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Histological observations on the intestinal immune response towards horseradish peroxidase in rats

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Summary. The intestinal immune response in rats following immunization with horseradish peroxidase (HRP) was studied morphologically. Various routes of immunization (intraperitoneal, intravenous, intraenteric and intra-Peyer's patch) were tested. Single injections did not evoke any specific antibody-containing (anti-HRP) cells in Peyer's patches (PP) and lamina propria of the small intestine. Only an intraperitoneal or intraenteric booster injection following intraperitoneal priming induced the formation of anti-HRP cells in PP and lamina propria. In the initial period of the immune response most of the anti-HRP cells were blast cells which were located mainly within the thymus-dependent interfollicular area of PP. Later on in the immune response more than 90% of the anti-HRP cells were located in the lamina propria of the villi and had the morphology of plasma cells.

INTRODUCTION

The role of the gut-associated lymphoid tissue (GALT), especially that of Peyer's patches (PP), in the humoral immune response to enteric antigens is still unclear. It has been shown that PP cells cannot be induced to produce antibody against sheep red blood cells or to react cytotoxically to allogeneic cells *in vitro* (Kagnoff & Campbell, 1974). This defect was attributed to the lack of accessory cells, as the immune responsiveness *in vitro* can be restored by the addition of adherent peritoneal cells or 2-mercaptoethanol to a culture of PP cells (Kagnoff & Campbell, 1974). Enteric or oral immunization did not give rise to antibody-producing cells in PP (Crabbé *et al.*, 1969). Only intra-PP immunization seems to be able to induce the formation of specific antibody and antibody-producing cells in PP (Cooper & Turner, 1967; Andrew & Hall, 1982). In contrast to PP, the lamina propria of the intestine was found to be very rich in antibody-producing cells after enteric stimulation (e.g. Bienenstock & Dolezel, 1971; Bienenstock & Defus, 1980).

In a previous study it was shown that PP contain immunoglobulin-containing cells (Sminia & Plesch, 1982). Cells containing IgM and IgG were located primarily in the subepithelial compartment and to a lesser extent in the germinal centre. IgA-containing cells were found predominantly within the thymus-dependent interfollicular area. These results suggest that production of immunoglobulins does occur in PP. The present study was undertaken to unravel the role of PP in the synthesis of specific antibodies. For this purpose, PP were stimulated via diverse routes with horseradish peroxidase (HRP).

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MATERIALS AND METHODS

Animals

Young adult male Wistar rats, obtained from the

Central Institute for the Breeding of Laboratory Animals, T.N.O., Zeist, The Netherlands, were used.

Immunization procedure

Rats were primed with a dose of 0·2 mg horseradish peroxidase (HRP, Grade I; Boehringer, Mannheim, West Germany) and boosted with half this dose. Several routes of immunization were tested (see Table 1, Results). Intravenous and intra-PP immunizations were always carried out with HRP in phosphate-buffered saline (PBS). The intraenteric and intraperitoneal inoculations were done with HRP in PBS or with HRP emulsified (50:50) in Freund's complete adjuvant (FCA; Difco Lab., Detroit, USA).

Booster injections were given 2 and 3 weeks after priming. Groups of two or three rats were killed with CO_2 1, 2 and 3 weeks after priming and at various times (2–11 days) after booster injections.

Histology and immunocytochemistry

Pieces of the small intestine, including PP, were fixed in a sublimate-formaldehyde solution (Bosman *et al.*, 1977) or frozen in liquid nitrogen and stored at −20°. The fixed material was routinely dehydrated with ethanol and embedded in low melting point paraffin (about 43°). Serial sections (thickness 6 μm) were stained with methyl green pyronine for routine histological observations. Paraffin sections were also used for immunohistochemistry (detection of anti-HRP cells) after routine removal of paraffin and rehydration, but without any special treatment. Serial cryostat sections (thickness 8 μm) were mounted on slides, air dried for at least 30 min, fixed for 10 min in pure acetone, dried overnight and used for the immunohistochemical demonstration of anti-HRP cells.

For the demonstration of anti-HRP-containing cells, paraffin and cryostat sections were washed in phosphate-buffered saline (PBS) and covered with a 0·1% and a 0·005% solution of HRP in PBS respectively. After a thorough rinsing in PBS, the preparations were stained for 6 min at room temperature with a mixture of 0·05% diaminobenzidine-tetrahydrochloride (DAB; Sigma, U.S.A.) and 0·01% H_2O_2 in 0·05 M Tris–HCl buffer (pH 7·6). The sections were washed with PBS again and lightly counterstained with Haematoxylin. Endogenous peroxidase activity was blocked as a control by incubating sections before exposing to the DAB solution in methanol–H_2O_2 (Streefkerk, 1972).

RESULTS

After a primary intraperitoneal (ip) or intravenous (iv) immunization with horseradish peroxidase (HRP), a very weak reaction was seen in the mesenteric lymph nodes (MLN) and the spleen. A few anti-HRP cells could be found scattered in these organs 3 weeks after immunization. These routes of immunization did not result in the appearance of anti-HRP cells in PP and the lamina propria of the villi. Intra-Peyer's patch (iPP) or intraenteric (ie) priming did not evoke any reaction, as could be concluded from the fact that PP, villi, MLN and spleen were totally devoid of anti-HRP-containing cells.

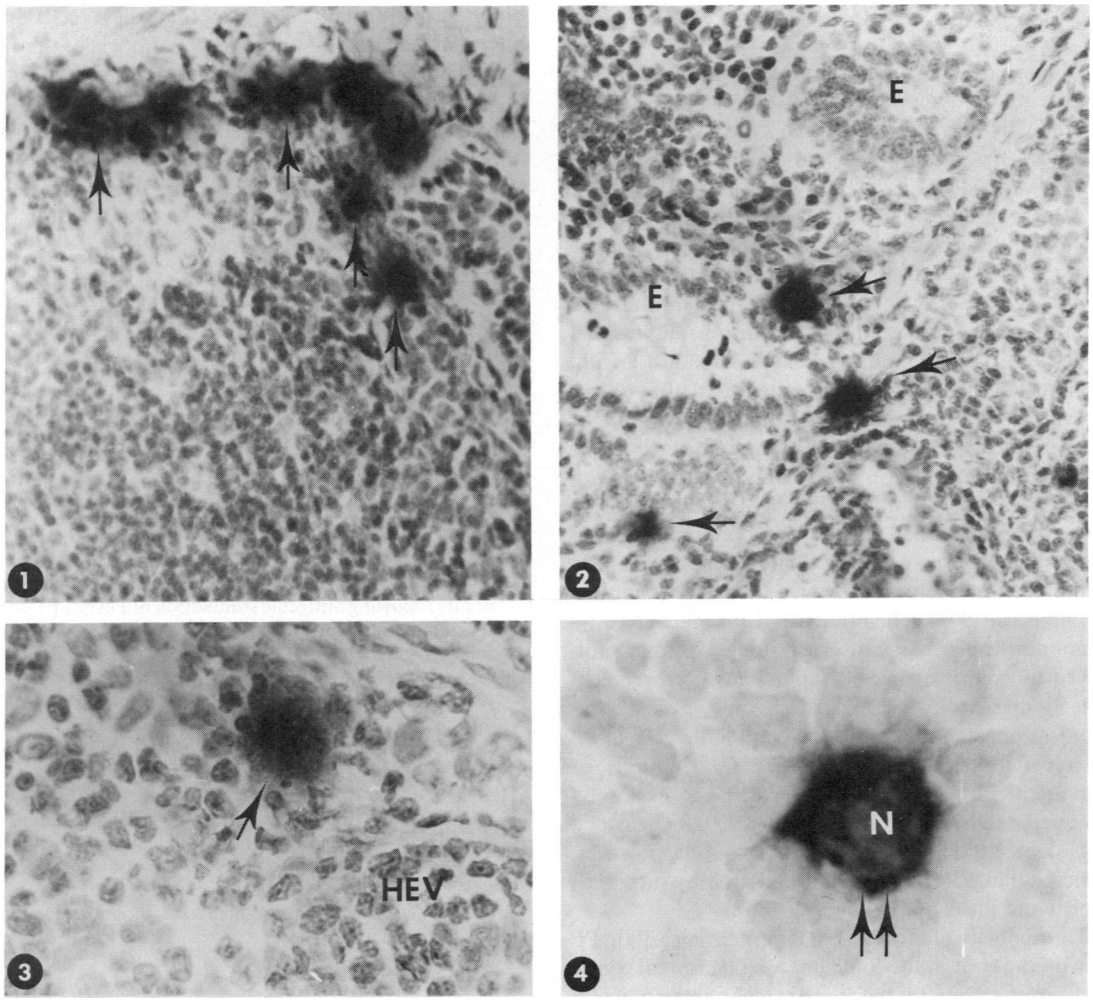
To study secondary responses in the intestinal immune system, rats were given booster injections 14 days after the primary immunization. The results of these booster injections are summarized in Table 1. All boosters, except those after ie priming, led to the formation of anti-HRP-containing cells in MLN and spleen, irrespective of the route of the booster injection. A reaction in PP, however, was only observed in rats primed ip and boosted via the same route or ie. The highest response was found in PP of rats primed ip and boosted ie. Other routes of administration of the

Table 1. The immune response to HRP in Peyer's patches (PP) and the lamina propria (L.P.) of the small intestine, mesenteric lymph node (MLN) and spleen

Immunization		Immune response in		
Priming	Booster	PP+L.P.	MLN	Spleen
ip	—	−	±	±
iv	—	−	+	+
iPP	—	−	−	−
ie	—	−	−	−
ip	ip	+	+	+
ip	iv	−	+	+
ip	ie	++	+	+
ip	iPP	−	±	±
ie	ie	−	−	−
iPP	iv	−	+	+

ip, intraperitoneal; iv, intravenous; ie, intraenteric; iPP, intra-Peyer's patch.

−, no reaction (no anti-HRP-containing cells found); ±, slight reaction (a few anti-HRP-containing cells); +, normal reaction (dispersed anti-HRP-containing cells); ++, strong reaction (numerous anti-HRP-containing cells).



Figures 1–4. Cryostat sections of Peyer's patches (PP; Figs 1, 3, 4) and villi of the small intestine (Fig. 2; magnification × 250) of the rat. Numerous anti-HRP-containing cells are present in the interfollicular area of PP 6 days after the booster immunization (*arrows*, Fig. 1; magnification × 300). These cells are found often around high endothelial venules (HEV; *arrow*; Fig. 3; magnification × 400) and have the morphological characteristics of blast cells (Fig. 4; *double arrows*; N, nucleus; magnification × 1000). 11 days after the booster injection most cells are located in the villi (Fig. 2; *arrows*; E, gut epithelium).

antigen did not evoke any reaction in PP and the villi of the small intestine.

With respect to the localization of anti-HRP-containing cells in the intestine the following observations were made. Until 6 days after the booster injection, almost all anti-HRP-containing cells were found in the interfollicular areas of PP, often in close association with high endothelial venules (Figs 1, 3). The anti-HRP-containing cells were intensely and homogeneously stained and had the morphological features of blast cells. About 11 days after boosting, more than 90% of the anti-HRP-containing cells were found in the lamina propria of the villi (Fig. 2). A few were seen in the subepithelial areas of PP and in the interfollicular areas (Fig. 3). No anti-HRP-containing cells were found in the follicles of PP. Morphologically the anti-HRP-containing cells found in the villi were plasma cells; occasionally an anti-HRP-containing blast cell was seen 11 days after booster injection (Fig. 4).

DISCUSSION

In this study the distribution and morphology of specific antibody-containing cells (anti-HRP cells) in PP and villi of the small intestine were investigated. In addition, the reaction on immunization by various routes was tested.

After a primary immunization with HRP no anti-HRP-containing cells could be found in PP and the villi; the spleen and MLN did contain anti-HRP-containing cells after iv and ip priming. Animals that had been primed and boosted ip or primed ip and boosted ie had anti-HRP-containing cells in PP and the villi. No anti-HRP-containing cells were found after boosting subsequent to ie and iv priming.

The appearance of antibody-containing cells in the villi after enteric or peritoneal immunization is not surprising. Several authors have reported the occurrence of high numbers of antibody-containing cells in the lamina propria of the small intestine after ip and ie immunization (Pierce & Gowans, 1975; Husband, Beh & Lascelles, 1979). The presence of specific antibody-containing cells in PP after peritoneal priming followed by enteric or ip boosting is, however, remarkable. It has been mentioned that only intra-PP immunization generates antibody-containing cells in PP *in vivo* (Cooper & Turner, 1967). Oral, enteric or parenteral stimulation did not give rise to antibody-producing cells in PP (Crabbé *et al.*, 1969; Bienenstock & Dolezel, 1971). The present observations, however, indicate that PP can also be stimulated by intraperitoneal and intraenteric immunization.

The majority of the anti-HRP-containing cells in PP was present not in the follicular area (germinal centre and corona) but in the T-dependent interfollicular area. A few were found in the area just beneath the lymphoepithelium, the subepithelial compartment (Parrott, 1976; Sminia, Janse & Plesch, 1983). The anti-HRP-containing cells in the interfollicular area had the morphological characteristics of blast cells. As they were mainly present in the proximity of the high endothelial venules it is possible that they enter PP via these specialized blood vessels. Later on in the immune response (11 days after infection) the majority of the anti-HRP-containing cells was present in the villi. This observation strongly suggests that these cells had migrated from the PP to the lamina propria of the villi during the course of the immune response. As most of the anti-HRP-containing cells in the villi were mature plasma cells, the migrating cells probably differentiate into plasma cells.

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