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Review

Biomarkers in canine inflammatory bowel disease diagnostics

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Abstract

Canine inflammatory bowel disease (IBD) is a heterogeneous group of chronic gastrointestinal disorders. The etiology, similar to human IBD, remains unknown. Canine IBD is diagnosed by exclusion, which is a long, time and money-consuming process due to the need of elimination of other diseases presenting with similar symptoms. Therefore, a search for a specific and sensitive marker is needed to overcome these difficulties.

The article is divided into 3 sections presenting up-to-date information about laboratory markers, immunohistochemical markers and changes in the neurochemical coding of the enteric nervous system, concentrating on their usefulness and future applications. Data concerning laboratory and immunohistochemical markers is based mainly on canine IBD, while the neuroimmunohistochemistry section presents knowledge from human IBD due to the lack of such studies in veterinary medicine.

Key words: canine IBD, laboratory markers, immunohistochemical markers, ENS, neurotransmitters

Introduction

Canine IBD is a group of idiopathic, chronic or recurrent inflammatory diseases of the gastrointestinal tract. The etiology is unknown, but it is presumed that genetic factors, intestinal bacteria and abnormal host response take part in the disease. The main symptoms are emesis, diarrhea, loss of body weight and histopathological changes in the small intestine and colon. IBD is probably the most common diagnosis in dogs with chronic gastrointestinal symptoms, but the true prevalence is unknown (Jergens 1999a). There are many forms of canine IBD such as: lymphoplasmacytic enteritis (predominant),

eosinophilic gastroenteritis, eosinophilic colitis, granulomatous colitis (GC), also known as histiocytic ulcerative colitis (HUC).

Etiology and pathogenesis

The etiology of canine IBD still remains unknown, similarly to human IBD. The fundamental assumption in case of Crohns disease (CD) and ulcerative colitis (UC) is a complex interaction between environmental factors (intestinal flora), deregulated host immune response and genetically predisposed individuals (Jergens and Simpson 2012). To support the idea of de-

regulated host immune response, an increase in the expression of membrane toll-like receptors (TLR) TLR-2, TLR-4 and TLR-9 have been noted in the duodenum and colon of dogs with IBD (Burgener et al. 2008). McMahon et al. (2010) found that TLR-2 was significantly increased in IBD dogs compared to control dogs and moderately correlated with clinical severity of disease. Genetic factors also play a role in canine IBD, for example immunoproliferative enteropathy in Basenji dogs, protein losing enteropathy in Soft-Coated Wheaten Terriers and HUC in Boxers. As in human medicine, there is a search for single nucleotide polymorphisms (SNPs), which are changes in the genome contributing to individual predisposition and the development of the disease. A change in these nucleotides may effect the intestinal barrier function, innate and adaptive immunity (Jergens et al. 2003).

Current diagnostic difficulties

Canine IBD is a diagnosis of exclusion, all other possible diseases must be eliminated in the differential diagnosis (DDx) (Jergens and Simpson 2012). The DDx includes not only diseases of the alimentary tract, but also illnesses that influence the gastrointestinal tract and cause secondary symptoms. The process of diagnosing canine IBD includes patient history and clinical examination, laboratory tests, medical imaging, intestinal biopsies and histopathological evaluation. The most common diseases symptomatically mimicking IBD are: infectious and parasitological diseases, liver and pancreas abnormalities, intestinal structural change, neurological, endocrinological, metabolic and neoplastic diseases. Presently, the diagnosis of IBD by the World Small Animal Veterinary Association (WSAVA) Gastrointestinal Standardization group is defined by the following:

1. Persistent or recurring chronic gastrointestinal (GI) symptoms (lasting more than 3 weeks).
2. Histopathologically confirmed intestinal mucosa inflammation.
3. Inability to find other causes of GI symptoms.
4. No response to dietary, antibiotic and antiparasitic trials.
5. A clinical response to anti-inflammatory and immunosuppressive therapy.

The histopathological examination of GI inflammation is limited due to the lack of evaluation standards of intestinal biopsy lesions. Many systems of classification have been developed depending on the type and intensity of cellular infiltration. The inflammation type is based on the dominant cell type in the lamina propria (lymphoplasmacytic, eosinophilic, suppurative inflammation). Such inflammatory cells may,

unfortunately, be found in many diseases and combinations. Eosinophils may indicate the presence of parasites or dietary intolerance, while lymphoplasmacytic enteritis is commonly associated with IBD and PLE (protein-losing enteropathy). Another problem are changes in the intestinal mucosa structures (villi morphology, dilatation of lymph vessels, changes in crypts and number of goblet cells), which is often overlooked due to emphasizing the cellular infiltration type (Simpson and Jergens 2011).

Other limitations of the histopathological evaluation are: various biopsate quality, the lack of generally accepted criteria for lesion interpretation and difficulties in histopathologic differentiation between inflammation and alimentary lymphoma (Jergens and Simpson 2012). WSAVA created histopathological standards for intestinal evaluation, but unfortunately, even these standards have limitations such as: the absence of goblet cells and the submucosal layer and the muscularis layer inflammation evaluation (Simpson and Jergens 2011). An additional factor hampering the unification of results is the different interpretation (subjective) of the same biopsy specimen by different histopathologists. There is variance in the use of the terms: normal, mild, moderate, severe and neoplastic in describing cellular infiltration. Generally, the alimentary tract is recognized as difficult to evaluate histopathologically (Willard et al. 2002). Due to all these difficulties there is a search for alternative methods.

Novel examination techniques in canine IBD

The modern diagnostic process of IBD has many disadvantages e. g. it is time-consuming, invasive and costly. Non-invasive markers may be helpful in estimating a diagnosis, prognosis and disease monitoring. Such biomarkers may be serological, tissue-derived or even present in urine and feces and have different functions in the organism (serum proteins, acute phase proteins, proinflammatory proteins). Biomarkers should be characterized by high sensitivity, specificity, low costs and simplicity and be able to dynamically evaluate disease severity. The applications of biomarkers are: screening, diagnostic, prognostic, regression prediction and therapy monitoring. Presently there are no biomarkers with all these features.

Laboratory markers

Serological markers

Perinuclear antineutrophilic cytoplasmic antibody (pANCA) and anti-*Saccharomyces cerevisiae* antibodies (ASCA).

pANCA positive samples are characterized by a coloration pattern around granulocyte nucleus (Berghoff and Steiner 2011). Antigens for pANCA are found in neutrophil granules (most frequently myeloperoxidase – MPO, but also the antibodies targets are proteinase, lactoferrin, elastase and lysozyme). pANCA is used in human medicine to differentiate IBD forms (UC patients are 50%-80% positive, CD patients 70%-90% negative)(Nakamura et al. 2003, Sandborn 2004). In a study conducted on dogs 62% of the patients with food-responsive diarrhea (FRD) were pANCA positive, and only 23% dogs with IBD were positive before treatment. It is presumed that the pANCA status may be helpful in differentiating dogs with FRD from IBD dogs before treatment (Luckschander et al. 2006). Allenspach et al. (2004) found that assays for pANCA and ASCA in IBD dogs had a sensitivity of 51% and 44%, respectively. The specificity for pANCA ranged from 82% to 95% and for ASCA 56% to 79%. In another study, pANCA was found as a helpful diagnostic tool due to high specificity when comparing IBD dogs to dogs with other gastrointestinal diseases. An additional finding was that most pANCA-positive dogs were not ANA (antinuclear antibody) positive (Mancho et al. 2010).

Acute phase proteins (APPs) serum concentration.

APPs are proteins produced by the liver during acute inflammation. The acute phase response is highly nonspecific, but sensitive e. g. ceruloplasmin and haptoglobin are 6 times more sensitive in detecting inflammation than white blood cells (WBC)(Cerun et al. 2005). In human medicine APPs are used as intensity indicators of infectious, neoplastic and rheumatoid disease.

Increased serum concentrations of C-reactive protein were noted in dogs with CIBDAI ≥ 5 (mild disease severity or greater) and also CRP strongly correlated with disease severity. (Jergens et al. 2003). Additionally, CRP was found to be a disease indicator during immunosuppressive therapy, because corticosteroids do not influence it (unlike WBC). Unfortunately, CRP is nonspecific and an increase in concentration is noted also in other inflammation states, pregnancy, neoplastic disease, necrosis, trauma, etc.

However, McCann et al. (2007) noted a deficiency in correlation between serum CRP concentrations and the CIBDAI as well as the histopathological lesion intensity. Also the same study found no use for tumor necrosis factor alpha (TNF α) and microalbuminuria in the evaluation of disease activity in canine IBD.

A positive correlation was also found between alpha-1-acid glycoprotein (AGP) and the CIBDAI

score in dogs, but this association became significant when CIBDAI ≥ 6 (Jergens et al. 2003).

Also haptoglobin (HAP) and serum amyloid A (SAA) have been investigated, but no application nor correlation in canine IBD have been found. In a study conducted by Jergens et al. (2003) the concentration of HAP after treatment was increased compared to the concentration before treatment. This may have been due to glucocorticoid induction.

Urine and fecal markers

Urine and fecal markers are a heterogenic group of substances that are synthesized, excreted or lost by the alimentary and urinary tract in inflammation states.

Alpha 1-proteinase inhibitor (α_1 -PI).

α_1 -PI is a serum protein with a molecular mass similar to albumins, which, in case of intestinal barrier dysfunction, can reflect the degree of albumin loss into the digestive tract (Batt 2000, Berghoff and Steiner 2011). α_1 -PI is synthesized in the liver, macrophages and the intestinal mucosa. Contrary to albumins, the properties of α_1 -PI protect it from proteases in the intestines and degradation does not occur (Batt 2000, Berghoff and Steiner 2011). Thus, α_1 -PI can be measured in fecal samples and used to determine gastrointestinal protein loss. Murphy et al. (2003) has shown that fecal α_1 -PI concentrations were significantly higher in dogs with gastrointestinal diseases associated with histologic abnormalities (IBD and lymphangiectasia), compared to dogs with gastrointestinal symptoms and no histological changes.

Calgranulin C (S100A12).

S100A12 is a calcium binding protein abundantly found in neutrophils and in smaller amounts in macrophages and monocytes (Berghoff and Steiner 2011). S100A12 has proinflammatory properties and is secreted in inflammatory states of various etiology. An increase of this protein has been noted in humans with IBD and a correlation with CRP and SAA was also noted. Research also indicates the usefulness of S100A12 in differentiating IBD form IBS (irritable bowel syndrome) and disease severity evaluation (Manolakis et al. 2010). Unfortunately, in dogs and cats, the inflammatory infiltrate is mainly lymphoplasmacytic, rarely eosinophilic or granulomatous, and this marker is of lesser value in these species.

Calprotectin (S100A8/S100A9).

S100A8/S100A9 is a heterogenic complex, belonging to the calcium binding protein family S100. It is

a sensitive marker of inflammation states. An increase of these markers in IBD patients and a correlation with endoscopic, clinical and biochemical markers of IBD activity have been noted. There is also a correlation between calprotectin and CRP, CDAI (Crohn's disease activity index), CDEIS (Crohn's disease endoscopic index of severity) and MDAI (Mayo Disease Activity Index) (Vieira et al. 2009, Neubauer and Dudkowiak 2012).

Calprotectin may be used as a screening test for more invasive examinations. Heilmann et al. (2008) described a radioimmunoassay for the quantification of canine calprotectin in canine serum and feces. Later, the same author stated that serum calprotectin concentrations may be a useful biomarker in canine IBD with a sensitivity of 82,4% and specificity of 68,4%. (Heilmann et al. 2012). However, the inability to identify the inflamed organ when measuring only canine calprotectin, the effect of treatment on canine calprotectin concentrations and no data concerning canine fecal calprotectin concentrations warrants further studies.

N-Methylhistamine (NMH).

NMH is a stable metabolite of histamine and may be used as a marker of mast cell degranulation and GI inflammation (Berghoff and Steiner 2011). Mast cells can take part in intestinal inflammation by releasing multiple inflammatory mediators, including histamine. An increase of NMH in urine was observed in UC and CD patients and also a correlation with endoscopic examination, clinical activity index and CRP was noted (Winterkamp et al. 2002). Similar studies concerning NMH in urine and feces of dogs were recently conducted, but the usefulness of NMH in diagnosing IBD and enteropathies needs further investigation (Berghoff and Steiner 2011).

Leukotriene E4 (LTE4).

LTE4 are proinflammatory derivative products of lipoxygenase. During inflammation they increase vessel permeability, chemotaxis and mucous secretion. The measurement of LTE4 may be useful as a noninvasive evaluation of the leukotriene cascade. Concentrations of urine LTE4 were higher in dogs with IBD compared to healthy dogs. Further investigations are required, but LTE4 may be a potential marker in canines with chronic enteropathies (Im Hof et al. 2012).

Immunohistochemical markers

Immunohistochemical studies are based on marking the distribution of T lymphocytes, MHC class II

cells, immunoglobulins (Ig) and protein cell structures mainly in the epithelium and lamina propria of ill dogs and comparing them to healthy ones for the purpose of recognizing quantitative and qualitative disorders. Disorders of such markers may be used for diagnostic, differential diagnostic, prognostic and monitoring purposes.

Jergens et al. (1996) documented the decrease in T cells and IgG+ plasma cells in the canine small intestine affected with IBD. Afterwards, the same researcher studied the large intestine of canines with IBD and noted an increase of IgA+ and IgG+ plasma cells and T cells (Jergens et al. 1999b). Similar results were obtained by Stonehewer et al. (1998) who described the percentage of plasma cells and T cells in the lamina propria. Healthy dogs had significantly lower numbers of T cells and B cells in the lamina propria and also lower numbers of T cells in the glandular epithelium.

German et al. (2001) noted an increase of IgG+ plasma cells, T cells (CD3+), T cells CD4+, macrophages and neutrophils in the lamina propria of dogs with IBD. Also, a decrease of mast cells count was observed compared to the control group and in the epithelium an increase in CD3+ T cells was noted.

Apoptosis markers were also evaluated immunohistochemically. The expression of caspase 3 (Casp3), poly (ADP-ribose) polymerase (PARP) and an antiapoptotic marker Bcl-2 were evaluated in the duodenal villous tips, the duodenal villous base and in the colonic mucosa. Expression of Casp3 in the duodenal villous tip was greater in control dogs than in IBD dogs before and after treatment with prednisolone. Expression of Bcl-2 in the duodenal villous tip, duodenal villous base and colonic mucosa was greater in control dogs than in IBD dogs. Furthermore, in the duodenal villous tip the expression of Bcl-2 was significantly greater in IBD dogs before treatment than in IBD dogs after treatment. Thus, Bcl-2 could be a potential early marker of therapy success (Dandrieux et al. 2008).

The Boxer breed, affected by HUC, was also evaluated immunohistochemically and by computer morphometry. In focal lesions an increase of IgG+, IgG3+, IgG4+, plasma cells, CD3+ T cells, MHC class II cells, L1+ and PAS+ cells in the lamina propria was observed compared to the control group and regions of the colon unaffected by HUC (German et al. 2000).

P-glycoprotein (p-gp) was also analyzed because studies in human medicine indicate that p-gp may play a role in prognosing response to corticosteroid treatment (Allenspach et al. 2006). Dogs with low p-gp concentrations in lamina propria lymphocytes found before treatment indicate positive response to

Table 1. Immunohistochemical studies summary.

Cell/Protein	Immunohistochemistry (IHC)		
	Change	Localization	Source
T Cell	↓	Small intestine	Holzer 1998
IgG ⁺ Plasma Cell	↓		
T Cell	↑	Colon	Holzer 1998; Jergens 1999b
IgG ⁺ Plasma Cell	↑		
IgA ⁺ Plasma Cell	↑		
IgG ⁺ Plasma Cell	↑	Duodenal lamina propria	Geboes and Collins 1998
T Cell (CD3 ⁺); (CD4 ⁺)	↑		
Macrophages	↑		
Neutophils	↑		
Mast cells	↓		
Caspase 3	↓	Lymphocytes located on the villus peak of the duodenum	Collins et al. 1992
IgG ⁺ ; IgG3 ⁺ ; IgG4 ⁺	↑	HUC in boxers. Colon, focal lesions	German et al. 2001
Plasma cells	↑		
T Cell CD3 ⁺	↑		
MHC class II cells	↑		
L1 ⁺ cells	↑		
PAS ⁺ cells	↑		
P-glycoprotein	↑	Lamina propria lymphocytes. Up – regulation is related to immunosuppressive therapy resistance.	Allenspach et al. 2006
NF-κB	↑	Lamina propria	Luckschander et al. 2006
CD11c ⁺ integrin	↓	Duodenum, Ileum and Colon	Jergens and Simpson, 2012

corticosteroid treatment. Dogs with high p-gp concentrations may require more aggressive treatment (Allenspach et al. 2006).

The quantitative estimation of nuclear factor kappa B (NF-κB) of activated B cells showed that in the lamina propria of dogs with chronic enteropathy there are more NF-κB-positive macrophages compared to the control group. An increase in NF-κB-positive epithelium cells was found in dogs with FRD as compared to those with IBD (Luckschander et al. 2010).

Recently, the number of cells which express CD11c+ integrins in the duodenum, ileum and colon of IBD dogs has also been evaluated (Kathrani et al. 2011). The researchers noted a significant decrease in CD11c+ cells in the above mentioned intestines in dogs with IBD compared to the healthy control group and they presume that this may play a role in mucosal tolerance breakdown in IBD.

A summary of immunohistochemical studies is presented in Table 1.

Neuroimmunohistochemical markers

This section is mainly based on evidence from human medicine, due to the lack of similar studies in veterinary medicine. Nevertheless, studying ENS plasticity may help to elucidate the

pathophysiology of IBD, support diagnostics or monitor treatment.

The enteric nervous system (ENS):

The alimentary tract possesses a complicated enteric nervous system which receives efferent signals from the central nervous system (CNS) and transmits afferent signals to the CNS. It starts in the esophagus and ends in the anus and contains plexuses: myenteric plexus (Auerbach plexus found between the longitudinal and circular muscle layers) and submucosal plexuses (Meissner and Schabadascha). The Auerbach plexus controls mainly peristalsis and the submucosal plexuses control fluid transport, secretion, blood supply and the smooth muscle of the muscularis mucosae. The chemical regulation of nerves in the alimentary tract is executed by many peptides and also nonorganic neurotransmitters such as; acetylcholine, noradrenaline and nitric oxide. The intestinal functions cannot be examined separately (secretion, motor function, sense, immunology) because they are closely linked with each other.

During intestinal inflammation there is a complex interaction between the immune system and the ENS. Activated immunocytes induce an inflammatory state by secreting cytokines, which induce chemotaxis, proliferation and activation of other immunocytes.

Table 2. Neuroimmunohistochemical studies summary.

Changes of neurotransmitter chemical coding in the intestines			
Neurotransmitter	Change	Disease	Source
Serotonin	↑	CD	5
	↑	UC	4; 8; 9; 12; 17
SP	↓	CD	4
	None	UC; CD	15
	↑	UC; CD	1; 7
VIP	↓	UC; CD; IBD (feline)	10; 11; 14; 16
	None	UC; CD	15
	↑	CD	19
CGRP	↓	UC; CD	18
	↑	UC; CD	2
Gal	↑	UC; CD	2
NOS	↑	CD	1; 7
Ach	↓	Enteritis/rat	6
Somatostatine	↓	UC i CD	3; 13; 20

Source: 1. Belai et al. 1997, 2. Benya et al. 1998, 3. Bergeijk and Wilson 1997, 4. Bernstein et al. 1993, 5. Coates et al. 2004, 6. Collins et al. 1992, 7. Geboes and Collins 1998, 8. Goldin et al. 1989, 9. Keranen et al. 1995, 10. Kimura et al. 1994, 11. Kleinschmidt et al. 2011, 12. Koch et al. 1987, 13. Koch et al. 1988, 14. Kubota et al. 1992, 15. Lee et al. 2002, 16. Mazumdar and Das 1992, 17. Neunlist et al. 2003, 18. Reinshagen et al. 1997, 19. Schneider et al. 2001, 20. Yamamoto et al. 1996.

Cytokines affect the nervous system of the intestinal wall and induce structural (neuronal necrosis, hypertrophy and hyperplasia) and functional (hyperactivity) changes (Taylor and Keely 2007). Plasticity of the ENS is a feature of inflammation which alter neurochemical coding of enteric neurons, change intestinal motility patterns, cause defects in the neural control of epithelial secretory response, increase excitability of neurons and alter synaptic transmission within the ENS. Also, it is presumed that localized changes may affect non-inflamed sites of the GI tract (Lomax et al. 2005). If we consider sustained nervous disorders, absence of autonomic balance and clinical symptoms in UC patients after colectomy, the enteric nervous system plays a role not only in the pathophysiology of intestinal inflammation in UC, but also in the development of the disease and predisposition to postoperative recurrences (Geboes and Collins 1998).

Neurochemical changes and receptor disorders:

In IBD there is a rapid change in the ENS as a result of inflammation and this correlates with motility disorders. Animal IBD models show change in the morphology and architecture of nerve ganglions and nerve cells and a subtle change in neurotransmitter expression and their receptors (Lakhan and Kirchgessner 2010). Intestinal cells taking part in inflammation (dendritic cells, lymphocytes, macrophages and mast cells) express receptors for neurotransmitters and neuropeptides and also enteric neurons are

receptive for cytokine signals from secretion cells. Change in the immunoreactivity of particular neurotransmitters according to various researchers are presented in Table 2, while a short description of the most studied neurotransmitters in IBD can be found below:

Serotonin.

Serotonin is the main GI paracrine hormone and an enteric neurotransmitter known for its ability to initiate peristalsis activity, recently identified as a proinflammatory neurotransmitter showing increased secretion from enterochromaffin cells in CD patients (Coates et al. 2004).

Substance P (SP).

SP, belonging to the tachykinin family, is a 11-aminoacid peptide secreted by neurons and inflammation cells such as: monocytes, macrophages, eosinophils and lymphocytes that acts by binding to the neurokinin-1 receptor (NK-1) (Gross and Pothoulakis 2007). SP has many functions, not only physiological (smooth muscle contraction, vessel dilatation and epithelial ion transport), but also plays a role as a neurogenic inflammation mediator in respiratory, gastrointestinal and musculoskeletal inflammation. SP and NK-1 expression increase was observed in the colon and rectum of IBD patients. The degree of density of SP nerve fibers in the lamina propria correlates with the degree of disease activity in UC patients (Taylor and Keely 2007). In pathologi-

cal states there is an increase of SP participating in gut sensitivity and motility (Holzer 1998).

The role of SP as a proinflammatory factor in IBD pathogenesis is confirmed by the fact that specific NK-1 receptor antagonist alleviate inflammation in animal models (Bernstein et al. 1993). Proinflammatory SP from neurons stimulate macrophages, mast cells and the endothelium to release cytokines which contribute and sustain the inflammatory infiltration, causing tissue damage and neurodegeneration.

Vasoactive Intestinal Peptide (VIP):

Evidence points that also VIP may play a role in IBD pathophysiology (Gross and Pothoulakis 2007). VIP is a peptide built of 28 amino acids. It is found in every layer of the colon, with the highest concentration in myenteric plexus. The functional role of VIP is to inhibit the peristaltic reflex in the circular muscle layer, to control of blood flow in the intestines and to modulate the immune system by binding to VPAC1 and VPAC2 receptors. VIP is released from nerve endings containing nitric oxide synthase (NOS). Together, these two transmitters are regarded to be a part of non-adrenergic non-cholinergic (NANC) transmission mechanism in the intestines. In CD patients change in neurochemical coding in muscular ganglion neurons was noted: increase in VIP, NOS and PACAP (pituitary adenylate cyclase-activating peptide) immunoreactivity (Belai et al. 1997).

Immunocytochemical studies have proven that in CD patients there is a VIP nerve hypertrophy in the intestines compared to healthy people and even UC patients. This suggests that nerve evaluation for VIP expression in intestinal endoscopic biopsies can be useful in differentiating IBD forms and diagnostics when the clinical and histopathological examinations are unclear (O'Morain et al. 1984).

Some evidence points out that VIP takes part in colitis and IBD. VIP therapy in mice with induced IBD decreased the clinical and histopathological severity of colitis. Unfortunately, VIP has many side-effects like hypotension and diarrhea when administered in high doses (Arranz et al. 2008).

Changes in SP and VIP immunoreactivity in mucosal nerves of IBD patients are inconsistent. Some researchers noted an increase, others found the decrease and sometimes, no change in the immunoreactivity of these neurotransmitters were found in CD and UC patients when compared to control groups (Lee et al. 2002).

Calcitonin Gene-Related Peptide (CGRP).

CGRP plays a part in intestinal smooth muscle relaxation and inhibits the propulsive motoric activ-

ity of the intestines. It also affects iron transport and fluid secretion (inhibition) and has vessel dilatation properties. A high expression of CGRP is noted in external afferent nerve fibers after the influence of a damaging factor (generally of inflammatory or infectious etiology)(Holzer 1998).

Neurokinin A (NKA).

NKA and CGRP have low activity in the normal intestine, but are capable to correct motility, hypersecretion, tissue homeostasis and intestinal pain caused by inflammation (Holzer 1998). Inflammatory processes of various etiology, including IBD, cause an increase in NK-1 receptor expression in intestinal blood vessels and lymphatic tissue.

Galanin (Gal).

Gal is responsible for smooth muscle contraction, motoric function regulation and ion secretion. Studies conducted on humans with IBD show an increase in GAL1R receptor expression in the colon of UC and CD patients (Benya et al. 1998).

Nitric oxide synthase (NOS), acetylcholine (Ach), and somatostatin have also been investigated, but not so thoroughly as the forementioned neurotransmitters. Information about changes in chemical coding can be found in Table 2.

The inconsistent information concerning certain neurotransmitter increase/decrease is a result of disease (CD, UC), species (human, animal models), localization (small intestine, large intestine), layer of the intestine affected (mucosa, submucosa, muscularis layer), time of biopsy collection (exacerbation, remission) and study method differences.

The assumption of neuroimmunohistochemical studies is to recognize changes in chemical coding in inflamed ENS biopsies. The interaction between the ENS and immune system is suspected in IBD pathophysiology. Chemical messengers take part in these interactions between enteric neurons and immunological effector cells. Chronic inflammation of the intestinal mucosa causes qualitative and quantitative changes in the ENS. This phenomenon is known as nervous system "plasticity" and is the ability of neurons to adapt to new circumstances. The aim of the changes in intestinal neuronal coding is to augment, inhibit or initiate neurotransmitter synthesis.

The disadvantages of neuroimmunohistochemical studies are: 1) poorly researched neuroplasticity changes in canine IBD; 2) no standards in neurotransmitter marking making studies non-repeatable; 3) difficulties in research and the necessity of specialized laboratories.

Summary

Simple, economical and dependable tests alternative to current IBD diagnostic procedures are still being searched for. The test should be analytically sensitive, specific, linear, precise and repeatable. The problem with most of the described biomarkers is low, or no, specificity. APPs and white blood cell markers increase in the early stages of inflammation, but give no information about the etiology of the disease because of low specificity, and the increase may be induced by various intestinal disorders and even disorders of other organs e.g. liver and joints. Fecal markers inform about inflammatory or intestinal permeability disorders, but also have low specificity and can only aid in differentiating IBD from IBS. The biomarkers described in this review cannot independently diagnose IBD, but may be useful in the elimination of non-inflammatory enteropathies or as screening tools for more invasive testing. The present problems in IBD diagnostics are a result of the idiopathic nature of the disease. The incomplete knowledge of etiopathogenesis hampers the understanding of IBD and, as a result, the development of specific examinations, methods and markers for IBD diagnostics.

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