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Lectin Histochemistry of Foam Cells in Tissues of Cattle Grazing *Brachiaria* spp.

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With 2 figures and 1 table

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Summary

Brachiaria decumbens and *B. brizantha* (signal grass), which occupy millions of acres in Brazil, are an important source of fodder for ruminants. Sporadic outbreaks of photosensitization in ruminants grazing on signal grass have been reported. Intoxicated animals showed the presence of foamy cells in the liver, spleen, intestinal submucosa and lymph nodes. These foamy cells are macrophages. They are very difficult to distinguish with haematoxylin and eosin stain, especially in the case of isolated cells. The purpose of the present study was to detect specific carbohydrate residues of storage material in the foamy cells in tissues of cattle exposed to *Brachiaria* spp. The characterization of glycoconjugates provides clues to the pathogenesis of these cells. Besides, the lectin peanut agglutinin was found to be an excellent marker to differentiate and quantify the foam cells, and could be used as a specific marker.

Introduction

Brachiaria decumbens and *B. brizantha* (signal grass) are an important source of fodder for ruminants which occupies millions of acres in the centre-east and south-east of Brazil (Hutton, 1975; Döbereiner et al., 1976; Baber, 1989). Sporadic outbreaks of photosensitization in ruminants grazing on signal grass have been reported in several countries (Nobre and Andrade, 1976; Mazni et al., 1985; Opasina, 1985; Zamri-Saad et al., 1987; Graydon et al., 1991; Miles et al., 1994; Lemos et al., 1997).

Considerable evidence has been accumulated indicating that this grass belongs to a group of plants that induce hepatogenous photosensitization (Meagher et al., 1996). All the species of this group are known to contain steroidal saponins which have been associated with the deposition of crystalloid material within the biliary system (Miles et al., 1994; Meagher et al., 1996; Cruz et al., 2000).

Besides the photosensitization, several alterations have been linked to *Brachiaria* spp. toxicity. More important are ruminal changes in the growth and activity of micro-organisms (Abdullah et al., 1992) and changes in motility and pH of reticulo-rumen (Abdullah et al., 1988). An important histological change is the presence in liver, lymph nodes and intestine of cells displaying cellular enlargement and fine cytoplasmatic vacuolation. They are very difficult to distinguish with haematoxylin and eosin stain, especially in the case of isolated cells (Driemeier et al., 1998).

Lectins are proteins or glycoproteins of non-immune origin with one or more binding sites per subunit, which can reversibly bind to specific sugar residues (Lis and Sharon, 1998). They have been used to define the types and distribution of glycoconjugates on normal embryonic and adult cells (Damjanov, 1987). They have also been applied to study alterations in cell growth and differentiation in metaplastic and neoplastic conditions (Hsu and Ree, 1983).

The purpose of the present study was to detect specific carbohydrate residues of storage material in foamy cells in tissues of cattle exposed to *Brachiaria* spp.

Material and Methods

Experimental design

Two different groups of materials were used

Group A: Fragments of liver, duodenum and hepatic and mesenteric lymph nodes of 100 adult Nelore cattle, 3–4 years old, from the state of Mato Grosso, were fixed in 10% neutral formalin. The animals were grazing in pastures for almost all their life span. *Brachiaria decumbens* and *B. brizantha* were the predominant plants in those pastures.

Group B: Fragments of liver, duodenum and hepatic and mesenteric lymph nodes of five adult Holstein oxen, 3 years old, from the state of Rio Grande do Sul, were collected and fixed in 10% neutral formalin. They were grazing in pastures without *Brachiaria* spp. They served as negative controls.

Tissue sections

After 24–48 h fixation, the fragments were dehydrated with alcohol, cleared in xylene, embedded in paraffin wax, sectioned at 5 μm and stained with haematoxylin and eosin (HE) by routine processing methods.

Lectin histochemistry

Representative sections from liver and lymph nodes embedded in paraffin were incubated with the following biotinylated lectins: *Canavalia ensiformis* agglutinin (Con A; α-D-Man/α-D-Glc-specific); *Dolichos biflorus* agglutinin (DBA; α-D-GalNAc-specific); *Glycine max* agglutinin (SBA; α-D-GalNAc/β-Gal-specific); *Arachis hypogaea* agglutinin (PNA; β-D-Gal/(1–3)GalNAc-specific); *Ricinus communis* agglutinin-I (RCA – I; β-D-Gal/α-D-Gal-specific); *Ulex europaeus* agglutinin-I

(UEA-1; α-L-Fuc-specific); and *Triticum vulgaris* agglutinin (WGA; α-D-GlcNAc/NeuNAc-specific) (Goldstein and Hayes, 1978) (Lectin Kit Biotinylated BK 1000; Vector Laboratories Inc., Burlingame, CA, USA). The optimal concentration for each lectin, which allowed maximum staining with minimum background, was at a dilution of 30 μg/ml in PBS, except for PNA, which was applied at a concentration of 10 μg/ml.

After deparaffination, the sections were incubated in 0.3% hydrogen peroxide in methanol for 30 min at room temperature, rinsed several times in 0.01 M phosphate-buffered saline (PBS), pH 7.2, and treated with 0.1% bovine serum albumin in PBS for 15 min. They were then incubated with biotinylated lectins for 1 h, followed by incubation with avidin–biotin–peroxidase complex (Vector Laboratories Inc.) for 45 min. The horseradish peroxidase was activated by incubation for 4–10 min with a buffered Tris-HCl 0.05 M pH 7.6 solution containing 0.02% diaminobenzidine and 0.05% H_2O_2. All sections were counterstained with Mayer's haematoxylin.

The following controls were performed: the lectins were omitted or blocked by incubating them with their blocking sugars (0.1–0.2 M in PBS) for 1 h at room temperature before application to the sections.

Results

Gross findings

The livers from animals in group A were firm and with white yellowish discolouration. There were whitish spots of multifocal distribution scattered throughout the parenchyma. Hepatic and mesenteric lymph nodes had on the cut surface whitish grooves extending from the cortex to the medullary area, and multiple small white nodules in the medullary area. Additionally, there were red zones. No gross alterations were observed in the duodenum. No macroscopic changes were found in group B.

Microscopic findings

In group A all the animals showed hepatic changes. They presented diffusely distributed hepatocellular cloudy swelling, and marked multifocal cholangitis in the portal triads with bile duct proliferation and infiltration of macrophages and lymphocytes.

All animals had cells with foam cytoplasm in the liver (Fig. 1) and in the hepatic and mesenteric lymph nodes, often forming multinucleated cells. In the lymph nodes these infiltrates were adjacent to necrotic, haemorrhagic areas. In the liver, they were irregularly disseminated throughout the parenchyma or forming nodules around the central vein. In the duodenum all foamy cells were found in smaller number and scattered heterogeneously in the submucosa. No lesion were observed in tissues belonging to group B.

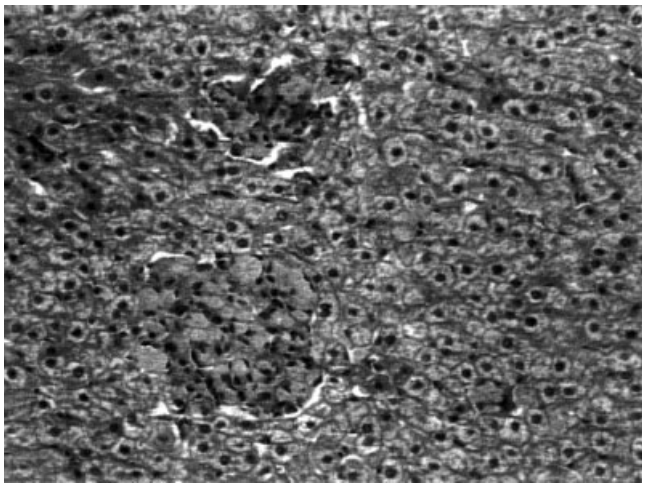

Fig. 1. Liver of an intoxicated animal. Hepatic parenchyma showing diffuse hepatocytic swelling. Foam cells grouped (arrows) or isolated (arrowhead) can be observed. (HE 400×).

Lectin histochemical findings

Results of the lectin-binding pattern for affected and control animals were summarized in Table 1. The lectin-binding pattern of the studied lectins to normal and intoxicated animals were compared. PNA, WGA and SBA lectins were the ones that bound better with the foamy cells. Especially PNA, in lymph nodes and liver, showed high affinity with the foam cells in contrast to its low affinity with the rest of the cells (Fig. 2).

Discussion

The presence of the foam cells was showed by retrospective studies in liver and lymph nodes of cattle grazing *Brachiaria* spp. in Brazil since 1976. This coincided with the introduction of the plant in this country (Driemeier et al., 1998, 1999). In 1992, a relationship between the macrophages and the consumption of *Brachiaria* spp. (P.V. Peixoto and C.H. Tokarnia, unpublished data) was established. The presence of these cells seems to be an evidence of chronicity because they do not appear during short periods of consumption (< 150 days) (Driemeier et al., 1998).

Similar foam cells, with accumulation of lipid substances, have been described in different tissues and disease states (Watanabe et al., 1982; Kleinert and Radner, 1987; Satti et al., 1990; Kamiya et al., 1991; Chen et al., 1997; Kuriwaki and Yoshida, 1999; Taraszewska et al., 2000; Iuliano, 2001).

The foam cells have been found in liver, mesenteric and hepatic lymph nodes but not in other lymph nodes outwith the

Table 1. Lectin binding to foam cells of liver and lymph node of cattle grazing *Brachiaria* spp. and controls

LECTIN	UEA-I		PNA		RCA-I		SBA		DBA		WGA		Con A	
	N	I	N	I	N	I	N	I	N	I	N	I	N	I
Foam cells in the liver	–	0	–	3	–	0	–	3	–	0	–	2	–	1
Foam cells in lymph nodes	–	2	–	3	–	2	–	2	–	1–2	–	3	–	0

N, control; I, affected.
Numbers indicate staining intensity on a subjective estimated scale from 0 (unreactive) to 3 (most reactive).

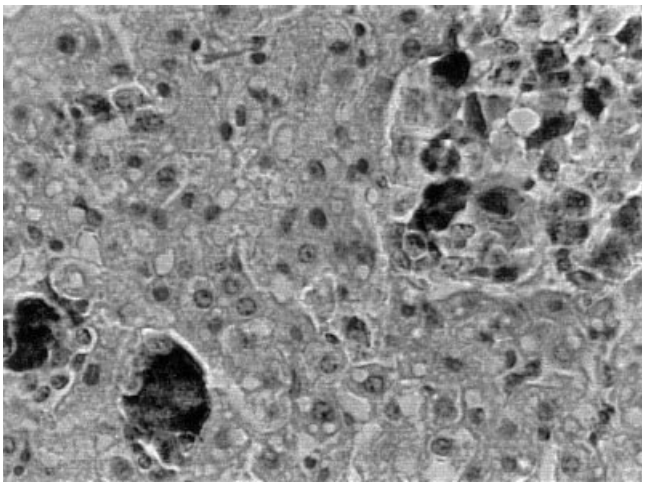

Fig. 2. Liver of an intoxicated animal. Section stained with peanut agglutinin (PNA) and counterstained with Mayer's haematoxylin. Foam cells are clearly distinguished either in groups or isolated (400×).

digestive tract, suggesting that the substances accumulated in these cells are probably absorbed by the enterohepatic cycle of bile by the intestines (Driemeier et al., 2002). Accumulation of foam cells was also found in the submucosa of the duodenum. However, light infiltration of foam cells in the submucosa of the duodenum can also be found in healthy cattle. A wasting syndrome was associated with the intestinal granulomatous lesions of cattle grazing *B. decumbens* (Riet Correa et al., 2002).

The composition of the material stored in the foam cells presented in animals intoxicated with *Brachiaria* spp. is still unknown. They contain crystals in its cytoplasm that are birefringent when examined with polarized light (Driemeier et al., 2002). Ultrastructurally crystalloid structures have been found within the hepatocytes and foam cells. These structures seem to be phagolysosomes (Driemeier et al., 1998). They also presented hyperplasia of smooth endoplasmic reticulum (Driemeier et al., 2002).

Considering that obstruction of biliary ducts in humans induces infiltration of macrophages (Patrick, 1983), there could be a relationship between the grade of biliar obstruction and the foam cells in this intoxication.

In the present study, we found that foam cells in liver and lymph node bind PNA, WGA and SBA in a selective way. The staining is particularly clear in liver, probably because hepatocytes, as a rule, do not react with lectins, most probably because of loss of the more soluble glycoconjugates during fixation (Faraggiana et al., 1982). Our results suggest that the foam cells contain large amounts of D-galactose, N-acetyl-D-galactosamine and N-acetyl-D-glucosamine.

The observed staining pattern of stored material is in line with histiocytic and xanthoma cells studied in human meningiomas (Kleinert and Radner, 1987). It is interesting to point out that foamy cells in papular xanthoma showed also other similarities to the foam macrophages of bovine grazing *Brachiaria*, for e.g. they are positive for oil red and macrophage markers, and negative for PAS (Chen et al., 1997). The ultrastructural features are also similar (Chen et al., 1997; Taraszewska et al., 2000).

The lectin staining is also partially comparable to the liver and spleen foamy cells found in human Hurler's disease, positive to PNA, WGA and TPA (*Tetragonolobulus purpureus*) (Faraggiana et al., 1982) and in human sphingomyelinosis, which were stained by PNA, SBA and Con A (Lageron, 1987). However, in cases of feline sphingomyelinosis, the stored material was positive for Con A, RCA-I and WGA in non-nervous tissues (Kamiya et al., 1991).

The molecules having the sugar residues reactive to PNA, WGA and SBA are unknown. These molecules could be undegraded substrates, such as N-linked oligosaccharides which accumulate due to deficient activity of the particular hydrolase causing a lysosomal storage lipidosis. They could also be the result of the formation of complex lipids resistant to the metabolism rather than an enzymatic lysosomal defect, the underlying molecular mechanism being similar to the so-called drug-induced lipidosis (Drenckhahn and Lullmann-Rauch, 1979; Halliwell, 1997). Although changes in cell carbohydrate components have been reported (Ruben et al., 1991), as far as we know, no lectin-binding studies have been conducted in this kind of lipidosis. The material produced by modifications of endoplasmic reticulum could become later on included in lysosomes as occurred in drug-induced lipidosis (Drenckhahn and Lullmann-Rauch, 1979; Robison et al., 1985).

The participation of steroidal sapogenins, mainly diosgenin, in *Brachiaria* spp. induced photosensitization has been established (Abdullah et al., 1992; Cruz et al., 2000). Anyway, the molecular mechanisms involved in *Brachiaria* spp. toxicosis are not know. It has been communicated that the steroidal saponins of *B. decumbens* produces lipid peroxidation (Zhang et al., 2001). It could be interesting to mention that other vegetal toxins, like the alkaloid sanguinine inhibit different lysosomal hydrolases, among them beta-galactosidase and *N*-acetyl-D-galactosaminidase (Balyaeva et al., 2003). These inhibitions would probably led to accumulation of material positive to PNA, SBA and WGA.

Morphological and histochemical characteristics suggest that an induced inhibition of lysosomal acidic lipase could also be involved in the pathogenesis of foam cells in *Brachiaria* spp. toxicity. A congenital deficiency of this enzyme, known as Wolman's disease, induces the development of similar foam cells with accumulation of cholesterol esters and triglycerides (Kuriwaki and Yoshida, 1999). Wolman's disease is a lipid storage disorder characterized by the formation of the already mentioned xanthoma cells (Takahashi and Naito, 1983).

Further histochemical and biochemical studies would be necessary to fully characterize the disease. Lectin histochemical studies merit further detailed study in conjunction with other histochemical tests as possible to clarify the genesis of foam cells in *Brachiaria* spp. grazing cattle. Besides, PNA was found to be an excellent marker to differentiate and quantify foam cells in the tissues of *Brachiaria* spp. grazing animals.

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