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Localized Gut-Associated Lymphoid Tissue Hemorrhage Induced by Intravenous Peptidoglycan-Polysaccharide Polymers

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A hemorrhage into gut-associated lymphoid tissue developed as early as 3 min after the intravenous injection of group A streptococcal peptidoglycan-polysaccharide polymers into rats. Extravasated erythrocytes were specifically located in the lamina propria and organized lymphoid follicles of the intestines and mesenteric lymph nodes and did not occur in the lungs, kidneys, liver, spleen, adrenal glands, or submandibular and popliteal lymph nodes, as determined by gross and histologic observations and measurement of radiolabeled erythrocytes. Petechial hemorrhage was preferentially located within the intestine to the distal ileum, Peyer's patches, and lymphoid aggregates of the colon. The hemorrhage was transient and occurred in a dose-dependent fashion. It was maximal 5 min after injection and resolved completely by 3 days. A unique feature of this altered vascular permeability was the absence of polymorphonuclear leukocytic infiltration, edema, vasculitis, and tissue necrosis.

Increased vascular permeability is one of the earliest manifestations of the acute inflammatory response initiated by a variety of agents (3, 10). Under most experimental conditions, increased vascular permeability and congestion are accompanied by the accumulation of polymorphonuclear leukocytes (PMN) and interstitial fluid, followed by vasculitis and extravasation of erythrocytes (RBC), with or without tissue necrosis (15). Biochemical mediators implicated in the enhanced vascular permeability of acute inflammation include complement, vasoactive amines, oxygen radicals, prostaglandins, leukotrienes, and kinins (20, 26, 30). Few studies of microvascular alterations in intestinal inflammation have been done. Increased vascular and mucosal permeability occurs during intestinal anaphylaxis (16) and ischemia (24), and we have demonstrated hemorrhage and edema in the early phase of intestinal inflammation induced by the intramural injection of purified peptidoglycan-polysaccharide polymers (PG-APS) isolated from group A streptococcal cell walls (28). Sterile PG-APS can initiate acute and chronic inflammation not only at the site of local injection but also in distant organs, such as joints in rats (5, 29) or the heart in mice (23), after parenteral injection. The mechanism of location of injury to specific organs after systemic PG-APS injection is not known.

While investigating the localization of antigen after the intravenous (i.v.) injection of PG-APS, one of us (S.K.A.) observed a hemorrhage in the intestine and mesenteric lymph nodes (LN). In this paper we describe a transient hemorrhage confined to the gut-associated lymphoid tissue (GALT) and occurring as early as 3 min after the i.v. injection of PG-APS polymers. A unique feature of this derangement of vascular permeability was the absence of PMN infiltration, edema, and tissue necrosis. In this paper we define GALT broadly to include the lymphoid population of the loose connective tissue of the lamina propria, organized lymphoid nodules of the small intestine (Peyer's patches) and colon, and mesenteric LN (12).

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MATERIALS AND METHODS

Animals. Female outbred Sprague-Dawley rats with a mean weight of 150 g at the time of injection were obtained from Zivic Miller Laboratory (Allison Park, Pa.). Female inbred Lewis rats with a mean weight of 145 g were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, Mass.) and used for the ^{51}Cr-labeled RBC study so that a syngeneic source of RBC could be used. Rats were fed Purina rat chow ad libidum and housed together, with two or three animals per cage.

Cell wall preparation. PG-APS polymers were isolated from the cell walls of group A, type 3, strain D58 streptococci (*Streptococcus pyogenes*) as previously described (8). Briefly, streptococci were grown in Todd-Hewitt broth (BBL Microbiology Systems, Cockeysville, Md.), collected by centrifugation, and washed with sterile phosphate-buffered saline. Cells resuspended in phosphate-buffered saline were disrupted in a Braun MSK shaker (Bronwill Scientific Inc., Rochester, N.Y.). Intact cells were removed by centrifugation at 2,000 × *g* for 30 min, and the cell walls were collected by centrifugation at 10,000 × *g* for 30 min. The crude cell wall preparation was sequentially treated with 0.025% RNase, 0.025% trypsin, and 0.020% papain, extracted with chloroform-methanol, washed, and lyophilized. Cell wall fragments were prepared by subjecting 400 mg of purified cell wall suspended in 20 ml of phosphate-buffered saline or pyrogen-free saline to 70 min of ultrasonic vibration in a model 350 sonifier (Branson Sonic Power Co., Danbury, Conn.) and then centrifugation at 10,000 × *g* for 30 min to remove large particles. Immediately before injection, the cell walls were diluted to appropriate concentrations in phosphate-buffered saline or pyrogen-free saline and sonicated for 3 min with a 9-kc sonic oscillator (Raytheon Co., Waltham, Mass.) to disperse aggregates. Aseptic techniques were followed, and sterility was confirmed by culturing 0.1 ml on sheep blood agar. Cell wall fragments prepared by this method are composed of PG-APS polymers with molecular weights ranging from 5×10^6 to 5×10^8 (8). The purity of the cell wall preparations was assessed by amino acid, amino

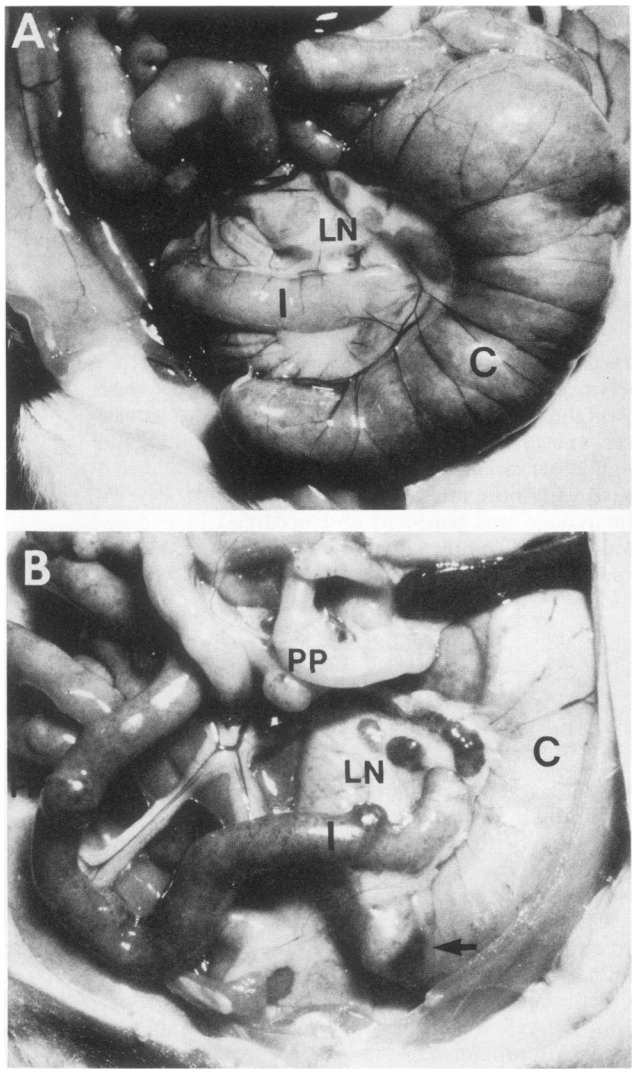

FIG. 1. (A) Gross appearance of a control rat injected with pyrogen-free saline. No hemorrhage is seen in the distal ileum (I), cecum (C), or mesenteric LN (LN). (B) Petechial hemorrhage of the distal ileum (I) in a rat injected i.v. with 15 μg of PG-APS per gbw 5 min before necropsy. The Peyer's patches (PP) of the small intestine are preferentially involved. Confluent hemorrhage is present in the lymphoid aggregate at the tip of the cecum (arrow) and the mesenteric LN (LN). No hemorrhage is seen in the parenchyma of the cecum (C).

sugar, and neutral sugar analyses (8). Rhamnose was assayed by the method of Dische and Shettles (7).

Radiolabeled-RBC preparation. EDTA-anticoagulated cardiac blood (11 ml) was obtained from two inbred Lewis rats, and RBC were separated from plasma by centrifugation at 500 × *g* for 10 min. The RBC were incubated with 500 μCi of $Na_2{}^{51}CrO_4$ (New England Nuclear Corp., Boston, Mass.) for 30 min at room temperature and washed three times with phosphate-buffered saline (pH 7.1). ^{51}Cr-labeled RBC were diluted with phosphate-buffered saline to a specific activity of 0.82 μCi/ml and injected within 30 min.

Experimental procedures. To investigate the gross and microscopic responses at different times, we injected 32 Sprague-Dawley rats i.v. with 15 μg of PG-APS per g of body weight (gbw). PG-APS suspended in pyrogen-free saline were diluted so that a final volume of 0.4 ml was injected into a tail vein of each rat. Groups of four rats were sacrificed with ether at 5 min, 1.5 h, 4 h, 8 h, 1 day, 3 days, 7 days, and 14 days after injection. Groups of two control rats were sacrificed at 5 min, 1.5 h, 24 h, and 14 days after i.v. injection of 0.4 ml of pyrogen-free saline. At necropsy the intestines, the mesenteric, popliteal, and submandibular LN, and the solid organs of the abdominal and thoracic cavities were examined and scored grossly, and specimens were collected and fixed in 10% Formalin. Paraffin-embedded sections were stained with hematoxylin and eosin (HE) and by the periodic acid-Schiff digest method.

To document selective vascular permeability, we injected 13 Lewis rats i.v. with 0.5 ml of ^{51}Cr-labeled RBC (0.41 μCi). After 30 min five rats received an i.v. tail vein injection of 15 μg of PG-APS per gbw, and eight control rats received an equal volume of phosphate-buffered saline. Rats were sacrificed with ether at 30 min after injection of PG-APS or saline. While the heart was still beating, the right ventricle of each of the PG-APS-injected and of five of the eight saline-injected rats was pierced with an 18-gauge needle and cannulated with sharpened polyethylene tubing, allowing the blood to flow by gravity into collecting tubes. The left ventricle was cannulated with an 18-gauge needle attached to standard i.v. infusion tubing with sterile 5% glucose in water (D5W; Travenol Laboratories, Deerfield, Ill.) with heparin (1 U/ml). The animals were perfused with the heparinized D5W until the liver became pale brown and perfusate from the right ventricle was clear. Tissues were then harvested and weighed, and the amount of ^{51}Cr was determined with a gamma counter (1197 series; Automatic Gamma Counting System; Searle Analytic Inc., Des Plains, Ill.).

Scoring criteria. At necropsy the jejunum, jejunal Peyer's patches, ileum, ileal Peyer's patches, cecum, ascending colon, rectum, and mesenteric LN were grossly scored on a 0 to 4+ scale in a coded fashion. The following criteria were used: 0, no hemorrhage seen; 1+, scattered petechiae; 2+, frequent but isolated petechiae; 3+, more than 50% of the surface hemorrhagic; and 4+, confluent hemorrhage. The maximal gross intestinal hemorrhagic score was 28 per animal. Coded slides of the jejunum, ileum, cecum, ascending colon, and rectum were similarly evaluated for a microscopic hemorrhagic score on a scale of 0 to 4+. The following criteria were used: 0, no extravasation of RBC; 1+, scattered RBC outside blood vessels; 2+, frequent RBC; 3+, small areas of confluent RBC; and 4+, large areas of confluent RBC. Each layer of the intestine (epithelium, lamina propria, submucosa, muscularis, serosa, and mesentery) and lymphoid aggregates were graded, so that the maximal microscopic score for each animal was 140.

Statistical evaluation. The sum of the hemorrhagic scores for all intestinal specimens was computed for each animal. The mean gross or microscopic hemorrhagic score or both and tissue counts per minute per gram were calculated for each group of rats and compared by Student's unpaired *t* test. Gross and microscopic hemorrhagic scores and tissue counts per minute per gram were correlated by linear regression analysis.

RESULTS

Gross observations. Rats injected with 15 μg of PG-APS per gbw developed petechial hemorrhages in the small intestines, with the distal ileum and Peyer's patches being most prominently involved (Fig. 1). In contrast to the small intestine, which had petechiae visible on the serosal (external) surface of the intestine as well as the Peyer's patches,

hemorrhages in the large intestines were confined to lymphoid aggregates of the cecum, ascending colon, and rectum. These lymphoid aggregates were normally not easily seen but became strikingly visible when outlined by the hemorrhage. The distal ileum was most reproducibly involved, with 19 of 20 rats sacrificed within 24 h after injection demonstrating a hemorrhage, compared with only 6 of 20 rats developing a jejunal hemorrhage. The mesenteric LN developed a petechial, stellate, or diffuse hemorrhage in 19 of 20 rats sacrificed within 24 h after injection with PG-APS. Although the intensity of the hemorrhage in the intestines and mesenteric LN correlated in most animals, three rats with a marked intestinal hemorrhage had limited mesenteric LN involvement. Rats examined 72 h or more after injection showed no evidence of intestinal or mesenteric LN hemorrhage. Only 3 of 32 PG-APS-injected rats developed a hemorrhage in the submandibular (1 rat), mediastinal (1 rat), or popliteal (1 rat) LN. Petechiae were not seen in the lungs, adrenal glands, skin, liver, spleen, or kidneys. An occasional hemorrhage was seen in the thymus at all times. There was no evidence of edema of the intestines or extremities. Eight saline control rats had no intestinal or mesenteric LN petechiae, although one rat had a hemorrhagic thymus and one rat had a hemorrhage in a submandibular LN.

In a separate experiment, ether-anesthetized rats whose intestines were exposed by laparotomy were observed for the appearance of hemorrhage. Petechial hemorrhage appeared in the intestines and mesenteric LN simultaneously as early as 3 min after the i.v. injection of PG-APS.

Microscopic observations. In tissues obtained from rats sacrificed 24 h or less after PG-APS injection, extravasated RBC were located in the lamina propria (Fig. 2) and lymphoid aggregates (Fig. 3) of the intestines. In the small intestines the hemorrhage was frequently found in the lamina propria away from the Peyer's patches, but in the colon the hemorrhage usually occurred adjacent to a lymphoid aggregate. Submucosal RBC extravasation was unusual except at the base of organized lymphoid tissue and never occurred without an adjacent lamina proprial hemorrhage. RBC were frequently seen between crypt epithelial cells (Fig. 4) but were very rarely seen within the lumen of the intestine. There was no evidence of edema, PMN infiltration, or an increased number of lymphocytes, macrophages, or eosinophils in the areas of hemorrhage. Only 1 of 32 PG-APS-injected rats had any evidence of vasculitis in the intestinal or mesenteric blood vessels. There was no evidence of fibrin accumulation or intravascular clotting. Extravasated RBC were found in focal areas of the mesenteric LN of the PG-APS-injected animals. RBC were most commonly found within medullary sinusoids but also frequently occurred within the parenchyma of the cortex and within the subcapsular sinuses. RBC were occasionally seen within afferent lymphatics. A common finding within mesenteric LN was the aggregation of RBC around macrophages (Fig. 5). RBC appeared to be within macrophages in some sections, possibly representing erythrophagocytosis. Sections from animals sacrificed 72 h or more after injection demonstrated almost no evidence of an intestinal or mesenteric LN hemorrhage. Control animals failed to show any significant intestinal or mesenteric LN hemorrhage at any time point. No hemorrhages were seen in the liver, spleen, kidneys, adrenal glands, lungs, or popliteal LN in either group of animals, but occasional focal hemorrhages were seen in the submandibular LN and thymus in both the PG-APS-injected and control rats.

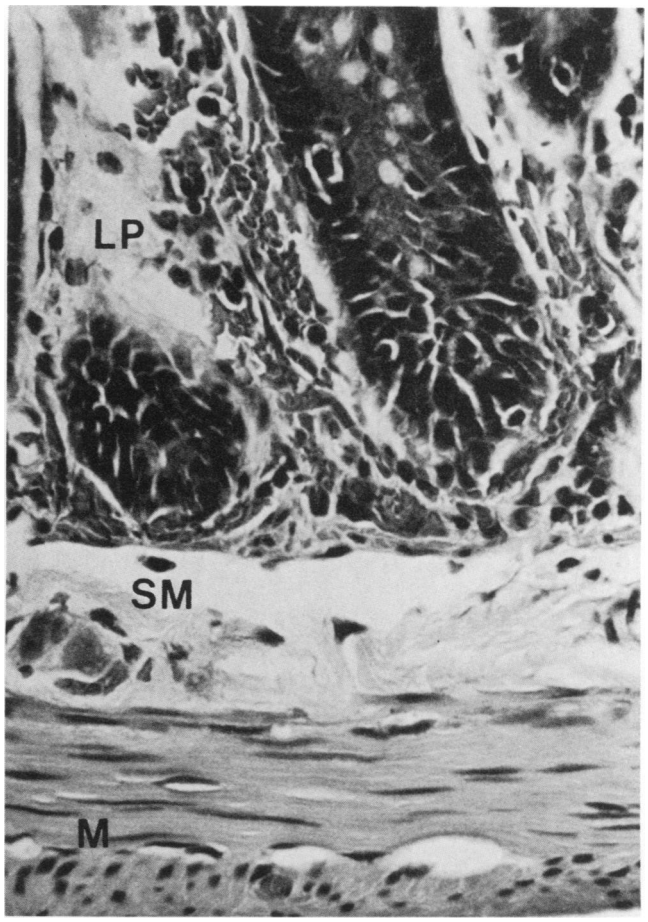

FIG. 2. Photomicrograph of the distal ileum of a rat sacrificed 90 min after the i.v. injection of 15 μg of PG-APS per gbw. Extravasated RBC are confined to the lamina propria (LP) of the ileum and surround the longitudinally sectioned crypt. PMN and edema are not present, and no hemorrhage is seen in the submucosal (SM) or muscle (M) layers. HE stain. Magnification, ×450.

Quantitation of hemorrhages. The gross and microscopic hemorrhagic scores decreased at similar rates (Fig. 6) and had a high correlation ($r = 0.87$, $P < 0.0001$). The gross hemorrhagic score also correlated closely with the counts per minute per gram for each area of the intestine in the ^{51}Cr-RBC-injected rats (Fig. 7; $r = 0.86$). The gross and microscopic hemorrhagic scores slowly decreased from 5 min to 24 h after PG-APS injection and were no different from those in controls 3 days or more after injection (Fig. 6). In our subjective system, the ileum had the highest gross and microscopic hemorrhagic scores, followed by the cecum, ascending colon, rectum, and jejunum.

Dose response. The gross hemorrhagic score progressively increased after the injection of increasing amounts of PG-APS from 0.3 to 6 μg/gbw (Fig. 8). An injection of more than 6 μg/gbw produced no further increase in the total hemorrhagic score, although the colon appeared to be more responsive to high doses (Fig. 8).

Localization of hemorrhages to intestines and mesenteric LN. To confirm the gross and microscopic observations that hemorrhages were specifically localized to the intestines and mesenteric LN, we measured the extravasation of ^{51}Cr-labeled RBC after the i.v. injection of PG-APS or phosphate-buffered saline. The circulatory system was perfused with

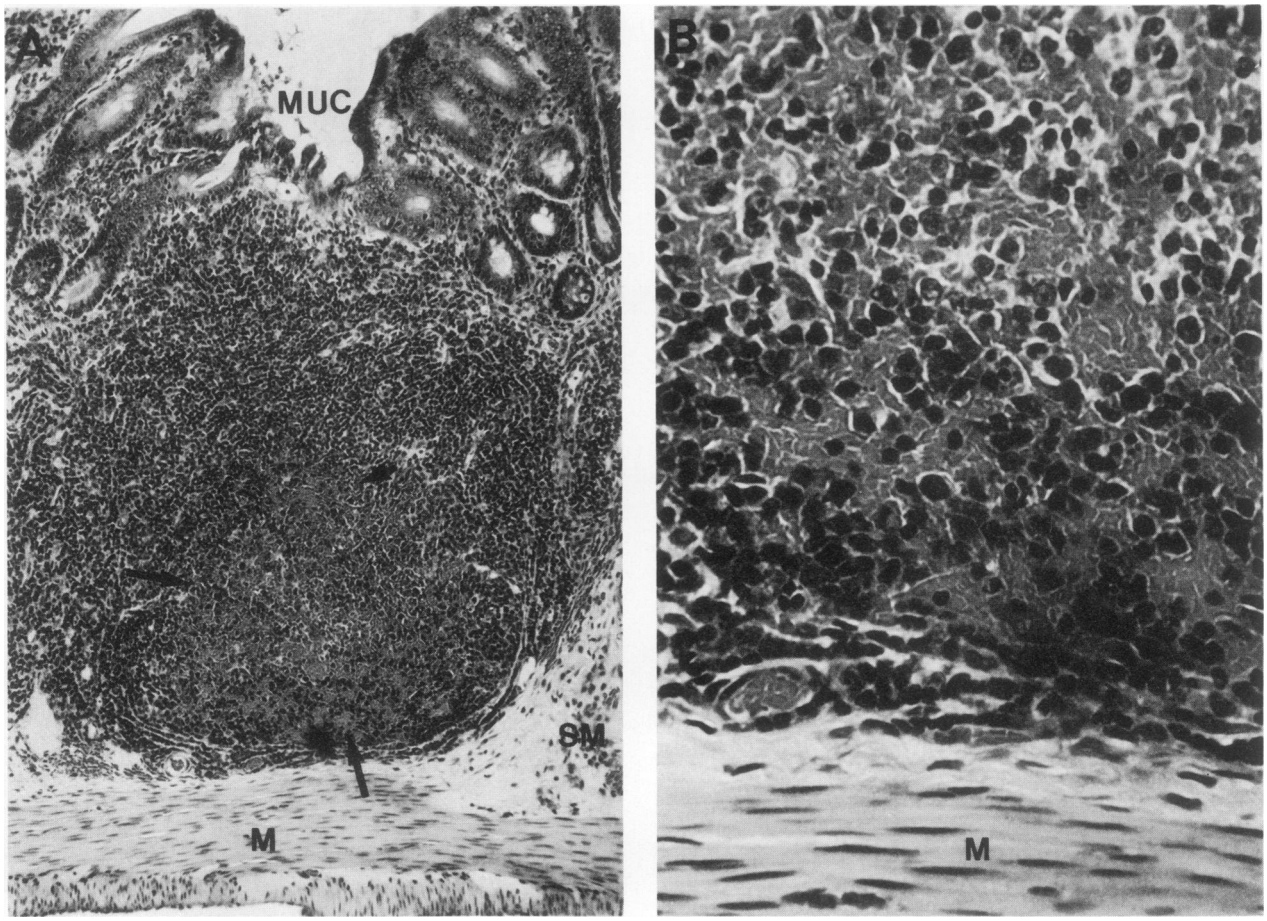

FIG. 3. (A) Hemorrhage confined to an organized lymphoid aggregate at the tip of the cecum 5 min after the i.v. injection of 15 μg of PG-APS per gbw. Amorphous pale areas (arrows) represent RBC. No hemorrhage is seen in the muscle (M), submucosa (SM), or mucosa (MUC) of the cecum. HE stain. Magnification, ×100. (B) Higher magnification of the hemorrhage within the same cecal lymphoid aggregate as that shown in panel A. Confluent extravasated RBC lie between lymphocytes. M, muscle. HE stain. Magnification, ×450.

D5W to clear radiolabeled RBC from blood vessels, so that RBC extravasation from blood vessels could be measured independently of vascular congestion. One saline-injected animal was excluded because of difficulty in perfusing owing to blood clotting. The jejunum, ileum, cecum, ascending colon, and mesenteric LN of the PG-APS-injected rats had significantly higher counts per minute per gram than those of the control rats (Table 1). The 5-cm jejunal and ileal segments each contained at least one Peyer's patch, and the ascending colon, rectum, and tip of the cecum each contained a large lymphoid nodule. There were no differences in the solid organs or extraintestinal lymphoid tissues examined, except for the liver and adrenal glands, which had higher counts per minute per gram in the control group. Although the difference in the adrenal gland counts appeared to be impressive because of low tissue weight, the actual counts were only slightly above the background (45 and 3 cpm for the control and PG-APS groups, respectively). Perfusion of the intestines was comparable in the two groups, as indicated by the slightly lower counts per minute per gram of the grossly normal ileum in the PG-APS-injected rats than in the control rats. The hemorrhagic (distal) portion of the ileum had higher values than the grossly normal ileum in the PG-APS-injected group ($P < 0.0001$). The mean counts per minute per gram of the distal ileum, cecal tip, and ascending colon in the PG-APS-injected rats perfused with D5W were even higher than those in the three control rats that were not perfused, indicating that locally defective perfusion was not responsible for the increased counts. This experiment confirmed a pilot study with similar results.

DISCUSSION

Increased vascular permeability is one of the earliest and most consistent features of the acute inflammatory response (3, 10, 30). In our model grossly apparent hemorrhages occurred in the lamina propria and lymphoid nodules of the intestines and mesenteric LN (GALT) as early as 3 min after the i.v. injection of PG-APS, with maximal hemorrhage present at 5 min. Extravasation of RBC within the GALT after i.v. PG-APS injection differed from previously described hemorrhagic inflammatory models in several important respects. First, it was a transient phenomenon that completely resolved by 72 h after injection, with no evidence of PMN infiltration, edema, thrombosis, tissue necrosis, or vasculitis. Thus, it is not a localized Schwartzman reaction (22), Arthus reaction (4), or Auer phenomenon (13), in which perivascular PMN infiltration and subsequent vasculitis accompany vascular permeability changes. Similarly, the GALT hemorrhage model differed from previously described inflammation induced by local or parenteral PG-APS

injection, in which acute edema, hemorrhage, PMN infiltration, and fibrinoid necrosis evolve into chronic, relapsing granulomatous inflammation (5, 28, 29). Second, the hemorrhage was specifically localized to the GALT, as determined by gross and microscopic examinations and with radioisotope-labeled RBC without prior intestinal manipulation or injury. In the Shwartzman reaction an area is primed by the local injection of endotoxin before i.v. endotoxin injection (22). The Arthus reaction occurs when antigen is instilled locally after systemic immunization (4), and in the Auer reaction circulating immune complexes are deposited at a site after local injury has occurred (13). The lungs and kidneys, which are particularly susceptible to circulating toxins (6) and immune complexes (11), showed no evidence of RBC leakage or injury. It is possible that the intestine was selectively involved because it was primed by mucosal absorption of cell wall components from the normal luminal bacteria of the distal small intestine and colon. Alternate explanations may relate to specific intestinal effector cells, endothelial receptors, or vascular morphology. For example, intestinal mast cells respond to different stimuli than do mast cells recovered from other tissues (18). Intestinal venules have been postulated to have endothelial receptors that are important in immunoglobulin A lymphocyte homing (1). Similar intestinal endothelial receptors may allow the recognition of circulating RBC which have membrane-bound antigen or complement. Postcapillary venules of Peyer's patches differ morphologically from mucosal vessels. They contain high columnar endothelial cells that permit the leakage of colloidal carbon and ferritin much more readily than do intestinal mucosal venules, whereas mucosal venules leak more carbon than do vessels in the muscularis or serosa (9).

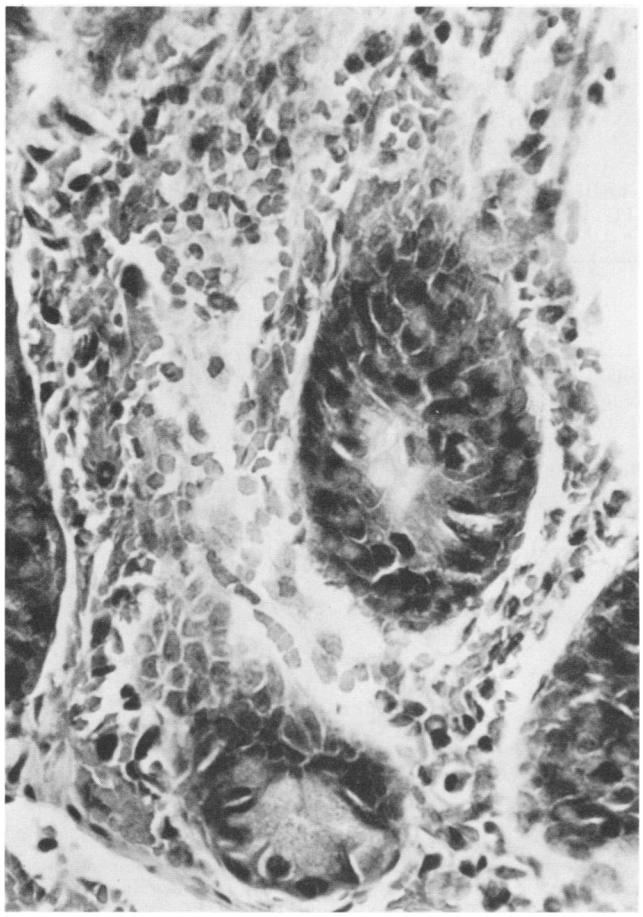

FIG. 4. Photomicrograph of the distal ileum of a rat sacrificed 90 min after the i.v. injection of 15 μg of PG-APS per gbw. RBC are present within the epithelium of the central crypt in addition to the surrounding lamina propria. HE stain. Magnification, ×450.

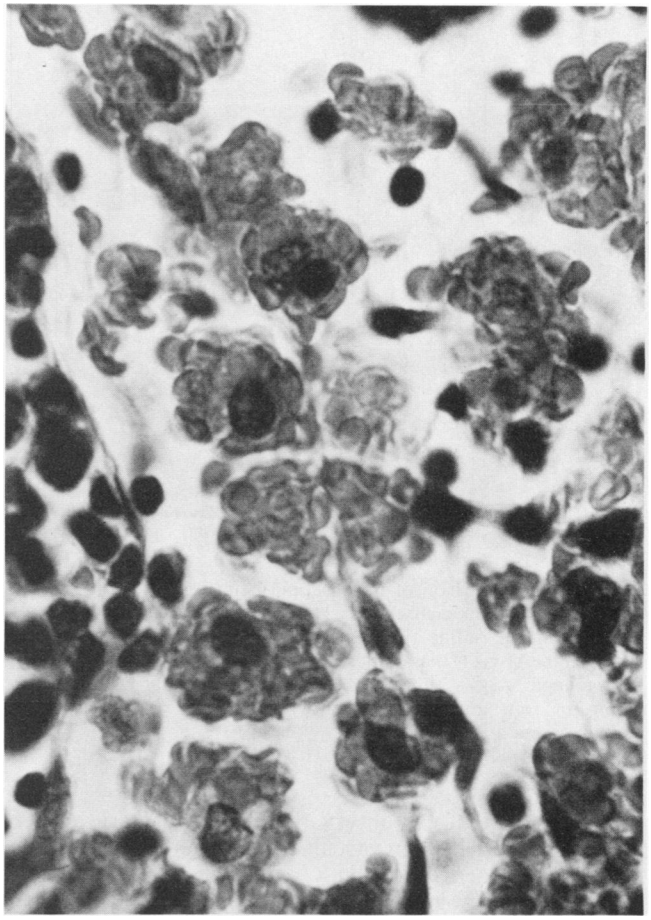

FIG. 5. RBC adherent to macrophages within the sinusoid of a mesenteric LN 4 h after the i.v. injection of 15 μg of PG-APS per gbw. Some RBC appear to be within the cytoplasm, indicating erythrophagocytosis. HE stain. Magnification, ×1,000.

RBC consistently appeared within the cortex and medullary sinuses of the mesenteric LN but only rarely in popliteal or submandibular LN. Evidence supporting a specific extravasation of RBC within the mesenteric LN rather than a passive accumulation from intestinal hemorrhage is as follows: (i) discrepancies occurred between the amount of hemorrhage in the intestines and mesenteric LN, (ii) mesenteric LN hemorrhage occurred simultaneously with intestinal petechiae, and (iii) RBC were not routinely seen in the afferent lymphatics and subcapsular sinuses.

The hemorrhage was not only specifically localized to the GALT but was also preferentially located within different areas of the intestine. The distal small intestine (distal ileum) was hemorrhagic more frequently than was the jejunum (95 versus 30%). The hemorrhage occurred diffusely in the lamina propria of the ileum as well as within Peyer's patches but, in the colon and cecum, the hemorrhage was confined to the lymphoid aggregates.

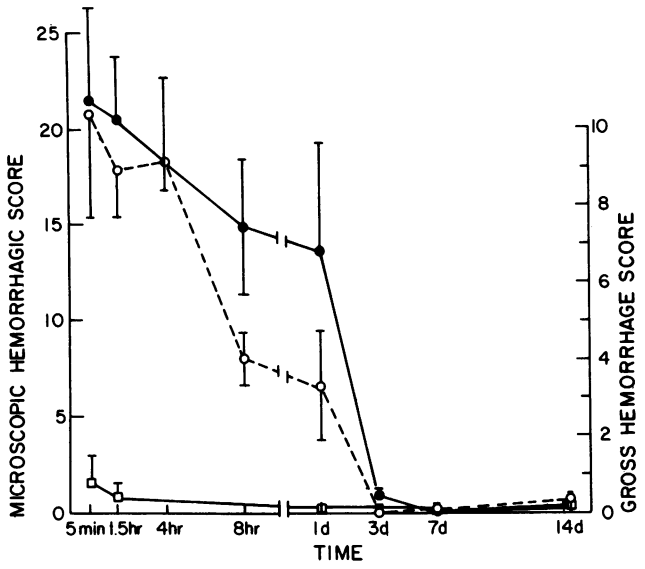

FIG. 6. Comparison of microscopic hemorrhagic scores of rats killed at various times after the i.v. injection of 15 μg of PG-APS (●) or pyrogen-free saline (□) per gbw. There is a close correlation between the gross (○) and microscopic (●) hemorrhagic scores. The hemorrhagic scores represent the mean ± standard error of the mean for four animals injected with PG-APS and two control animals at each time. d, Day(s).

We believe that our observations are a result of a selective, transient change in vascular permeability leading to the extravasation of RBC in the GALT rather than a regional redistribution of blood flow induced by PG-APS. Petechial hemorrhages were not connected with visible blood vessels, did not disappear when touched, and remained after vascular perfusion with D5W. The predominent histological findings were extravasation of RBC rather than vasodilation and

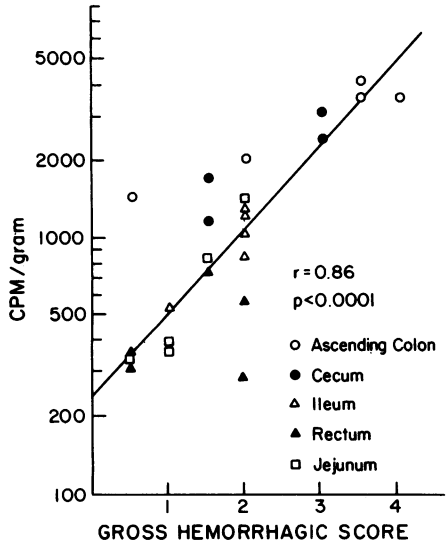

FIG. 7. Linear regression analysis of the gross hemorrhagic score and counts per minute per gram of intestinal segments of rats sacrificed 30 min after the injection of 15 μg of PG-APS per gbw and ^{51}Cr-labeled RBC. Each point represents an intestinal segment from each of five rats.

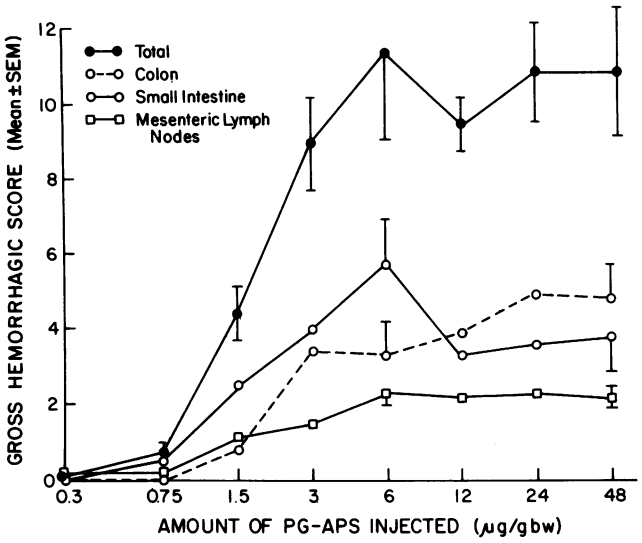

FIG. 8. Dose response of PG-APS in rats sacrificed 30 min after i.v. injection. Each point represents the mean ± standard error of the mean (SEM) for five rats, and the total score (●) is a sum of the scores of the small intestine (○——○), colon (○----○), and mesenteric LN (□).

^{51}Cr-labeled RBC remained in the hemorrhagic intestines and mesenteric LN after vascular perfusion. There was no morphological evidence of selective vasoconstriction or ischemia and no mortality, suggesting that shock was not present.

TABLE 1. Specificity of hemorrhages after the i.v. injection of PG-APS or phosphate-buffered saline (controls) measured as the amount of ^{51}Cr-labeled RBC in various tissues

Organ	cpm (mean ± SD)/g of tissue[a]		P value
	Control rats (n = 4)	PG-APS-treated rats (n = 5)	
Blood	86,020 ± 6,930	84,570 ± 4,630	NS[b]
Jejunum	197 ± 65	669 ± 478	<0.05
Ileum (normal)	361 ± 445	149 ± 56	NS
Ileum (hemorrhagic)[c]	349 ± 193	1,012 ± 331[d]	<0.005
Ileal contents	109 ± 122	85 ± 180	NS
Cecal tip	255 ± 205	1,848 ± 998	<0.01
Ascending colon	124 ± 25	2,955 ± 1,135	<0.0001
Rectum	270 ± 273	449 ± 195	NS
Mesenteric LN	237 ± 100	736 ± 248	<0.0005
Popliteal LN	876 ± 534	950 ± 1,889	NS
Submandibular LN	243 ± 150	175 ± 175	NS
Thymus	147 ± 57	317 ± 188	NS
Lungs	3,220 ± 750	4,585 ± 2,395	NS
Liver	1,525 ± 512	984 ± 232	<0.05
Spleen	13,861 ± 1,616	12,165 ± 1,482	NS
Kidneys	5,457 ± 577	4,653 ± 1,240	NS
Adrenal glands	1,412 ± 453	111 ± 129	<0.005
Joints	457 ± 454	356 ± 273	NS
Skin	186 ± 82	114 ± 60	NS
Muscle	282 ± 54	225 ± 46	NS

[a] The rats were sacrificed 30 min after the i.v. injection of the test suspension and 1 h after the injection of ^{51}Cr-RBC. Rats were perfused with 5% D5W to clear the vascular system of ^{51}Cr-RBC.

[b] NS, Not significant.

[c] In control animals, the terminal ileum, corresponding to the usual area of grossly evident hemorrhage in PG-APS animals, was harvested.

[d] $P < 0.0001$ as compared with grossly normal ileum from PG-APS-injected rats.

Biochemical mediators which enhance vascular permeability include complement, vasoactive amines, prostaglandins, leukotrienes, and kinins (20, 26, 30). Although the mechanism of GALT hemorrhage induced by PG-APS is unknown, complement fixation may be important. RBC were frequently observed to be surrounding and sequestered within macrophages of the mesenteric LN (Fig. 5). This finding is similar to the in vitro aggregation of phagocytic cells to RBC via PG-APS–complement (C3b) bridging (25). PG-APS activates the alternate complement pathway in vivo (17), and endothelial cells are capable of binding complement components (19), particularly after injury (27). However, endotoxin also activates the alternate complement pathway and produces intravascular RBC clumping and adherence of RBC to damaged epithelium (21), but i.v. doses up to 100 μg do not induce intestinal hemorrhages (R. B. Sartor, manuscript in preparation). PG-APS has recently been demonstrated to induce the production of prostaglandin E_2 and leukotriene B_4 in rats (31), suggesting that these vasoactive substances could produce GALT hemorrhages.

The ability to induce inflammation and vascular permeability changes in remote organs is an interesting feature of systemic PG-APS administration. PG-APS in a sterile aqueous suspension can induce chronic, relapsing erosive arthritis in rats after intraperitoneal or i.v. injection (5, 29). Chetty et al. (2) described transient edema of the extremities of rats induced by the i.v. or intraperitoneal injection of group A streptococcal polysaccharide or small fragments of PG-APS (molecular weight, 4×10^3 to 5×10^4). No hemorrhage was evident in the extremities or intestines, there was no increased vascular permeability within the ascending colon and mesenteric LN, and the edema resolved within 4 h. As in our model of GALT hemorrhage induced by PG-APS polymers of molecular weight 5×10^6 to 5×10^8, no PMN infiltration was seen. The reason for the different localization and characteristics of vascular permeability changes with different-sized PG-APS polymers is unknown.

The pathophysiologic significance of the selective alteration of vascular permeability in the GALT in response to i.v. PG-APS injection is unknown. It is possible that transient increased vascular permeability may result in the selective deposition of circulating antigen or enhanced mucosal absorption of luminal antigen and toxins in certain areas of the gut. The accumulation of antigen or toxins could then lead to more protracted inflammation in these specific areas of the intestine. Thus, one could postulate that this phenomenon might be important in the pathogenesis of Crohn's disease, which involves the distal ileum and colon (14). Certainly, further study of this model could improve our understanding of specific intestinal endothelial receptors or effector cell, e.g., mast cell, function.

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