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Distribution of Small Intestinal Lesions in Anaphylaxis of Rats

Key Words

Anaphylaxis
Small intestine

Abstract

Hemorrhage and congestion were not uniformly distributed along the small intestine during systemic anaphylaxis in rats. Duodenum had little hemorrhage and congestion and the terminal ileum had even less. Maximum involvement occurred in jejunum of 13% of rats, in ileum of 47%, and in both jejunum and ileum of 40%. Sequential scoring along the entire length of small intestine revealed different patterns of distribution with more than one peak of intensity in some of the rats. Within a lesioned area, banding was common except where hemorrhage and congestion were so severe as to obliterate all patterns. Banding represented a gradient of injury with respect to the vascular supply. The pale stripes (less severe hemorrhage) contained the penetrating vessels derived from the terminal mesenteric arcades. The dark stripes (more severe hemorrhage) did not contain such vessels. In addition, the mesenteric side of the intestine was less affected than the antimesenteric side. This constituted a second gradient that might also be related to the distance from the vascular supply. In both instances, proximity to larger blood vessels had a protective effect. These two gradients and the variable sites and patterns of distribution have not been described previously in intestinal anaphylaxis. Gradients and the variability of the distribution of lesions in intestinal anaphylaxis should be considered in experiments on pathogenesis and altered function.

Introduction

The small intestine is a prominent site of congestion, edema and hemorrhage during anaphylaxis in rats. The various parts of the normal small intestine differ in gross and microscopic structure, in function, in luminal content and in distance from major arteries and veins. Nevertheless, little has been recorded on the distribution of gut lesions in systemic anaphylaxis. The distribution of lesions has been described as 'complete peppering' but 'most extensive in the upper intestinal tract' [1], 'patches' (early) and 'whole alimentary canal' (late) [2], along the whole small intestine or some fraction thereof or scattered small areas [3]. Most investigators have not specified the sites of lesions. Many

studies involve sampling of intestinal tissue without any assurance that the sample is representative of the whole. In view of the sophisticated methodology currently applied to functional studies of intestinal anaphylaxis, it seemed appropriate to examine more closely the distribution of lesions using the unsophisticated but nonetheless essential methods of macroscopic and microscopic observations.

Materials and Methods

Lewis rats, 7–10 weeks old, were kept in hanging shoe-box-type cages with hardwood bedding and Purina Formulab Chow 5008 and tap water freely available. The rats were sensitized to chicken ovalbumin (0.01 mg) in eleven experiments and to mouse serum (0.001 ml),

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azovalbumin (2 mg) and casein (sodium salt, 10 mg) in one experiment each. The antigen dissolved in 0.1 ml saline was mixed with Alhydrogel (3% aluminum hydroxide adjuvant, Superfos Biosector, Vedbaek, Denmark; 0.1 ml) and injected by subplantar route in the anesthetized rat's right hindfoot. In three of the ovalbumin experiments, the Alhydrogel was omitted. Three fourths of the rats also received pertussis vaccine (2×10^{10} organisms) in the dorsum of the same foot. Thirteen days later, the rats were challenged with 10 mg of ovalbumin or azovalbumin, 100 mg casein, or 0.1 ml mouse serum intravenously in male rats and by subplantar route in female rats [for other details, see 4–6].

Anaphylaxis caused prostration and cyanosis that appeared within 5–60 min. The present report concerns necropsies on 140 rats that died or were killed after challenge. The small intestine was examined in entirety immediately after death. Among the rats with severe, fatal anaphylaxis, 33% died within 15 min after challenge, 35% died in the second quarter hour, 10, 9, 3 and 3% died in the third, fourth, fifth and sixth quarter hours, and 7% after the sixth quarter hour. Rats with milder, nonlethal anaphylaxis were killed by inhalation of carbon dioxide at 90 min, 2 and 3 h after challenge; their symptoms had reached a peak and started to subside. The lesions of anaphylaxis were scored sequentially along the entire length of the small intestine of every rat by one observer (SL). A score of 1+ represented just detectable congestion while 4+ indicated dark red or purple discoloration of the gut wall caused by intense hemorrhage and congestion; 2+ and 3+ represented intermediate grades. In this manner, increase and decrease in the severity of the lesions along the length of the intestine were recorded as a consecutive series of scores for each rat. The data were recorded separately for duodenum, jejunum, ileum and terminal ileum in that order. The latter was defined as the last 2 cm of ileum. The jejunal-ileal junction was halfway from the ligament of Treitz to the cecum. For presentation in the tables 1 and 2, the data for each rat were studied for the pattern of lesions along the entire length (one, two or three or more peaks of severity) and for the part of small intestine with the highest score. Inasmuch as the scores were subjective, there could be variability from one experiment to another. However, observations of differences in congestion from one part of the gut to another part were reliable, and this report is concerned only with such changes.

For microscopic study, 2-cm-long segments of small intestine were fixed by immersion in 10% formalin or in Bouin's fluid. Blocks were cut in cross sections as well as in the long axis of the gut. Tissues were embedded in paraffin, sectioned and stained with hematoxylin and eosin.

Results

The distribution of congestion and hemorrhage was not uniform or even close to uniform over the length of the small intestine. The lack of uniformity applied to severe lethal anaphylaxis as well as to mild or moderate nonlethal anaphylaxis, regardless of the antigen, adjuvant and route of challenge. Inasmuch as this study concerned distribution of lesions, not severity, all the data were combined for presentation in tables 1 and 2. The duodenum usually had little or no congestion and hemorrhage; the terminal ileum had even less and was most often entirely normal in gross appearance (table 1). The peak severity was always in jejunum and/or

Table 1. Site of maximum lesions in intestinal anaphylaxis of rats

Site of greatest severity	Number of rats
Duodenum	0
Jejunum	18
Ileum	66
Jejunum and ileum	56
Terminal ileum	0
Total	140

These data represent distribution of lesions regardless of severity, so that rats with maximum lesions in jejunum (for example) are listed together regardless of whether that maximum was 1+ or 4+ in severity.

Table 2. Distribution of lesions along entire length of intestines in anaphylaxis of rats

Patterns of lesions	Number of rats
One peak	99
Two peaks	33
Three or more peaks	8
Total	140

These data represent patterns of distribution regardless of severity, so that rats with only one peak (for example) are listed together regardless of whether that peak located in jejunum or ileum, and regardless of whether that peak was 1+ or 4+ in severity.

ileum. The peak of severity was in the jejunum of 13% and in the ileum of 47% of the 140 rats, and the peak extended over both jejunum and ileum of 40% of the rats. Sequential scoring along the entire length of the intestine also revealed different patterns in the distribution of lesions. In 71% of the rats, the lesions increased from proximal to distal, to a single peak of intensity in jejunum or ileum or both, and then the severity decreased further distally. This common pattern occurred in mild anaphylaxis where only part of the small intestine was involved as well as in severe anaphylaxis, where most of the small intestine was affected. In contrast, 24% of the rats had two peaks of severity separated by a normal or less affected zone (table 2). The two peaks were both in jejunum or both in ileum, or one peak was in jejunum and the other in ileum. In 5% of the rats, there was even greater variability: either three distinct peaks with lesser involvement between peaks, or so much alternation of severity that it could only be described as 'patchy'.

Banding was detected in only 20% of the rats even though it was specifically sought in every necropsy. This

1

2

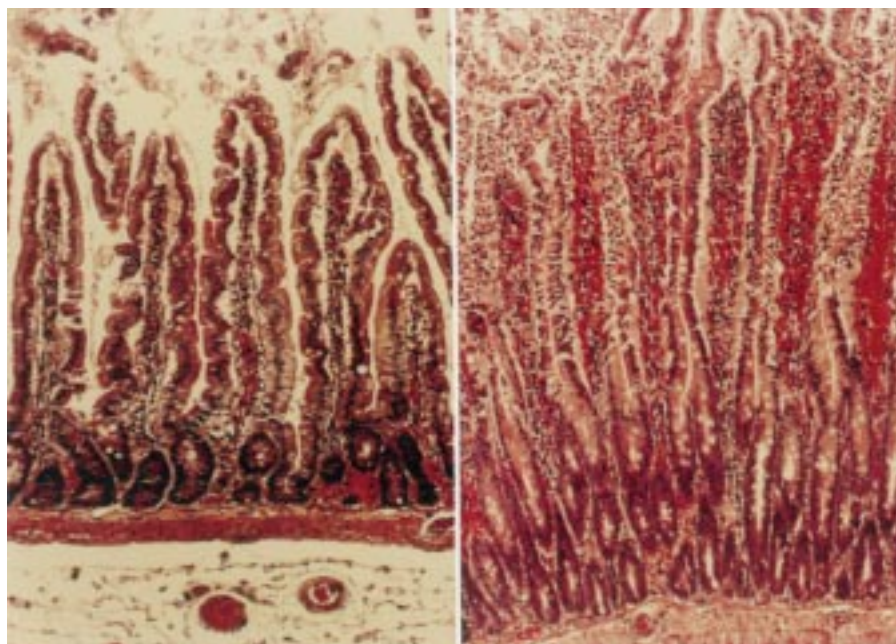


Fig. 1. Longitudinal section of jejunum of rat with severe anaphylaxis. The vessels in the mesenteric fat are congested (bottom of figure) and there are small hemorrhages in the lamina propria but not in the villi. HE. $\times 100$.

Fig. 2. The antimesenteric side of the same segment of jejunum has much more severe lesions with hemorrhage in the villi. HE. $\times 100$.

term refers to a gridiron-like pattern of alternating light and dark lines, each 1–2 mm in width, in the lesions. Banding differed from the variable congestion described above in the narrow width of its component stripes and in its relation to the vascular system. The pale stripe usually contained in its center one of the terminal branches (vasa recta) of the last mesenteric arterial arcade as it entered the gut wall. Banding was not observed in severe lesions; perhaps it was overshadowed by the intense congestion and hemorrhage in such zones. The relatively high incidence of banding in duodenum (6 instances) may be related to the lesser severity of congestion in that part of the gut. Banding was negatively correlated with the use of pertussis vaccine; it occurred in only 5% of rats given pertussis vaccine in contrast to 47% that did not receive this adjuvant. This observation correlates well with the infrequency of banding in severely congested areas, as mentioned above, because pertussis vaccine increased greatly the severity of anaphylaxis [2].

The microscopic study revealed congestion of mucosal vessels with hemorrhages in the lamina propria. Frequently there was an asymmetrical distribution of the lesions. The antimesenteric side was more affected than the mesenteric side (fig. 1, 2). The reverse was not observed.

Discussion

Current literature emphasizes the functional effects of intestinal anaphylaxis as a model for food allergy. The small intestinal tissues selected for such studies have been, for example, the first 15 cm starting from the pylorus [7, 8], 10 or 15 cm of jejunum beginning 5 or 10 cm distal to the ligament of Treitz [9–11], the entire jejunum and ileum divided into three equal parts [12], an unspecified segment of proximal jejunum [13], or separate duodenum, jejunum and ileum [14]. Assuming a similar distribution pattern in our Lewis rats and in the strains of rats used in functional studies, the choice of duodenum and even proximal jejunum may have provided only minimally susceptible tissue. The preference of investigators for jejunum over ileum may have caused them to miss the most susceptible area in many rats. And regardless of the site chosen for study, the variability of distribution of lesions described in the present work might detract from uniformity of observations and accuracy of quantitative evaluations in different samples and in different subjects.

These considerations do not detract from the excellent studies cited above. However, they do show the need for specification of study sites and raise the question of how to improve uniformity and quantitation. The utilization of the entire small intestine to provide an integrated evaluation may have some advantages. For example, intestinal anaphylaxis was found to increase the release of goblet cell mucus

into the lumen [14] and to decrease the absorption of salicylic acid perfused in situ through the entire small intestine [15]. Alternatively, multiple specified sites along the entire intestine, or the entire intestine divided into portions should be studied. This would allow a comparison of regional differences under various conditions [for example, see 16].

It is possible that the patterns of lesions and the variability of distribution are related to the local content of mast cells and to the local release of mediators in response to antigen challenge. Mast cell numbers and histamine content in small intestine have been studied [8, 11, 12, 17–19] but there are no detailed data to substantiate this hypothesis. Variations in blood flow and in blood vessel numbers have been reported [20] but there are no correlations with anaphylaxis. Variation in vascular permeability is not a likely explanation because a study of passive anaphylaxis revealed incomplete correlation between sites of increased permeability and the known sites of hemorrhage and congestion in anaphylaxis [19].

The lesions of intestinal anaphylaxis, including banded congestion, are very similar to the lesions produced in rat

intestine by histamine-liberating agents [21], by immune complexes [22, 23] and by human serum [24]. In the case of the lesions induced in rats by human serum, as in the present work, the pale (less affected) bands contained terminal vessels while the dark (more affected) bands did not, and the antimesenteric side (further from arterial blood supply) was more affected than the mesenteric side (closer to arterial blood supply). These similarities suggest some common factor in pathogenesis. These two gradients in respect to blood vessels might be related to the ability of IgE antibodies or mediators of anaphylaxis to leave the bloodstream.

The present work seems to be the first recognition of these two vascular gradients as well as the variability in the distribution and pattern of lesions in intestinal anaphylaxis of the rat.

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References

- Pratt HN: Studies of anaphylaxis in the albino rat with reference to diet and histamine. *J Immunol* 1935;29:301–317.
- Sanyal RK, West GB: Anaphylactic shock in the albino rat. *J Physiol* 1958;142:571–584.
- Keller R, Jones VE: Immunological and pharmacological analysis of the primary and secondary reagin response to *Nippostrongylus brasiliensis* in the rat. *Immunology* 1971;21:565–574.
- Levine S, Saltzman A: Method for systemic anaphylactic challenge in the rat using subplantar route. *J Immunol Methods* 1994;173:203–206.
- Levine S, Saltzman A: The balance between lymphatic and systemic absorption determines the outcome of sensitization for anaphylaxis in rats. *Int Arch Allergy Immunol* 1994;105:91–95.
- Levine S, Saltzman A: Anaphylaxis is enhanced by increased absorption of antigen in postinflammatory state. *Int Arch Allergy Appl Immunol* 1991;95:207–211.
- Byars NE, Ferraresi RW: Intestinal anaphylaxis in the rat as a model of food allergy. *Clin Exp Immunol* 1976;24:352–356.
- Freier S, Eran M, Goldstein R: The effect of immediate-type gastrointestinal allergic reactions on brush border enzymes and gut morphology in the rat. *Pediatr Res* 1985;19:456–459.
- Perdue MH, Gall DG: Intestinal anaphylaxis in the rat: Jejunal response to in vitro antigen exposure. *Am J Physiol* 1986;250:G427–G431.
- Crowe SE, Sestini P, Perdue MH: Allergic reactions of rat jejunal mucosa. Ion transport responses to luminal antigen and inflammatory mediators. *Gastroenterology* 1990;99:74–82.
- D'Inca R, Ramage JK, Hunt RH, Perdue MH: Antigen-induced mucosal damage and restitution in the small intestine of the immunized rat. *Int Arch Allergy Appl Immunol* 1990;91:270–277.
- Curtis GH, Patrick MK, Catto-Smith AG, Gall DG: Intestinal anaphylaxis in the rat. Effect of chronic antigen exposure. *Gastroenterology* 1990;98:1558–1566.
- Gould SS, Castro GA: Suppression by *Trypanosoma brucei* of anaphylaxis-mediated ion transport in the small intestine of rats. *Immunology* 1994;81:468–474.
- Lake AM, Bloch KJ, Sinclair KJ, Walker WA: Anaphylactic release of intestinal goblet cell mucus. *Immunology* 1980;39:173–178.
- Nakamura J, Yamamoto A, Takada S, Kimura T, Sezaki H: Antigen-induced decrease of salicylic acid absorption from the small intestine in actively immunized rats. *J Pharmacobiodyn* 1982;5:278–284.
- Prieto RM, Ferrer M, Fe JM, Rayo JM, Tur JA: Morphological adaptive changes of small intestinal tract regions due to pregnancy and lactation in rats. *Ann Nutr Metab* 1994;38:295–300.
- Saavedra-Delgado AMP, Turpin S, Metcalfe DD: Typical and atypical mast cells of the rat gastrointestinal system: Distribution and correlation with tissue histamine. *Agents Actions* 1984;14:1–7.
- Selye H: The Mast Cells. Washington, Butterworths, 1965.
- Leng W, Chang K, Williamson JR, Jakschik BA: Increased regional vascular albumin permeation in the rat during anaphylaxis. *J Immunol* 1989;142:1982–1985.
- Megison SM, Horton JE, Chao H, Walker PB: A new model for intestinal ischemia in the rat. *J Surg Res* 1990;49:168–173.
- Fell BF, Boyne R, Cuthbertson DP: Intestinal lesions following histamine liberation in the rat. *J Pathol Bacteriol* 1961;82:445–452.
- Kirkham SE, Bloch KJ, Bloch MB, Perry RP, Walker WA: Immune complex-induced enteropathy in the rat. I. Clinical and histological features. *Digest Dis Sci* 1986;31:737–743.
- Hassell LA, Bishara SM, Bloch KH: Immune complex-induced enteropathy. Effects of repeated injections of immune complexes on the small intestine of the rat. *Am J Pathol* 1989;134:193–201.
- Levine S, Saltzman A: New observations on the distribution of an old and forgotten model of enteritis in rats. *J Exp Pathol* 1992;6:89–92.

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