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Canine Lymphocytic Plasmocytic Enteritis: An Immunopathological Investigation of Intestinal Plasma Cells

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Vibe-Petersen, G.: Canine lymphocytic plasmacytic enteritis: An immunopathological investigation of intestinal plasma cells. Acta vet. scand. 1991, 32, 221–232. – The numbers of IgA-, IgG- and IgM-containing plasma cells in the lamina propria of duodenum, jejunum, ileum, and coecum were quantitated in 3 patients suffering from lymphocytic plasmacytic enteritis and 3 normal control dogs. A great increase was found in all 3 types of plasmacells and at all levels of the intestinal tract. Especially the IgG-containing cells showed a remarkable increase particularly in 2 patients. The possible reasons for these heavy infiltrations of immunologically competent cells are discussed, and chronic antigenic stimulations of food origin is suggested.

IgA; IgG; IgM; immunofluorescense; food antigens; chronic coeliac disease.

Introduction

Chronic inflammatory bowel disease is a common cause of intermittent diarrhoea in dogs. The inflammatory changes may be associated with various degrees of malabsorption and protein loosing enteropathy leading to debilitation and weight loss. Histologically, the chronic inflammatory bowel diseases are characterized by diffuse mucosal infiltration of inflammatory cells such as lymphocytes, plasma cells, eosinophils and neutrophils. The lymphocytic plasmacytic enteritis is found to be far the most common inflammatory bowel disease (*Hayden* 1982, *Tams* 1987).

The purpose of the present study was to investigate and characterize the different types of plasma cells involved in the lymphocytic plasmacytic enteritis compared to normal control dogs. This was achieved by immunopathological methods that are described in the following.

Materials and methods

In a group of 65 dogs referred to the clinic because of chronic gastrointestinal problems, 3 patients with a histologically confirmed diagnosis of lymphocytic plasmacytic enteritis were selected. These three patients were euthanized at the owner's request allowing total necropsy taking biopsies from all parts of the gastrointestinal tract. The purpose of this was to compare the chronic histological changes and numbers of plasma cells in the various parts of the gut.

Dog nr. 1 was a 3-year-old Labrador who had suffered from prolonged intermittent diarrhoea resulting in minor weight loss.

Dog nr. 2 was a 9-year-old German Shepherd with long lasting diarrhoea, pronounced weight loss and ascites.

Dog nr. 3 was a 9-months-old Labrador who had been suffering from profuse diarrhoea since 3 month of age. This dog was emaci-

ated and growth retarded in spite of a voracious appetite.

Normal control dogs. 3 clinically healthy dogs euthanized for non-medical reasons were examined in a similar way and used as normal control dogs.

In this clinic all patients with a history of chronic diarrhoea and/or vomiting are subjected to a standardized examination procedure as follows:

Clinical investigation

A complete clinical examination is performed, checking general conditions, body weight and weight loss, mucous membranes, hair coat and skin (enteritis patients often have skin problems too), thorough abdominal palpation, perineal region and digital rectal exploration and auscultation of heart and lung sounds. A faecal sample is tested for parasitic ova and coccidial oocysts, and a rectal swab is submitted for culturing of specific pathogenic bacteria such as Salmonella and Campylobacter.

Hematology and blood-chemistry

The following blood values are recorded: Sedimentation rate (SR), total leucocyte count, differential leucocyte count, total erythrocyte count, hematocrit, hemoglobin, alanine-amino-transferase (ALAT), alkaline-phosphatase (BASP), creatinine, carbamide, glucose, total serum protein and albumin. This can identify patients with chronic hepatic or renal dysfunctions as well as diabetic patients.

Digestion/absorption studies

A regular fat-absorption test is performed. After 12 h of fasting a heparinized blood sample is collected. Maize-oil 3 ml per kg body weight is fed, and blood samples are collected after 2 and 3 h. The blood samples are centrifuged and plasma turbidity is estimated. By low fat-absorption the plasma will stay clear and translucent.

Simultaneous evaluation of pancreatic exocrine function and intestinal absorption function can be achieved by BT-PABA and D-Xylose testing.

N-benzoyl L-tyrosyl-paraaminobencoic-acid (BT-PABA)

This is given orally in a dose of 35 µmol/kg BW. Chymotrypsin decomposes the peptide, and paraaminobenzoic acid is readily absorbed from the intestine. Unstabilized blood samples are collected before and 90, 120 and 180 min after the administration and their content of PABA is analyzed. Normal peak values of PABA are above 25 µmol/l at 90 min.

D-Xylose

This is administered as a 5 % solution immediately after the BT-PABA in a dosage of 10 ml/kg BW. NaF/heparin stabilized blood-samples are collected before and 30, 60, 90 and 120 min after the administration, and their content of D-Xylose is analyzed. Normal peak values of Xylose is above 3 mmol/l at 60 min. Malabsorption will result in a slow and incomplete absorption of Xylose.

Trypsin in serum

As a new test for pancreatic exocrine insufficiency we have introduced the trypsin in serum test. After 12 h fasting an unstabilized blood sample is collected and submitted to a laboratory (Medical Laboratory, Copenhagen), where the content of trypsin is estimated using a radioimmune assay (*Williams & Batt* 1983, *Williams* 1987).

This assay estimates anodic trypsin and the normal values for the laboratory, are above 30 µg/l (*Vibe-Petersen et al.* 1989).

X-ray-contrast studies

An X-ray contrast study of the gastrointestinal tract is performed in order to estimate the passage time and adherence of the contrast media (Micropaque Standard) to the mucous membrane. Radiographic pictures are taken before and 1/2, 1, 3, 5 and 24 h after the contrast media is fed.

Intestinal biopsy

Biopsy specimens are obtained either orally through a flexible endoscope or through an exploratory laparotomy. The 3 selected patients in the present study were submitted to an exploratory laparotomy and enterotomy. The freshly harvested enteric biopsies were fixed in buffered formaldehyde. After the diagnosis of lymphocytic plasmacytic enteritis was confirmed by histopathology the dogs were euthanized at the owners request as previously mentioned. A total necropsy was performed within 5 min after euthanasia. Biopsies were collected from the duodenum, jejunum – approximately 20 cm after the jejuna-colic ligament, ileum – approx. 30 cm before the ileo-coecal junction and coecum. The biopsies were readily transferred to 4 % buffered formaldehyde for subsequent histopathological and immunopathological investigations.

Immunopathological methods

The method used in the present study was based upon normal paraffine embedded tissue sections and provided good morphology and clear fluorescence. As previously mentioned a 4 % buffered formaldehyde was used as fixative. By this method antigens are retained in the tissue, but cross linking between antigens and other proteins causes masking of the antigen determinants and makes them inaccessible to the antibodies.

The cross linking is getting more pronounced the longer the fixation continues. Therefore, the fixation-time is reduced to 3–5 h.

Following this short fixation period the issue is processed through a number of ethanol baths and finally cleared with xylene and paraffine embedded. The tissue is then sliced into 5 μm tissue sections which are placed upon slides coated with poly-L-lysine (see later).

The sections are now deparaffinized by immersion through baths of xylene, absolute alcohol, 93 % alcohol, distilled water and phosphate buffered saline (PBS), pH 7.2. In order to unmask the antigenic determinants, the cross linkage can be digested using proteolytic enzymes. In this study trypsin-digestion at 37°C for 15 min was used (Trypsin 0.1 % in 0.1 % calciumchloride at a pH of 7.8) (Sigma – chemical company).

Coating the slides with Poly-L-lysine (Sigma) prevents the tissue sections from slipping off the slides during trypsin digestion.

The excess of trypsin is washed away and the sections are now ready for antibody incubation.

To avoid non-specific staining due to non-immunological binding of antibodies to tissue components, the sections are incubated with normal serum from the same animal that produced the secondary antibody – in this case, the rabbit.

After this, the sections are incubated with the primary antigen = goat anti-dog immunoglobulin A, G or M. (The antibody solutions were diluted 1:20 (Nordic Laboratory)). This is done in a humid chamber at room temperature for 30 min. After rinsing with PBS the secondary antibody is added: FITC-conjugated rabbit anti-goat immunoglobulin diluted 1:20 (DACOPAT). This was also incubated for 30 min at room temperature in a humid chamber.

After rinsing in PBS the slides were mounted

in a special mounting media called "No-Fade"[1] (The pharmacy produced this). "No-Fade" delays the normal fading of fluorescence considerably so that the slides can be kept for several months in a refrigerator and still show a clear fluorescence.

Microscopy and counting of cells

A conventional Leitz fluorescence microscope was used and color photographs were taken of all sections. The number of plasma cells were determined counting them at 500 ×, using a square ocular reticule (10 mm × 10 mm, with 100 squares) placed in the eyepiece. This means that each large square measured 0.02 × 0.02 mm = 0.0004 mm^2. In each tissue section the immunoglobulin containing plasma cells were counted in 2 or 3 such squares, placing the squares in 2 or 3 corresponding zones of the lamina propria: 1) immediately above the basement membrane 2) in the middle of the villi and 3) at the top of the villi.

Each group of plasma cells – IgA, IgG and IgM containing – were counted separately in 3 consecutive sections. The sections were marked with code numbers, so that the person counting did not know from which animal the section was taken.

Results

Clinical examinations

The results of the standard examination procedure are presented in Table 1.

It is remarkable that, in spite of the manifest clinical symptoms and pathological changes in the gut, the absorption of fat, xylose and PABA is fairly normal. All 3 dogs, however, showed hypoproteinaemia.

The histopathological investigations confirmed that the patients suffered from varying degrees of lymphocytic plasmacytic enteritis: The lamina propria were thickened and packed with cells, the villi were shortened and blunted, and the lymph vessels of the villi were slightly distended, especially in case 2, as a result of a low degree of intestinal lymphangiectasia.

The number of different types of plasma cells in the 3 patients and the normal control dogs are listed in Table 2. The differences are visualized more clearly in the diagrams of Figs. 1 and 2.

The number of IgA containing plasma cells was increased by approximately 50 % especially in the duodenum, jejunum and coecum compared to the normal dogs, Fig. 3.

IgG containing plasma cells in the normal dogs were very scarce, scattered along the basal lamina propria.

In the patients, however, numbers og IgA containing cells were considerably higher. Thus, in comparison with the controls, the numbers of IgG containing cells in dog no. 1 showed a more than doubling, and dogs no. 2 and 3 a more than 10 times increase. This is clearly visualized in Figs. 1, 2 and 4.

The number of IgM cells also showed increases in most of the sections from the patients compared to the normal dogs. The increase varied from a few percent to more than 100 percent (Fig. 5).

Discussion

The results of this study show that there is a marked stimulation of the antibody production in patients suffering from lymphocytic plasmacytic enteritis. The local IgA response is enlarged but especially in patients 2 and 3 the IgG response is also remarkable. Other research groups have previously shown a si-

[1] Mounting medium: "No Fade"
10 ml phosphate buffered saline (PBS)
100 ml paraphenylenediamine
90 ml glycerol (Glycerine 87 %)
pH is adjusted to 8.0 with 0.5 m carbonate-bicarbonate buffer
pH = 9.0

Table 1. Summary of standard examination procedure.

Patient	Abdom. palpation	Fecal examination	Blood test	Fat absorption	Xylose test	PABA test	Serum Protein Normal 60–82 g/l	Contrast X-ray	Explor. laparotomi	Clinical diagnosis	Histopathological diagnosis
1. Male Labrador, 3 years old. Diarrhoea for 1 year. Good general condition. Thin – BW – 22 kg. Normal hair coat. Good appetite	Slightly tender abdomen. Palpable thickening of the intestine	Yellow watery putrid. No parasites	Normal except diff. count = Lymfocytosis 60 %	Normal	Not done	Not done	57	Normal passage but adhesion of contrast to the pyloric and intestinal mucosa	Enlarged mesenteric lymph nodes Universel thickening of the intestinal wall	Chronic hyperplastic enteritis	Lymfocytic plasmacytic enteritis
2. Female German Shepherd. 9 years old. Diarrhoea for 1 year. Slightly depressed. Thin – 26 kg. Lusterless shedding hair coat. Good appetite	Slightly distended but indolent abdomen	Yellow watery-soft. Normal smell. No parasites	Normal except Hypoproteinamia: 54 g/l	Normal	Normal 60 min: 3.87 mmol/l	Normal 90 min: 42.6 µmol/l	54	Normal passage but adhesion of contrast to the pyloric and intestinal mucosa	Thickening of the intestinal wall. Excessive fluid in abdomen	Chronic hyperplastic enteritis Ascites	Lymfocytic plasmacytic enteritis with a slight lymphangiectasia
3. Female Labrador. 8 months old. Diarrhoea for 5 months. Happy and playfull. Emaciated, 16 kg. Normal hair coat. Extremely good appetite	Indolent abdomen yet tenesmi by rectal finger exploration	Light brown soft. Normal smell. No ova yet coccidae	Normal except leucocytosis: 23 mia/l and hypoproteinaemia: 44 g/l	Normal	Normal 60 min: 3.81 mmol/l	Normal 90 min: 37.4 µmol/l	44	Normal passage but some adhesion of contrast to the gastric and intestinal mucosa	Thickening of the intestinal wall	Chronic hyperplastic enteritis	Lymfocytic plasmacytic enteritis

Table 2. Numbers of plasma cells/field (0.02 × 0.02 mm).

		Patient			Normal controls mean	Range
		1	2	3		
DUODENUM	IgA	186	184	195	116	91–163
	IgG	9	56	98	4	0– 8
	IgM	81	65	64	32	21– 67
JEJUNUM	IgA	207	131	155	104	82–115
	IgG	6	72	108	1	0– 1
	IgM	61	23	49	43	6– 75
ILEUM	IgA	70	51	63	52	48– 51
	IgG	–	43	19	6	0– 14
	IgM	48	27	26	12	6– 22
COECUM	IgA	97	80	112	60	20– 82
	IgG	20	6	64	2	0– 5
	IgM	101	31	30	18	15– 23

milar pattern in humans with chronic coeliac disease, especially children with gluten induced enteropathy (*Scott et al.* 1980) and patients with Crohn's disease (*Balien & Brandtzaeg* 1975, *Brandtzaeg & Baklien* 1976, *Brandtzaeg et al.* 1974). However, very little has been published on the distribution of immunoglobulin containing cells in canine small intestine. *Villard et al.* (1978), *Hart* (1979) and *Villard & Leid* (1981) found a distribution of IgA, IgM and IgG cells in a ratio of 2:1:1 at all level of the small bowel in normal adult dogs. The greatest total number of plasma cells was found in the duodenum, the number decreasing towards the distal portion of the small intestine. A study of plasma cells in primary intestinal lymphangiectasia (*Suter et al.* 1985) revealed a decreased total number of plasma cells in the cranial part of the small intestine, but an absolute and relative increase of IgG containing plasma cells in the caudal parts.

So far, no references have been found on the distribution of different types of plasma cells in dogs with lymphocytic plasmacytic enteritis.

The previously mentioned study of children with coeliac disease (*Scott et al.* 1980) revealed increasing numbers of immunoglobulin-producing cells. Per square unit the increase in IgA, IgM and IgG-producing cells was 2.1, 3.8 and 2.9 times the normal controls, resp. Moreover, high numbers of IgG cells in the mucosa were associated with an immediate risk of clinical relapse. In our study there was a similar correlation between the number of IgG cells and the severity of the clinical symptoms (Table 1). These findings suggest that locally produced antibodies of the IgG class are particularly involved in the pathogenesis of coeliac disease in children as well as in lymphocytic plasmacytic enteritis in dogs.

It is generally assumed that the IgA and IgM producing cells act as a first line of defence by providing secretory antibodies, whereas IgG producing cells act in a second line of defence (*Mowat* 1987). An aggravation of the mucosal lesions would therefore imply

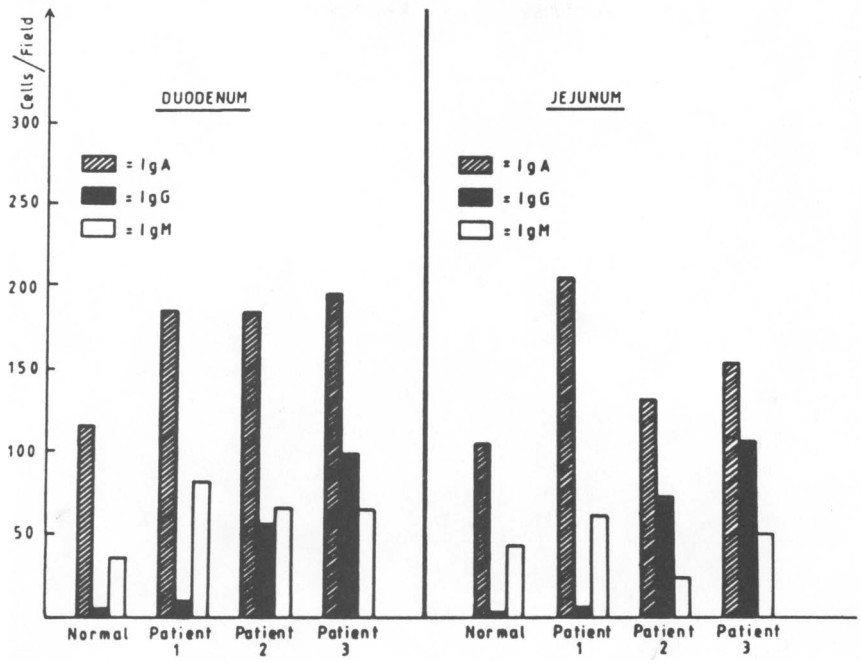

Figure 1. Numbers of plasma cells/field (0.02 × 0.02 mm) in duodenum and jejunum.

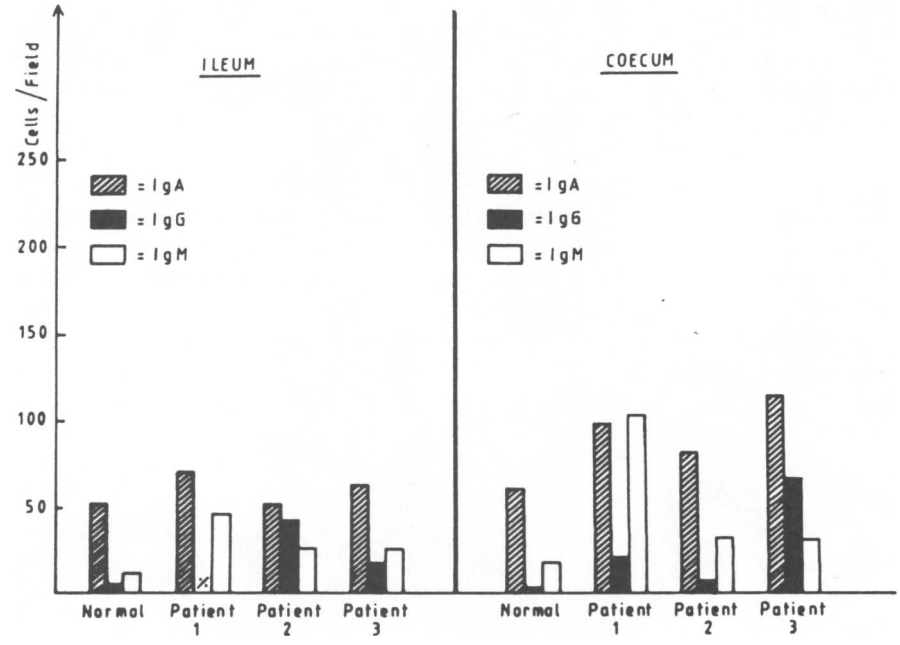

Figure 2. Numbers of plasma cells/field in ileum and coecum.

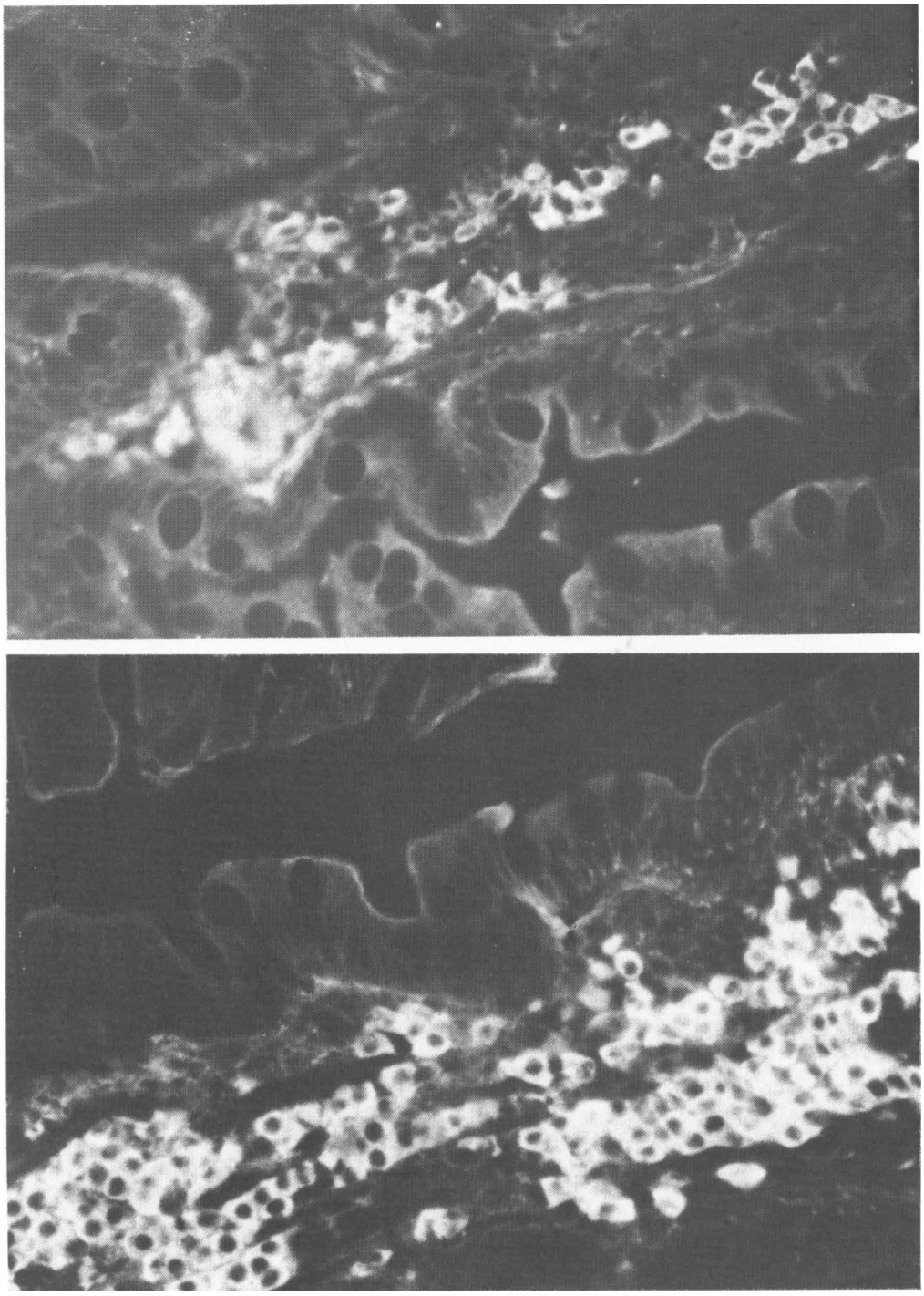

Figure 3. IgA containing plasma cells in duodenum of a normal dog (top) and patient no. 1 (bottom).

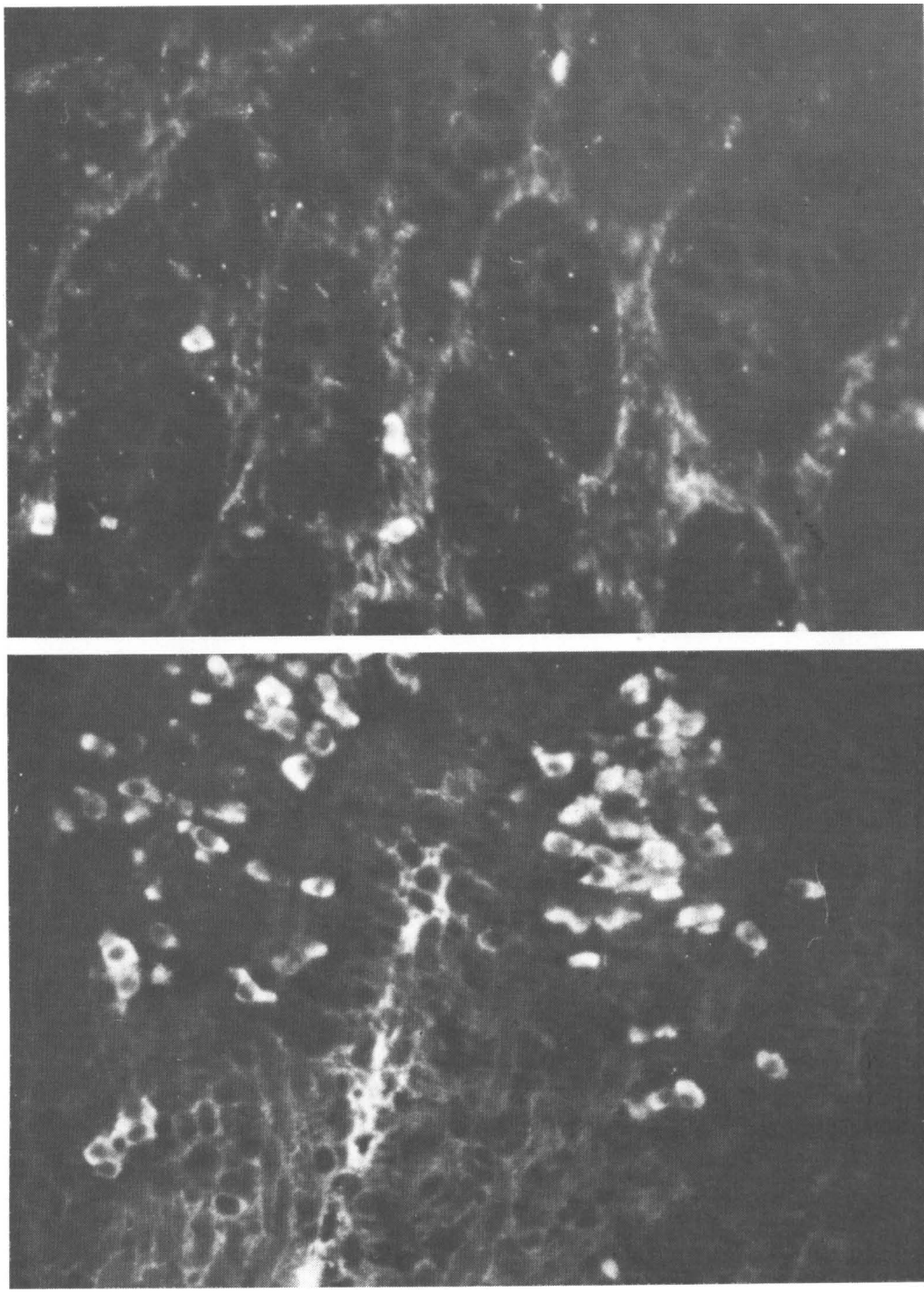

Table 4. IgG containing plasma cells in ileum of a normal dog (top) and patient no. 2 (bottom).

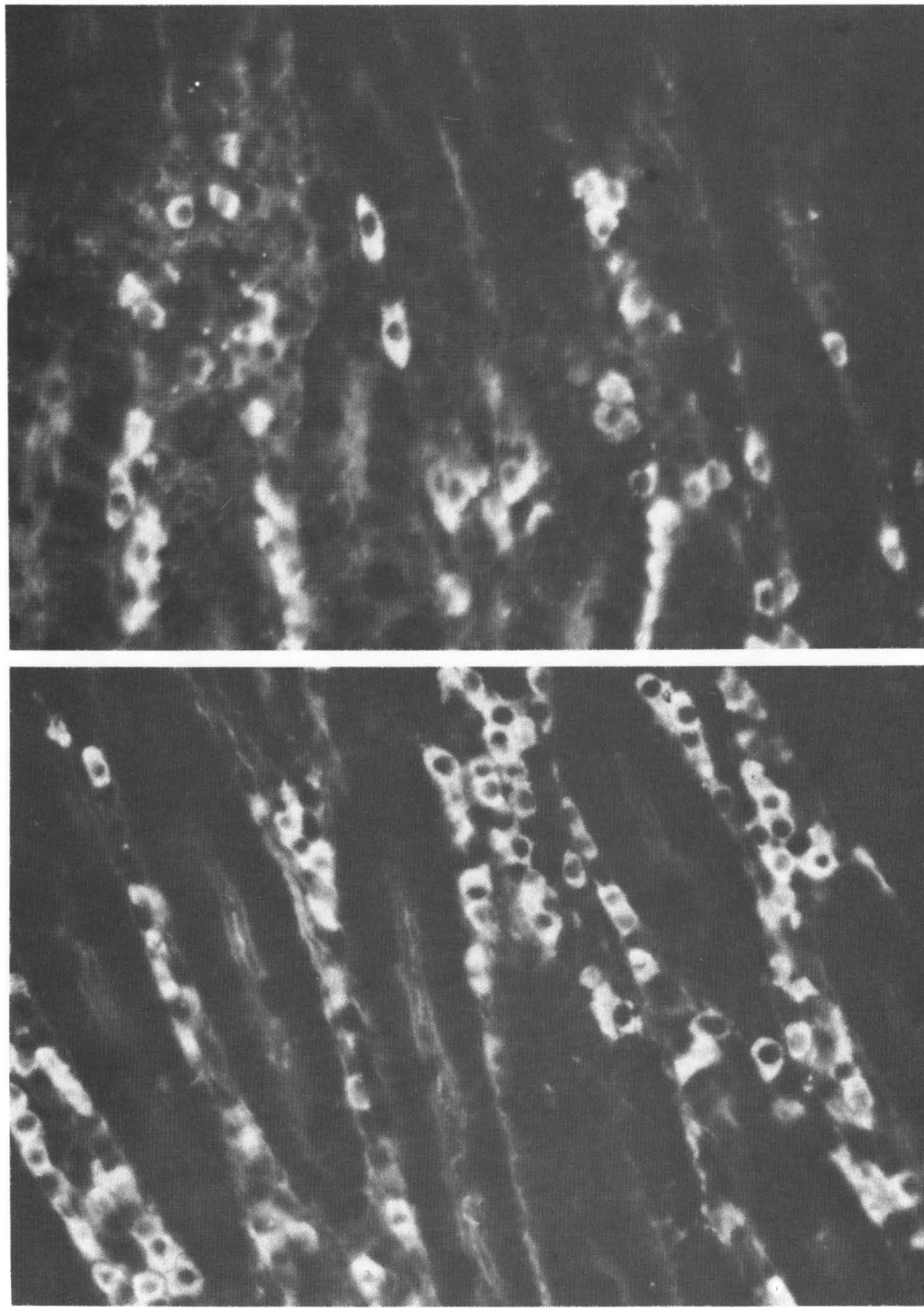

Figure 5. IgM containing plasma cells in duodenum of a normal dog (top) and patient no. 1 (bottom).

an increasing involvement of the second line of defence, leading to an increasing number of IgG producing cells in the lamina propria. The immunoglobulin produced in the lamina propria may be directed towards many different antigens in the ingesta: Food proteins, bacterial and fungal proteins and a great number of break-down products.

In humans it has been shown that patients suffering from chronic coeliac disease (CD) have larger levels of circulating antibodies against ovalbumin and lacto-globulin than normal control patients (*Husby et al.* 1986). Furthermore, these undegraded dietary proteins passed the intestinal barriers more easily in CD patients than in normal controls (*Husby et al.* 1985).

In dogs with chronic immunoproliferative enteropathies such as lymphocytic plasmacytic enteritis it is suspected that undegraded dietary antigens are presented to the immunological competent cells in a similar way. This results in an increase of first the IgA and IgM containing plasma cells and as the process aggrevates, also in the IgG containing plasma cells as has been demonstrated. This indicates that the secretory immune response is being gradually replaced by a systemic immune response. However, the increased numbers of lymphocytes may also be a result of a cellular immune response.

The nature of those antigens that may be responsible for the development of lymphocytic – plasmacytic enteritis in dogs remains to be established but research in progress will hopefully elucidate this point.

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Sammendrag

Lymfocytær-plasmacytær enteritis: En immunopatologisk undersøgelse af plasmaceller i tarmen.

Antallet af IgA, IgG og IgM producerende plasmaceller i lamina propria i duodenum, jejunum, ileum og coecum blev bestemt hos 3 patienter med lymfocytær-plasmacytær enteritis og 3 normale kontrol hunde.

Der fandtes en stor stigning i alle tre typer plasmaceller på alle niveauer af tarmkanalen. De IgG holdige plasmaceller viste en særlig markant stigning specielt hos 2 patienter. Mulige årsager til disse voldsomme infiltrationer af immunologisk kompetente celler diskuteres, og en kronisk fødemiddel antigen stimulation foreslåes.

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