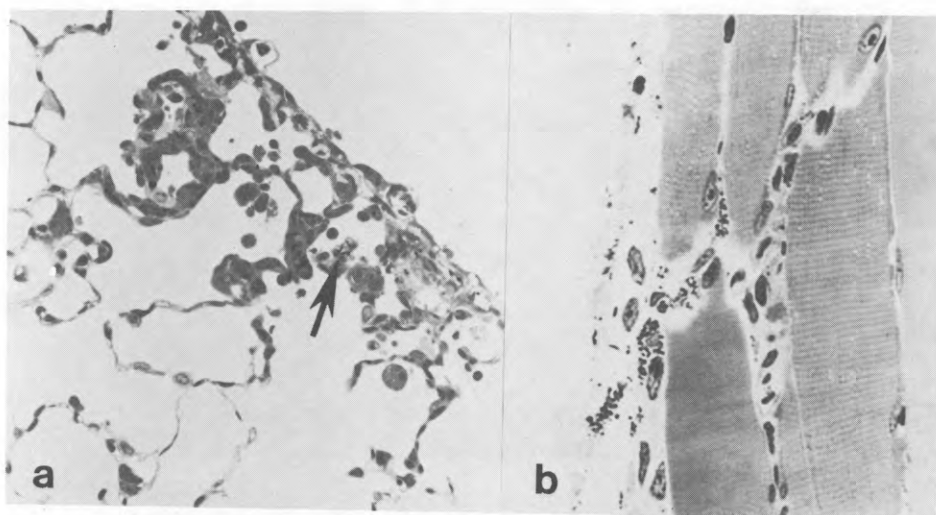


Figure 2. 1 μ m sections through (a) a lymphatic channel in the lung showing carbon in the macrophages of the potential lymphatic. A labelled macrophage (\blackleftarrow) can be observed in an alveolus, (b) a diaphragm showing macrophages containing carbon entering a channel on the peritoneal cavity side and moving through a channel. These were obtained from a mouse 18 hrs post i.p. inoculation.

and had reached the mediastinal and parathymic lymph nodes within 5 minutes of i.p. introduction. Eighteen hours post i.p. inoculation the carbon was also present in discrete areas of the lung pleura (Figure 1a & 1b).

Light microscopic examination of 1 μ m sections of the cleared lungs showed that the carbon was in fact present in macrophages which were delineating unique lymphatic channels leading from the visceral surface of the lung into interalveolar septae deep within the lobules (Figure 2a). Examination of cleared diaphragms showed macrophages containing carbon making their way in channels across the diaphragm (Figure 2b). Lymphocytes, small monocytes, macrophages and Langerhans-like cells were present in diaphragmatic lymphatics of both normal mice and mice inoculated i.p. with carbon (Figure 3).

Carbon introduced intravenously did not result in carbon-laden macrophages being present in discrete areas associated with the visceral pleura as with i.p. introduced carbon although free carbon was spread diffusely in the perivascular spaces throughout the lung. Carbon given intranasally resulted in macrophages containing carbon in the alveolar spaces while carbon injected into the pleural space resulted in carbon being situated in discrete areas similar to i.p. inoculation.

Transport of colloidal carbon in macrophages from the peritoneal cavity to the lung: Macrophages containing carbon were first observed in the pleural cavity 5 minutes post i.p. inoculation. The number of carbon-laden pleural macrophages increased with time to reach a maximum at 24 hours post injection (Figure 4a) while there was no significant change in the total number of pleural macrophages obtained throughout the study period (Figure 4b).

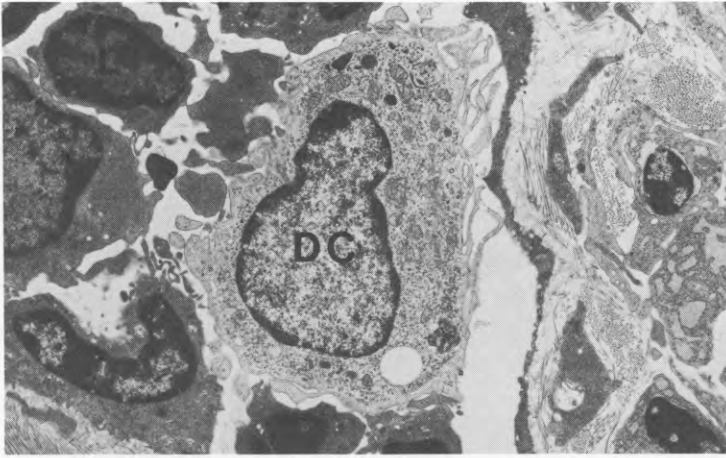


Figure 3. An electron micrograph of a diaphragmatic lymphatic showing a lymphoid cell (L) and a dendritic cell (DC).

Macrophages containing carbon were first encountered in bronchial lavage 1 hour post i.p. inoculation and in blood after 3 hours. While the number of carbon-laden macrophages present in bronchial lavage remained very low, less than 2%, throughout the time studied, the numbers in blood increased with time to 10% at 24 hours post i.p. inoculation (Figure 4a). No free carbon was found in the washings from the pleural cavity.

Intravenous injection of carbon did not lead to the presence of labelled macrophages in the pleural cavity and intranasally introduced carbon did not produce labelled macrophages in either the pleural or peritoneal cavities. Carbon introduced intrapleurally did not result in carbon-laden peritoneal macrophages although they were present in the bronchial lavage after 24 hours.

DISCUSSION

We have described in this study the unidirectional traffic of macrophages from the peritoneal cavity through the diaphragmatic lymphatics, across the pleural space to the lung. Introduction of carbon i.p. did not elicit this macrophage traffic because the total number of macrophages isolated from the pleural cavity did not increase during the time period studied. This suggests that the transition of peritoneal macrophages across the diaphragm to the pleural cavity is a normal ongoing movement. The dissemination of carbon through the bloodstream could not be responsible for the presence of macrophages containing carbon in channels leading from the visceral pleura into interalveolar septae as intravenous carbon introduction did not produce the same carbon deposition patterns in the lung as the i.p. introduced carbon. Also, carbon was not detected in the blood following i.p. inoculation until well after the appearance of labelled cells in the pleural cavity and lung. The appearance of carbon-labelled macrophages in the blood at 3 hours in the present study may have been due to passage up the mediastinal lymphatics or via thoracic duct as described by Drayson et al.³

The absence of carbon-labelled macrophages in the peritoneal cavity after intrapleural introduction and in both the pleural and peritoneal

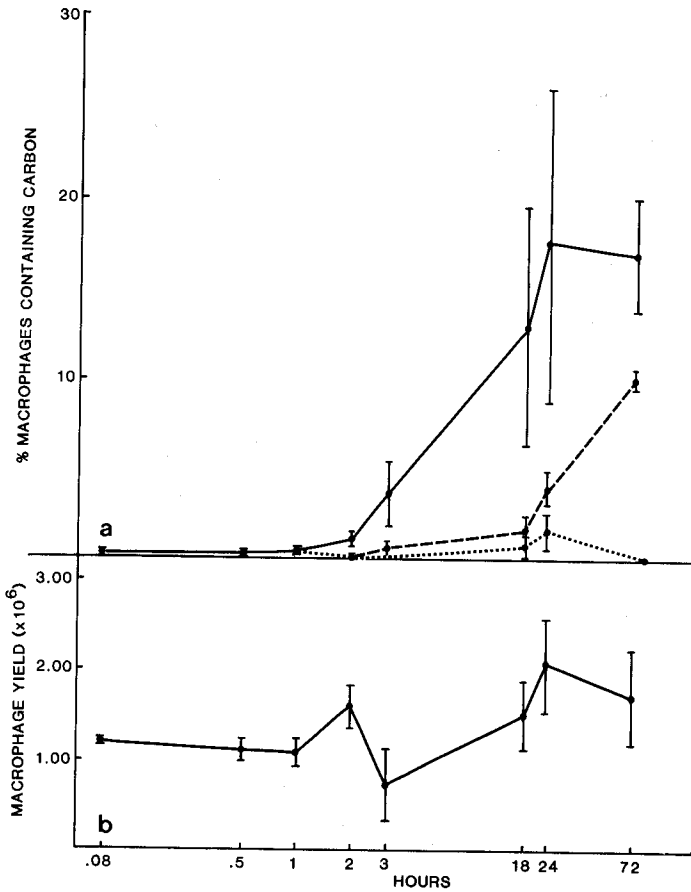


Figure 4. (a) Dissemination of i.p. inoculated carbon with time (hrs in log scale). The percentage of carbon laden macrophages in pleural cavity cells (—), blood (----) and bronchial lavage (.....) were estimated. (b) The total number of pleural cavity macrophages/monocytes isolated throughout the time period studied.

cavities after intranasal inoculation emphasizes the unidirectional movement of peritoneal macrophages to the lung. The finding of carbon-labelled macrophages in bronchial lavage following intrapleural instillation supports this.

The lack or very low numbers of carbon-laden macrophages found in the bronchial lavage suggests that the peritoneal macrophages that migrate to the lung are not a significant source of alveolar macrophages. Instead, they enter the interstitium. We propose that these macrophages are a significant source of long-surviving interstitial macrophages. This is supported by the observation that mice which were killed 5 months after receiving carbon i.p. still had distinct areas of carbon-labelled macrophages in channels associated with the periphery of the lungs.

Intraperitoneal introduction of antigen primes for a mucosal immune response. Pierce and Koster⁴ found the i.p. route primed for a mucosal IgA response to cholera toxin and in their opinion appeared to reflect enhanced encounter of the i.p. introduced antigen with IgA-committed lymphocytes in

extra-intestinal MALT. It is probable that the peritoneal macrophages which migrate to the lung carrying antigen could be antigen-presenting cells which prime T cells either in the lung or associated lymphoid tissue. The observations of Davies and Parrott⁵ support this. They detected increased cytotoxic T cell activity in the lungs after an i.p. injection of tumour cells compared to subcutaneous or i.v. injection. The presence of lymphocytes and Langerhans-like cells in diaphragmatic lymphatics as well as labelled and non-labelled macrophages, which appear to be moving together, provide evidence that these mononuclear cells could be antigen presenting cells which lodge and reside in the interstitium.

Macrophages laden with carbon in potential lymphatics dissect along interalveolar septae presumably within an extravascular compartment. Aggregates of lymphocytes and monocytes were found along the pathway intermingled with the labelled cells. The labelled macrophages may slowly migrate through the interstitium until they reach lymphoid aggregates including the bronchial-associated lymphoid tissue (BALT). Support for this theory comes from unpublished data by Potter et al.⁶, in their study they observed oil droplet-containing macrophages resulting from i.p. inoculation of pristane in the BALT. Green⁷ observed that pigment-laden macrophages, labelled by airborne exposure, move through neighbouring alveoli and interstitial locations through lymphoid nodules out into the bronchiolar mucosa at the level of broncho-alveolar junctions. The intraperitoneally labelled macrophages appear to take a similar route.

Carbon deposition in the macrophages of lung interstitium is much less than that in the mediastinal internal mammary and parathyroid lymphatics and nodes indicating that passage across the diaphragm to the pleural cavity and lung is not the predominant route of clearance of peritoneal lymph. However, the longevity of carbon-laden peritoneal macrophages in the lung interstitium points to a potentially important role of these cells in presenting antigens to lung-homing lymphoid cells.

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