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Post-capillary venules in the "milky spots" of the greater omentum are the major site of plasma protein and leukocyte extravasation in rodent models of peritonitis

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Abstract. Intraperitoneal injection of inflammatory agents in the mouse and rat causes plasma protein and leukocyte extravasation into the peritoneal cavity. Following an intraperitoneal injection of zymosan A, the milky spots of the omentum were the only abdominal sites detected where intravenously administered Monastral Blue labeled interendothelial cell gaps responsible for plasma extravasation. In addition, when colored microspheres were intraventricularly administered to quantify blood flow, the omentum was the only abdominal organ which showed an increase in blood flow during zymosan A peritonitis. A combination of light and electron microscopy, plus measurement of myeloperoxidase activity (a marker of neutrophil accumulation) demonstrated that the omental milky spots are the major route through which leukocytes migrate into the peritoneal cavity. Identical structures in the pleura likewise are the sites of protein leakage into the pleural cavity. In contrast, selective sites of protein and cellular extravasation could not be detected in the synovial lining of the inflamed knee joint.

Key words: Milky spots – Omentum – Pleura – Synovium and capillary permeability

Introduction

Extravasation of plasma and accumulation of leukocytes are hallmarks of inflammatory and allergic responses. It has been demonstrated in a variety of tissues (skin, muscle, mesentery) that the blood vessels which leak plasma proteins and are the sites of leukocyte extravasation are exclusively post-capillary venules; neither capillaries nor arterioles are involved unless subject to direct physical damage [1]. In order for post-capillary venules to leak, two conditions must be present: *1)* adequate local blood flow and intravascular pressure – achieved by dilation of the precapillary arterioles, usually in response to inflammatory mediators with vasodilator activity, e.g., prostaglandins, histamine, bradykinin, nitric oxide [2]; *2)* formation of gaps between adjacent endothelial cells [3, 4] so as to permit efflux of plasma proteins – achieved by contraction of endothelial cells in response to inflammatory mediators (e.g., histamine, bradykinin, peptidoleukotrienes) or by interaction of neutrophils with the endothelium [5]. Leukocyte extravasation is also dependent on local blood flow [6, 7] but in addition requires expression of the appropriate adhesion molecules on endothelial cells of the post-capillary venules [8].

In a number of clinical conditions, and animal models of inflammation, plasma proteins and leukocytes accumulate in the peritoneal cavity. In order for plasma proteins to reach the peritoneal cavity they must not only pass through blood vessel walls but also through the peritoneum. Therefore, there must be either a mechanism for reversibly opening the tight junctions between adjacent mesothelial cells of the peritoneum, or, a region in which the mesothelial barrier is permanently open. Cranshaw and Leak [9] have demonstrated that the greater omentum is a site of plasma leak and cell extravasation in peritonitis. The data reported here confirm these findings and demonstrate that the milky spots of the greater omentum are the primary, perhaps only, site of plasma protein and leukocyte extravasation in models of peritonitis in rodents. At these sites there is no mesothelial barrier to the movement of extravasated plasma proteins into the peritoneal cavity. The extravasation of plasma in the omentum utilizes similar mechanisms to those identified in other tissues i.e. vasodilation and formation of gaps between adjacent endothelial cells. In addition, these sites may be the major route for leukocyte migration into the peritoneal cavity.

Materials and methods

Induction of inflammation

Peritonitis was induced in mice as described previously, with minor

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modifications [10]. An intraperitoneal injection of an irritant (usually zymosan A, Sigma, 2 mg/ml suspended in saline, 0.5 ml/mouse; other irritants used are shown in Table 1) was administered to male, 25–35 g Swiss Webster mice (Taconic Farms). At various times after injection of irritants, groups of mice were killed by carbon dioxide inhalation and the peritoneal cavity lavaged with 3 ml of saline containing 10 U/ml heparin. The numbers of neutrophils present in lavage and a homogenate of the omentum were determined by measuring the myeloperoxidase activity and comparing it with a calibration curve constructed from known numbers of sodium caseinate (Eastman Chemicals) induced murine peritoneal neutrophils as described by Pettipher *et al.* [11]. Myeloperoxidase is present in neutrophils at high levels and is widely used to quantify these cells [12]. However, monocytes/macrophages also contain this activity albeit at much lower levels [13, 14]. In some experiments zymosan A induced peritonitis (2 ml of a 5 mg/ml suspension) or arthritis (0.05 ml of a 20 mg/ml suspension) was studied in rats (Male, Charles River CD outbred, 200–300 g) as described by Griffiths *et al.* [15]. Pleurisy was induced in rats by the injection of 0.1 ml of a 10 mg/ml suspension of zymosan A, using the injection technique described by Vinegar *et al.* [16].

Labelling of blood vessels

Animals were injected intravenously via the tail vein with either Monastral Blue (Sigma, diluted 1 : 1 with saline, 0.2 ml/mouse, 1 ml/rat [17] or a suspension of carbon particles ([18], Special India Ink, Pelikan, diluted 1 : 1 with saline, 0.2 ml/mouse, 1 ml/rat), either immediately prior to, or at various times after administration of the inflammatory stimulus. Thirty minutes after the intravenous injection the animals were killed by carbon dioxide inhalation, the inflamed tissues exposed and examined macroscopically for the characteristic color of the intravenously injected materials (blue or black respectively). Intravenously injected Monastral Blue circulates as a fine suspension. In regions of the vasculature undergoing an inflammatory or allergic response, gaps are formed between adjacent endothelial cells which permit plasma to escape from the vasculature. Particles of Monastral Blue present in the circulation at this time escape from the lumen of the vessel via these gaps to become trapped on the basement membrane. Over a thirty minute period, all of the circulating particles are removed from the circulation by fixed phagocytes in the liver and spleen so that the blood returns to its normal color and the liver, spleen and some lymph nodes are stained. Therefore, only blood vessels with inter-endothelial cell gaps are labelled with the colored particles when examined 30 minutes after intravenous injection. A suspension of carbon particles (India Ink) has the same ability to label leaking blood vessels as Monastral Blue.

Microscopic techniques

Light microscopy – Selected tissues were removed and fixed by immersion in 10% neutral buffered formalin for 12–18 hours. The fixed specimens were trimmed, processed and embedded in paraffin using standard techniques. Knee joints were processed as previously described [19]. Five micron thick sections were cut on a Reichert Jung Autocut microtome, stained with haematoxylin and eosin and viewed with a Nikon FXA microscope.

Electron microscopy – Small portions of omental tissue from mice and rats were removed at the 30 minute time point and fixed for one hour by immersion in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2, 4 °C). The tissue was then routinely processed and embedded in epoxy resin [20]. Thin sections were cut on a Reichert FC-4 ultramicrotome, stained with uranyl acetate and lead citrate and viewed on a JEOL 1200 electron microscope.

Vascular permeability

In some studies, Evans Blue dye (Sigma 10 mg/ml in saline, 0.2 ml/mouse) was injected intravenously instead of Monastral Blue or carbon particles. Thirty minutes after intravenous injection, animals were killed and the peritoneal cavity lavaged as described in the section "Induction of inflammation". The lavage fluid was centrifuged and the absorbance of Evans Blue in the supernatant measured by transferring 200 µl aliquots to a 96 well plate and measuring the absorbance at 650 nm with a Molecular Devices Thermomax plate reader. Evans Blue binds to plasma albumin and distributes with albumin throughout the animal. Therefore the accumulation of Evans Blue dye in the peritoneal cavity is a quantitative index of increased vascular permeability and extravasation of albumin [21].

Measurement of blood flow

Regional blood flow was measured in conscious rats using a reference microsphere technique [22] with 15 µm dye-extraction (Dye-Trak) microspheres [23]. Rats were anesthetized with a mixture of pentobarbital, chloral hydrate, and magnesium sulfate. A microsphere infusion catheter made from PE-50 tubing was advanced into the left ventricle through an incision in the right carotid artery. A second PE-50 catheter was placed in the caudal artery for withdrawal of the reference blood sample. Both catheters were ligated into position and exteriorized through the nape of the neck. The rats were allowed at least three hours recovery time before microsphere infusions. Heart rate and blood pressure were

Table 1. The vascular labeling achieved with Monastral Blue in response to intraperitoneal injection of inflammatory agents in mice.

Inflammatory agent	Omentum	Liver	Spleen	Lymph nodes	Muscle	Ureters
Zymosan A	+	+	+	±	−	−
Acetic acid*	+	+	+	±	±	−
Mustard oil**	+	+	+	±	−	+
Kaolin	+	+	+	±	−	−
Carrageenan	+	+	+	±	−	−
Bradykinin	+	+	+	±	−	−
Saline	−	+	+	±	−	−
Untreated	−	+	+	±	−	−

* slight, generalized labeling of muscle surrounding the peritoneal cavity, ** marked labeling of the ureters. + marked labeling, ± slight labeling, − no labeling.
Acetic acid, 0.5% in saline from Fisher. Mustard oil, 10% allyl isothiocyanate in 1% tween 80/saline from Aldrich. Carrageenan, 10 mg/ml in water, from Marine Colloids. Kaolin, 1 mg/ml in saline, from Sigma. Bradykinin, 2 µg/ml in saline, from Sigma.

monitored before and after infusion of microspheres. To establish control blood flows, the rats were given an intraperitoneal (i.p.) injection of 2.0 ml of phosphate buffered saline (PBS). Fifteen to thirty minutes later, about 200,000 dye-extraction microspheres, suspended in 0.01% Tween 80 in normal saline, were loaded into a coiled, 75 cm long piece of PE-50 tubing. One end of the coil was connected to the ventricular catheter; the other end was attached to a 1 ml syringe that had been loaded with 0.9% saline and mounted in a Harvard infusion/withdrawal pump. With the rat resting quietly in an acrylic restraint tube, withdrawal of the reference blood sample (0.5 ml/min) from the caudal artery catheter was started. Starting ten seconds after the withdrawal began, the microspheres were infused into the ventricle at a rate of 0.6 ml/min. Collection of the reference blood sample continued for another 15 seconds after the cessation of the infusion. After this first microsphere infusion, the rats were removed from the restraint tubes and given an intraperitoneal injection of 10 mg of zymosan A suspended in 2 ml of PBS. Fifteen minutes later, and again ninety minutes after the zymosan A injection, microspheres of a different color were infused according to the procedure described. In 2 rats, the second i.p. injection consisted of PBS without zymosan A to determine whether the first infusion of microspheres had any effect on omental blood flow, as measured by the two subsequent microsphere infusions.

After completion of microsphere infusions, the rats were killed with pentobarbital overdose. The heart, kidneys, and omentum were removed and weighed. The reference blood samples and tissues were digested and microspheres isolated as described [23]. Following the isolation procedure, the recovered microspheres were washed with ethanol, dried, and treated with dimethylformamide to extract the dyes. The concentrations of dyes in the extracts were quantified with a 96 well plate reader (Bio-Tek) using a quartz 96 well plate. Tissue flow rates were calculated as follows: $Q_s = A_s (Q_r/A_r)$, where Q_r and Q_s represent flow in the reference blood and sample tissue, respectively, and A_r and A_s represent the activity (Absorbance Units) of the reference blood and sample tissue, respectively. Blood flow was divided by tissue weight to yield ml/min/g. To ensure that the microspheres had been evenly distributed within the systemic circulation, if the blood flows to the left and right kidneys of any rat were not within 10% of each other, the rat was omitted from data analysis.

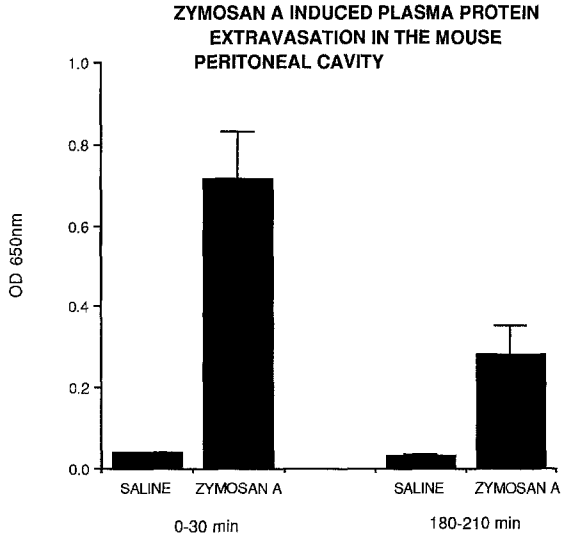


Fig. 1. Zymosan A induced plasma protein extravasation in the mouse peritoneal cavity. Mice were injected i.p. with zymosan A (1 mg) or PBS. Evans Blue dye was injected i.v. either immediately prior to the i.p. injection or 3 hours later. 30 minutes after the injection of Evans Blue dye the peritoneal cavity was lavaged and the absorbance of the cell free fluid measured in a microplate reader as an index of the Evans Blue content. Results are mean ± se, $n = 6$.

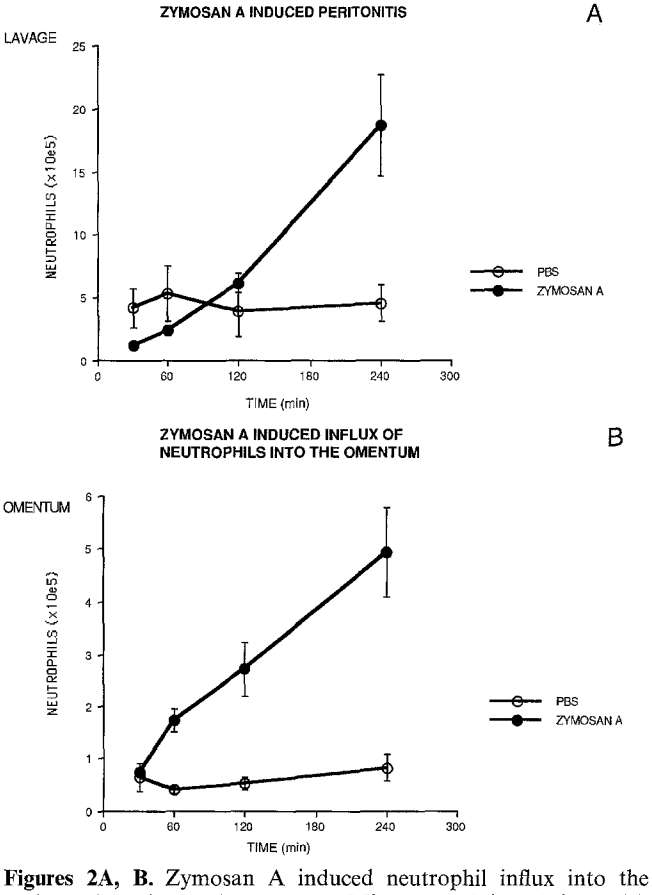
In a second set of studies, regional blood flows were determined 15 minutes after intraperitoneal injection of vehicle, and again 30 minutes after the i.p. injection of zymosan A. In these studies, in addition to heart, kidneys, and omentum, blood flow to the liver, pancreas, spleen, stomach (corpus), and sections of duodenum, ileum, and colon were also determined. Statistical differences in blood flow changes after the injection of zymosan A were determined with a paired *t*-test.

Results

Peritonitis

Characterization of the inflammatory response to zymosan A. Intraperitoneal injection of zymosan A, but not saline, induced in mice the characteristic pattern of responses reported previously [10, 24, 25], i.e.,

1. An early transient writhing response (0–15 minutes, not shown).
2. The rapid extravasation of plasma protein into the peritoneal cavity, as indicated by the accumulation of



Figures 2A, B. Zymosan A induced neutrophil influx into the peritoneal cavity and omentum. Mice were injected i.p. with zymosan A (●) or PBS (○). At the times indicated, the peritoneal cavity was lavaged. In a separate group of mice the omenta were removed. The content of myeloperoxidase in the cell free lavage fluid (A) and in homogenates (B) of the omentum was determined spectrophotometrically, and converted to neutrophil equivalents by reference to a standard curve prepared with known numbers of mouse neutrophils. Results are mean ± se, $n = 6$.

Evans Blue (Fig. 1). The plasma protein accumulation measured over the first half hour after zymosan A injection was approximately double the response measured between 2.5 to 3 hours after injection. No increase in the accumulation of plasma proteins was seen after an intraperitoneal injection of saline.

3. A transient decrease in the myeloperoxidase content of the peritoneal lavage fluid, followed by a marked and sustained increase (Fig. 2A). These data correspond to the initial decrease in cells (mostly resident macrophages) followed by an influx of neutrophils that has been reported previously in both mice [10] and rats [15]. The increased number of neutrophils in the peritoneal lavage was preceded by an increase in the omentum, as measured by myeloperoxidase content (Fig. 2B). This time course is consistent with the microscopic observations described below and suggests that the neutrophils leave blood vessels in the omentum then migrate into the peritoneal cavity. Intraperitoneal injection of saline produced no change in the myeloperoxidase content of the omentum or peritoneal lavage.

Identification of the leaking blood vessels in zymosan A induced peritonitis in mice

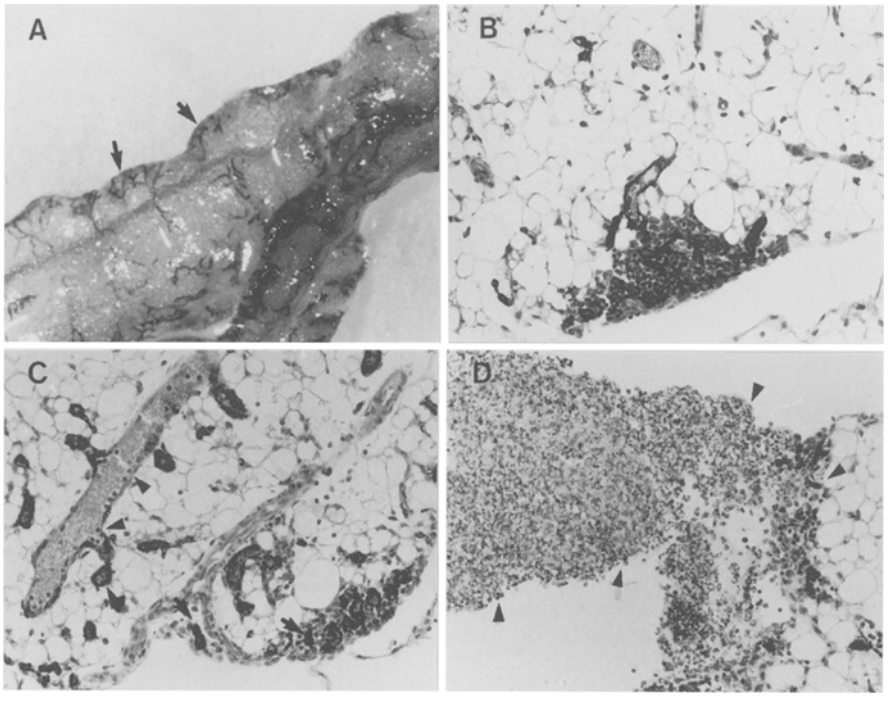
Macroscopic examination of the peritoneal cavity of mice injected with Monastral Blue immediately before the zymosan A and killed 30 minutes later showed the expected bluing of the reticuloendothelial system (liver and spleen – intense, uniform bluing; mesenteric and para-aortic lymph nodes – slight bluing). A fine but intensely blue speckling was also seen on the greater omentum (Fig. 4A). No bluing was discernible in any of the other abdominal organs, peritoneum or surrounding musculature. When the injection of Monastral Blue was delayed for 3 hours after zymosan A administration, a similar but less intense pattern was seen. Mice which were injected with Monastral Blue but received no intraperitoneal injection or were given an intraperitoneal injection of saline, exhibited labelling of spleen and liver plus slight labelling of lymph nodes but no labelling of the omentum or any other organs. An identical pattern of labelling was seen when India Ink was substituted for Monastral Blue in the study of zymosan A induced peritonitis.

Vascular leakage in peritonitis induced by other inflammatory stimuli and in rats

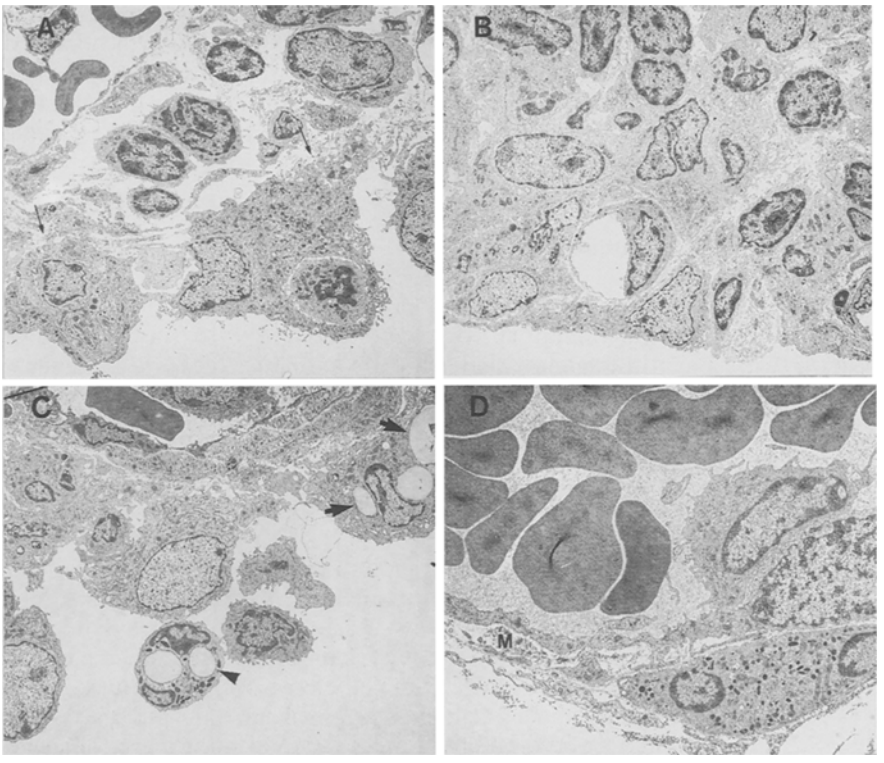
In order to determine whether similar labelling occurred when other inflammatory agents were administered, groups of four mice were injected intravenously with Monastral Blue immediately before an inflammatory agent was administered intraperitoneally. All animals were examined thirty minutes later. As shown in Table 1, in all cases the greater omentum and the reticuloendothelial system were intensely stained while other organs were unstained. The only exceptions were a marked staining of the ureters in animals injected with mustard oil and slight, generalized bluing of muscle surrounding the peritoneal cavity in animals injected with acetic acid. Staining of the omentum was never uniform, exhibiting a "speckled" pattern which was particularly evident when the omentum was spread. In zymosan A induced peritonitis in rats, the labelling pattern seen was similar to that described in mice (not shown).

Light microscopy

Monastral Blue stained omental tissue from zymosan A



Figures 3A–D. Mouse omentum labelled with intravenously administered colloidal carbon 30 min after zymosan A injection. A, In the gross omentum one can easily see that the numerous blood vessels labelled with colloidal carbon (arrows) are primarily at the edge of the organ (35×). B, A light microscopic section through one of these regions clearly shows numerous labelled vessels within an omental milky spot (450×). C, The post capillary venules draining a milky spot were all heavily labelled with colloidal carbon (arrows) and this labelling abruptly stopped at the junction of a venule. In addition, numerous marginated leukocytes can be seen adhering to the endothelium (arrowheads) in both the post-capillary venules and venules (450×). D, In this panel, a cellular mass can be seen extending from a milky spot (arrowheads) into the peritoneal cavity (450×).



Figures 4A–D. Ultrastructure of murine omental milky spots. A, A control omentum in which the discontinuous basal lamina (small arrows) beneath the lining macrophages is apparent (2500×). B, A control omental milky spot which demonstrates the typical clustering of leukocytes in this region (2500×). C, 30 minutes after IP zymosan A, numerous particles can be observed in the lining macrophages (arrows) and occasionally in other leukocytes (PMN-arrowhead) associated with the milky spot but in the peritoneal cavity. In addition, collections of Monastral Blue (M) can be seen trapped outside of the vessel in this field (2500×). D, At higher magnification, one can easily see some Monastral Blue trapped by the basal lamina (M) surrounding this blood vessel as well as an extravasated PMN (5000×).

injected mice was examined with a dissecting microscope. Labelling was exclusively in venules draining the large aggregate of mononuclear cells known as "milky spots". This localized distribution within the omentum resulted in the "speckled" appearance noted macroscopically (Fig. 3A). Light microscopy of stained sections from labelled omental tissue showed the deposits of Monastral Blue or India Ink on the abluminal surface of post capillary venules and some slightly larger venules (Fig. 3B). Very few venules outside the "milky spots" were labelled, nor were arterioles or capillaries labelled by either Monastral Blue or colloidal carbon. Thirty minutes after IP injection of zymosan A, marginating and migrating leukocytes were observed in many of the milky spot venules (Fig. 3C). In addition, large aggregations of extravascular leukocytes were observed extending from the milky spots into the abdominal cavity. Many of these aggregates included large amounts of amorphous, eosinophilic material resembling fibrin clots. The cells embedded in fibrin appeared to be undergoing degeneration since much of the structural definition in the cytoplasm and nucleus had been lost. While neutrophils appeared to be the primary leukocyte in these cell masses, other nucleated cells (lymphocytes, monocytes) as well as red blood cells could be seen (Fig. 3D).

Electron microscopy

Examination of the non-inflamed omentum at the EM level revealed no new structures from the description of the milky spots made by Beelen *et al.* [26]. Briefly, the omentum is covered by a continuous lining of mesothelial cells up to the region of the milky spots where the mesothelial cell lining changes to a quasi-continuous fixed macrophage cell lining. These lining macrophages do not have a continuous basal lamina beneath them (Fig. 4A). The milky spot itself is a collection of lymphocytes surrounded by a highly vascular loose connective tissue stroma (Fig. 4B).

Thirty minutes after IP injection of zymosan A, numerous phagocytized zymosan A particles were observed within the lining macrophage cells and occasional abutting neutrophils (Fig. 4C). Moreover, large collections of Monastral Blue were seen trapped between the endothelial cells of some post-capillary venules and the basal lamina (Fig. 4D). The basal lamina appeared to be a sufficient barrier to retard the free movement of the sub-endothelial Monastral Blue into the surrounding tissues or peritoneal cavity. In addition, red blood cells were occasionally trapped in these subendothelial collections of Monastral Blue.

Blood Flow

Within fifteen minutes of an IP injection of zymosan A in rats, blood flow to the omentum increased to more than twice that observed after the IP injection of vehicle (Fig. 5). The omental blood flow remained elevated for at least 90 minutes. The second set of blood flow experiments focused on the effect of IP injection of zymosan A on blood flow in a variety of tissues of the peritoneal cavity (Fig. 6). Thirty minutes after the injection of zymosan A, blood flow increased only in the omentum (+56%, $p < 0.05$). Blood flow was significantly ($p < 0.05$) reduced in the pancreas (−47%),

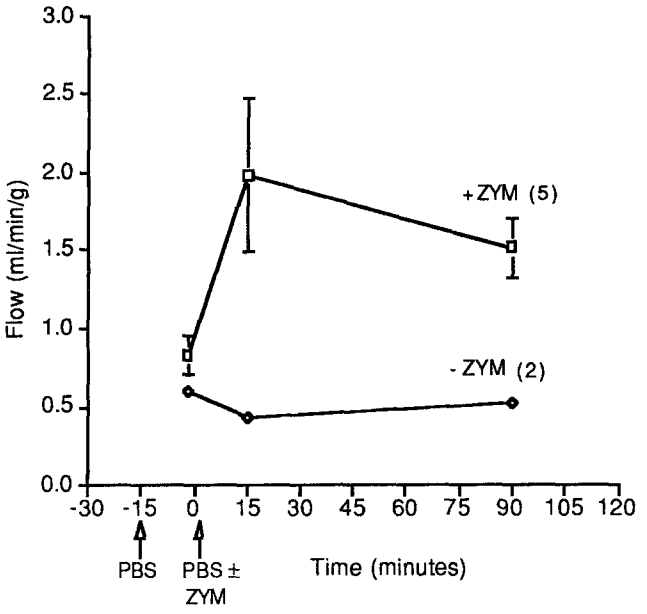


Fig. 5. Zymosan A induced increase in blood flow to the rat omentum evaluated by the use of dye-extraction microspheres. Rats were given an infusion of 15 μm microspheres to determine basal blood flow followed by an i.p. injection of zymosan A (□) or PBS (◇). A further two intravenous infusions of microspheres were given 15 and 90 minutes later. The content of microspheres isolated from the tissue was measured spectrophotometrically and converted to blood flow by comparison to a reference blood sample. Results are mean ± se of 5 zymosan A injected rats and mean of 2 PBS injected rats.

spleen (−50%), and gastric corpus (−60%). In the liver, duodenum, ileum, and colon, blood flow tended to be lower after the injection of zymosan A, but the differences were not statistically significant.

Zymosan A induced pleurisy in rats

When Monastral Blue was injected intravenously immediately after the intrapleural injection of zymosan A and the animals euthanized 30 minutes later, the only bluing observed in the pleural cavity was in a number of discrete regions of the mediastinal pleura near the root of the lung. These blue regions accounted for a very small fraction of the total surface area of the pleural cavity but presumably represent the only regions where vascular leak takes place. Light microscopy of these regions revealed great similarities with the milky spots of the greater omentum, i.e. interrupted basal lamina and non-contiguous mesothelial cells overlying aggregates of cells resembling lymphocytes and macrophages, Monastral Blue trapped in the subendothelial space of venules and aggregates of leukocytes (mostly neutrophils) embedded in fibrin extending into the pleural cavity (data not shown).

Arthritis

In zymosan A induced arthritis in the knee joints of rats, zymosan A particles were phagocytized by macrophages lining the synovial cavity. This induced a generalized Monastral Blue labelling of most venules subtending the synovium. No focal localization of this process was evident by either macroscopic or light microscopic examination (data not shown).

Discussion

In many tissues it has been clearly demonstrated that inflammatory edema is attributable to the opening of inter-endothelial cell gaps in dilated blood vessels; these gaps allow the extravasation of plasma proteins [3]. In these tissues the only blood vessels that leak are the post-capillary venules – arterioles, capillaries and venules do not leak unless directly injured. At first sight it might therefore be expected that an edema inducing agent injected into the peritoneal cavity would lead to the formation of inter-endothelial gaps in post-capillary venules of all the organs and tissues adjacent to the peritoneal cavity. However, for a range of stimuli injected into the peritoneal cavity, gap formation was restricted to a very localized region – the postcapillary venules of the milky spots in the greater omentum. This area accounts for a very small fraction of the total surface area adjacent to the peritoneal cavity and some explanation for this localization was sought.

The surfaces of most tissues adjacent to the peritoneal cavity are covered by a contiguous monolayer of mesothelial cells supported by a basement membrane. Unlike vascular endothelial cells, there have been no reports of the formation of intercellular gaps in such mesothelia in response to inflammatory mediators. It seems probable that, in most regions of the peritoneum, the mesothelium would provide a barrier to the movement of materials both out of, and into, the peritoneal cavity unless this barrier is physically damaged. Therefore,

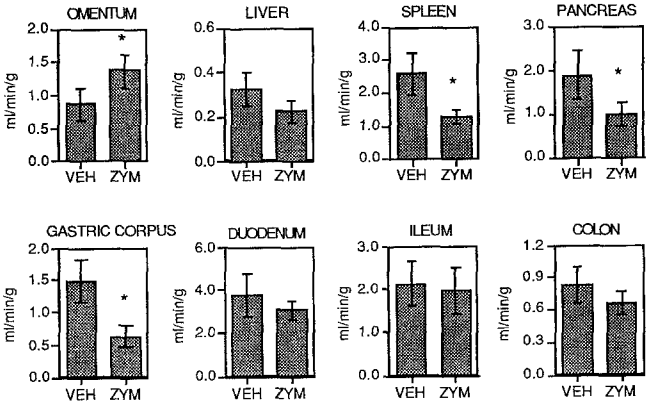


Fig. 6. Zymosan A induced changes in blood flow in various organs of the rat. Rats were given an infusion of microspheres to determine basal blood flow, followed by an i.p. injection of zymosan A (10 mg) or PBS. A further infusion of microspheres was given 30 minutes later. The content of microspheres isolated from the tissue was measured spectrophotometrically and converted to blood flow by comparison to a reference blood sample. * = $p < 0.05$. Results are mean ± se, $n = 8$.

the failure of most post-capillary venules in the peritoneal cavity to respond during peritonitis may be because they were not exposed to the inflammatory stimuli. However, in the milky spots of the omentum the contiguous mesothelium is absent, being replaced by clusters of macrophages and a discontinuous basement membrane [26]. Therefore, not only will the post-capillary venules in this region be exposed to inflammatory stimuli from the peritoneal cavity, but cells and plasma that extravasates in this region has direct access to the peritoneal cavity. Thus, the milky spots are ideally suited to a role in plasma leakage and cell migration in peritonitis.

This role for the milky spots appears to be of general significance since it was seen with a variety of inflammatory stimuli, both particulate and soluble, and was seen in two species, rats and mice. However, two apparently anomalous observations were made: *1*, Acetic acid, in addition to causing the formation of endothelial gaps in the omentum, also caused diffuse formation of gaps in the blood vessels of skeletal muscle of the abdominal wall. This phenomenon is most readily explained by assuming that the injected acid caused direct physical damage to the mesothelial cells and/or basement membrane of the peritoneum, thus breaking the barrier to the movement of materials. Acetic acid itself, or induced inflammatory mediators, could then gain access to the blood vessels of the surrounding musculature. *2*, Mustard oil induced marked vascular labelling both in the omentum and in the ureters. Selective induction of vascular leakage in the ureters is a characteristic feature of compounds which induce release of substance P and other neuropeptides, e.g. capsaicin [27]. Systemic administration of substance P itself causes the same phenomenon [27]. Mustard oil induces inflammation via neuropeptide release [28]. Therefore, it can be assumed that substance P released in the peritoneal cavity, or mustard oil itself, that reaches the general circulation (presumably via stomata into lymphatics then the general circulation) is responsible for the formation of endothelial gaps in the ureters.

Plasma leakage through such inter-endothelial cell gaps is dependent on adequate blood flow and hydrostatic pressure differential. It has been stated that peritonitis in mice results in increased omental blood flow [29] although the data to support this claim was not presented. In order to determine whether a vasodilator response to zymosan A could be detected, and whether it is also localized to the omentum, blood flow was monitored by measuring the tissue distribution of labelled microspheres which were infused into the cardiac ventricles of zymosan A injected rats. Rats were used for these experiments because larger animals are required in order to make cardiovascular monitoring possible. Previous studies indicate that the vascular leak in response to zymosan A is similar in rats and mice [10, 15, 24]. The microsphere studies demonstrated that a clear increase in blood flow occurred in the omentum following intraperitoneal injection of zymosan A while flow to other abdominal organs showed no change or decreased. This remarkable selectivity for the omentum can also be largely attributable to the microanatomy of the milky spots which are uniquely accessible to materials present in the peritoneal cavity. The combination of formation of endothelial gaps (as indicated by vascular labelling) and increased blood flow (indicated by distribution of microspheres) creates in the milky spots all the conditions required to produce extensive plasma extravasation which presumably accounts for most or all of the inflammatory edema in peritonitis.

One hour after zymosan A injection, the number of cells (measured by both direct cell counting and by myeloperoxidase activity) recovered from the peritoneal cavity of zymosan A injected mice and rats is less than that recovered from uninjected or saline injected animals, as reported previously [10, 15]. The occurrence of aggregates of cells and fibrin [30] in the lavage fluid and attached to the omentum at one hour suggests that cell–cell adhesion was increased resulting in increased interaction with peritoneal surfaces and reduced recovery of cells in the lavage fluid. Beyond two hours post zymosan A injection, a marked increase in the number of neutrophils in the lavage fluid occurs. In contrast, the number of leukocytes in the omentum (myeloperoxidase content) was increased at one hour and continued to rise for at least four hours. This early increase in myeloperoxidase activity in the omentum correlated with histologically detected margination of leukocytes in the venules of the milky spots, their extravasation and accumulation in large aggregates, mostly neutrophils, which remained attached to the omentum and would be resistant to removal by lavage. This early, localized cell accumulation would, therefore, not result in an early increase in cell number or levels of myeloperoxidase activity in lavage fluid. At later times, as more neutrophils accumulate and the degree of aggregation decreases [10], these neutrophils appear to dissociate from the omentum, move into the peritoneal cavity and result in an increase in the number of cells found in the lavage fluid. Although no evidence has been presented to exclude the possibility that cell trafficking may occur through other routes, given the scale of the response seen in the omentum, plus the absence of a mesothelial cell or basement membrane barrier and the fact that it is also the principal site of plasma extravasation, it seems likely that the omentum is the major, or perhaps the only, site of leukocyte traffic in peritonitis.

The data described here extend the range of roles attributable to the omentum. It has been shown by others to respond to peritoneal injury and infection by migrating to the involved area, enclosing and isolating it from other peritoneal tissues [31, 32]. In addition, it has been suggested that peritoneal resident macrophages originate in the milky spots of the omentum [29]. Although the efflux of fluid and cells from the peritoneal cavity most likely occurs via the draining lymphatics [33], clearance of particulate materials from the peritoneal cavity involves phagocytosis by omental macrophages. As reported here, large numbers of zymosan A particles were seen in omental macrophages and similar labeling of omental macrophages was seen with colloidal carbon [34] and radioactive latex beads [35].

Structures similar to the omental milky spots have been reported in the pleura [36, 37], pericardium [38] and meninges [39, 40], suggesting that the flux of extravasated plasma and leukocytes into body cavities commonly

occurs through such specialized regions. In our study of vascular labeling in the inflamed pleural cavity, i.v. Monastral Blue labelled only the milky spots in the mediastinal pleura, demonstrating the functional similarity of these milky spots to those found in the greater omentum (data not shown). In contrast, vascular leak occurred throughout the synovial surface of the inflamed rat knee, an observation consistent with its structure which resembles that of milky spots throughout its entire interface with the synovial cavity, i.e., a discontinuous basal lamina and aggregates of macrophages (type A synoviocytes). Recognition of the key role of specialized structures such as the milky spots in the inflammatory response in body cavities will allow studies of pathogenic mechanisms (e.g., adhesion molecule expression, regulation of blood flow, endothelial gap formation, leukocyte extravasation) and therapeutic agents to focus on the relevant tissues.

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