Gastric Eosinophilia in Rats Immunized with Stomach* (33910)

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The precise function of the eosinophil remains unknown, but eosinophils are known to accumulate at sites of antigen–antibody interaction. Advantage can be taken of this property to produce eosinophilia experimentally. Injection of antigen–antibody complexes or serial injection of soluble antigen alone will provoke eosinophilia consistently (1–4).

The capacity to provoke eosinophilia is also shared by at least some tissue antigens. Administration of homologous though apparently not isologous splenic tissue provokes eosinophilia (5), and eosinophilic infiltrates in the stomachs of dogs given autologous gastric juice in adjuvant have been described (6).

The present experiments were undertaken in an attempt to induce gastritis in rats. In the course of this work gastric eosinophilia was found to follow immunization with stomach.

Methods. Antigen. Rats or Hartley guinea pigs were exanguinated by cardiac puncture and their stomachs and livers were removed and washed in water. Gastric serosa was separated from the remainder of the stomach with a razor blade and discarded. The remainder (mucosa, submucosa, and muscularis) was emulsified in 3 vol (w/v) of Freund's adjuvant (containing 4 mg/ml of killed *M. Tuberc.* var. hominis) using a Thermovac homogenizer. Washed liver slices were similarly emulsified in 3 vol (w/v) of adjuvant.

Animals. Lewis and Sprague-Dawley rats 130–180 g were fed standard laboratory chow and water *ad libitum*. Each rat received 0.1 ml of the antigen–adjuvant mixture or adjuvant alone in each hind footpad.

Animals were sacrificed at 7, 10, 14, 21, 28, 35, or 40 days after immunization. At sacrifice the rats were bled out; and stomach, duodenum, ileum, ascending colon, lung, liver, and spleen were taken and fixed in 10% formalin. Hematoxylin–eosin and Dominici stained sections of the various organs were examined. Tissue eosinophilia was scored arbitrarily as 0 (few), + (moderate) or ++ (many). All sections were read blind. The results at 10, 14, 21, and 28 days were comparable and have been pooled in the tables.

Results. All groups remained healthy and gained weight comparably throughout the experiment. In normal rats, eosinophils were usually present in significant numbers in the mucosa, lamina propria, and muscularis mucosa, but were scant in the submucosa (Fig. 1A) except immediately adjacent to the point where squamous epithelium gave way to glandular. Eosinophils were scant in muscularis and serosa. This distribution is in accord with the findings of Räsänen (7).

Seven days after immunization with stomach there was no increase in eosinophilia in the stomach over that found in normal rats. At 10 days, and thereafter, most stomach-immunized rats showed increased eosinophilia throughout the tissues of the stomach wall. This was best perceived in the submucosa (Fig. 1B) and occurred whether the rats had been given isologous (Lewis), homologous (Sprague-Dawley) or heterologous (guinea pig) stomach. The findings are given in Table I. Liver-immunized controls showed no such response. The difference between stomach-immunized and control pools was significant at the 1% level of confidence (chi-square test with Yate's correction for continuity). Apart from the increased eosinophilia the stomachs appeared normal.

In the small number of stomach-immunized and control animals studied after 28 days, increased eosinophilia was no longer

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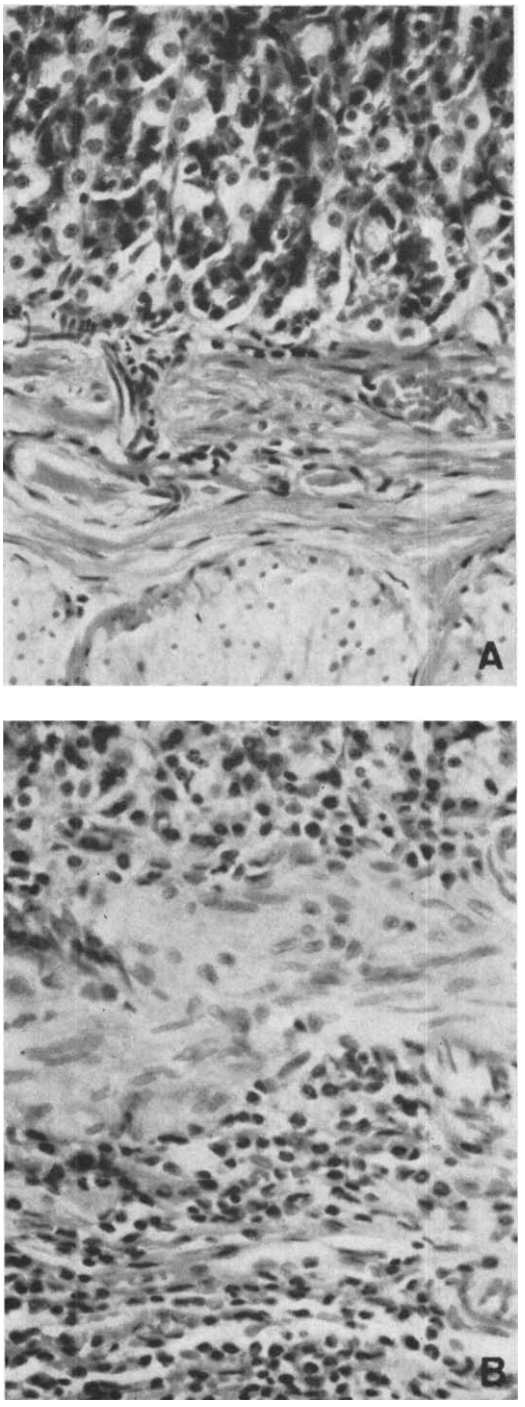

FIG. 1A. Lewis rat stomach 14 days postimmunization with isologous liver in Freund's adjuvant: a few eosinophils are shown in the basal layer of the mucosa but no eosinophils are in the remainder of the stomach wall; the section was scored as 0; hematoxylin and eosin ×590. (B) Lewis rat stomach 14 days post immunization with isologous stomach in Freund's adjuvant: an extensive collection of eosinophils are shown in the submucosa; in addition, numerous eosinophils are in the basal mucosa at the bases of the gastric glands; the ring-shaped nucleus characteristic of the rat eosinophil is readily identifiable; the section was scored as ++; hematoxylin and eosin ×590.

found. No eosinophilia was found in the liver, spleen, or lung at any time. The data for the duodenum, ileum, and colon 10 to 28 days after immunization are presented in Table II. The interpretation of these data is complicated by the finding of variable degrees of eosinophilia in control rats and considerable individual variation between rats in the experimental group. There was a trend toward more eosinophilia in the duodenum, ileum, and colon in the stomach-immunized group than in the control group, but the differences, though suggestive, were not statistically significant.

TABLE I. Eosinophils in Rat Stomach Following Immunization with Stomach or Liver in Adjuvant or with Adjuvant Alone.

Treatment	After immunization (days)	Eosinophil index		
		0	+	++
None[a]	0	7	0	0
Adjuvant only[b]	7	2	2	1
Liver[c]		3	0	1
GP stomach[d]		2	0	0
S.D. stomach[e]		3	1	2
Adjuvant only	10–28	5	2	0
Liver		10	2	0
GP stomach		4	3	3
S.D. stomach		3	4	7
Lewis stomach[f]		8	11	14
Adjuvant	>28	2	1	0
Liver		2	0	0
Lewis stomach		4	1	0

[a] Sprague-Dawley, 4; Lewis, 3.

[b] Lewis rats, 8; and S.D. rats, 7, received adjuvant.

[c] Lewis rats, 9, received Lewis liver; Lewis rats, 9, received GP liver.

[d] Lewis rats, 16, received GP stomach.

[e] S.D. rats, 39, received S.D. stomach.

[f] Lewis rats, 15, received Lewis stomach.

TABLE II. Eosinophils at 10–28 Days in Duodenum, Ileum, and Colon of Rats Immunized with Stomach.

Organ	Group	Eosinophil index 0	+	++
Duodenum	Stomach immunized[a]	18	14	24
	Control[b]	10	3	15
Ileum	Stomach immunized	11	13	32
	Control	7	4	8
Colon	Stomach immunized	17	26	13
	Control	7	11	0

[a] Ten Lewis stomach-immunized Lewis rats, 14 GP stomach-immunized Lewis rats, 32 S.D. stomach-immunized S.D. rats.

[b] Six Lewis liver-immunized Lewis rats, 6 GP liver-immunized Lewis rats, 6 adjuvant-immunized Lewis rats, 2 adjuvant-immunized S.D. rats.

Discussion. Injection of isologous, homologous, or heterologous stomach extracts in the rat is followed by increased stomach eosinophilia. Injection of isologous, homologous, and heterologous liver extracts does not evoke a comparable eosinophilia. The mechanism of this response is not established by these experiments, but it seems possible that relatively organ-specific stomach antigens are implicated.

It is now established that antigen–antibody complexes attract eosinophils (2, 3, 8, 9) and it is likely that tissue-bound complexes can evoke tissue eosinophilia. Sharp *et al.* (10) have shown in the guinea pig that passive transfer of rabbit antiserum to guinea pig thyroglobulin results in localization of antithyroglobulin antibody within the thyroid and a marked infiltration of this organ by eosinophils. In the present experiments it is possible that immunization of rats with organ-specific antigens induces autoantibody production, that these antibodies form complexes with organ-specific stomach antigens and that this somehow leads to eosinophilia in the stomach wall.

Hennes *et al.* (6) have demonstrated precipitating antibody and delayed-type hypersensitivity to gastric juice in dogs injected with isologous gastric juice in Freund's adjuvant, as well as gastric eosinophilia, findings in good agreement with our own since it is known that gastric juice and gastric mucosa share several antigens (11). Attempts by us to elicit delayed-type hypersensitivity to stomach antigens in the rat as well as studies of antistomach antibodies are continuing.

Summary. Eosinophilia in the rat stomach was produced by immunization with isologous, homologous, and heterologous stomach extracts in Freund's adjuvant. Immunization with isologous, homologous, and heterologous liver extracts failed to evoke stomach eosinophilia. It is possible that production of autoantibodies directed against organ-specific antigens in the stomach extracts and interaction of these antibodies with organ-specific antigen in the stomach wall may be responsible for the stomach eosinophilia.

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