

Relationship of Intestinal Eosinophilia and the Acid-fast Bacilli in Johne's Disease

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ABSTRACT

Intestinal and lymph node slides from 12 cows that had necropsy documented, advanced disease (Johne's disease) due to *Mycobacterium avium* subspecies paratuberculosis (MAP) were analyzed to assess a possible relationship between the presence of eosinophils within the lamina propria and the demonstrability of acid-fast bacilli. In three instances where significant eosinophilia was identified, an inverse correlation with the amount of demonstrable acid-fast bacilli present within intestinal slides and corresponding lymph node was identified. The role of eosinophils in the ultimate destruction of mycobacteria is discussed.

INTRODUCTION

Eosinophils are considered to be end-stage granulocytic immune cells that are considered as having a homeostatic role in the defense against selected parasitic diseases as well as participation in a number of inflammatory and allergic reactions. Eosinophilic infiltration of tissues is an important Th2 inflammatory response.¹ Once elicited, the cytokine IL5 has a central role in promoting eosinophil differentiation from bone marrow precursor cells. This priming of eosinophils with IL5 facilitates their chemotaxis to eosinophil chemoattractants. IL5 primed

eosinophils to degranulate and release eosinophil-derived neurotoxin, eosinophil peroxidase, and eosinophil cationic protein.²

In 2003, a cow whose serum agar immunodiffusion test and serum ELISA detecting antibodies had repeatedly tested positive for *Mycobacterium avium* subspecies paratuberculosis (MAP) and whose feces had cultured out the organism, developed profuse, progressive diarrhea characteristic of the terminal stages of Johne's disease, which is a chronic granulomatous enteritis.

Desiring additional quantities of high titer anti-MAP antibody serum, the animal was removed from the dairy herd maintained by the University of Florida College of Veterinary Medicine and placed on a supplemented feed regimen. When initially removed from the herd for study, the animal had a low body condition score and weighed an estimated 460-475 kilograms.

Instead of dying in the anticipated 2-4 week period based upon body condition score, the animal survived and eventually thrived. When necropsied 4 months later, the animal weighed 668 kilograms. On gross inspection, the gastrointestinal tract was totally normal. Fourteen sections of jejunum and ileum and six sections of mesenteric lymph node failed to demonstrate the presence of any acid-fast organisms.³ Within the paracortex of several sections of lymph

Table 1. Comparison of the Presence of Acid-fast Bacilli and Increased Eosinophils in Lamina Propria and in Regional Lymph Node from Animals with Johne's Disease

Case	AFB Presence (Lamina Propria)	Eosinophilis (Lamina Propria)	AFB Presence (Lymph Node)
Pilot Case	"none"	massive*	"none"
NO4-242	"occasional to frequent"	"occasional to frequent"	"occasional"
NO4-450	"rare"	"moderate to high numbers"	not described (rare*)
NO4-528	"few"	"occasional"	"few"
NO4-672	"rare"	"large numbers"	"low numbers"
NO5-779	"small to moderate"	"few eosinophils"	"small to moderate"
NO5-790	"small"	"occasional"	"large"

* assessment from second pathologist

node, small clusters of Langerhans-type giant cells were present. The intestinal lamina propria exhibited massive infiltration by sheets of eosinophils.

The purpose of this paper is to assess the validity of the inferred inverse correlation between the eosinophilia within the lamina propria and demonstrability acid-fast mycobacteria.

MATERIALS & METHODS

Study Population

Twelve cases of Johne's disease within the necropsy files of University of Florida's Veterinary Medical Teaching Hospital constituted the study population. All 12 necropsies had been done by a senior veterinary pathologist who had special interest in Johne's disease.

Study Design

To avoid investigator bias, the identification of the presence or absence of eosinophils within the lamina propria, as well as the crude quantitative assessment of the number of acid-fast staining mycobacteria (AFB), was derived from the original necropsy report. The slides had been previously stained using Mayer's hematoxylin and eosin and Ziehl-Neelsen stains. The presence or absence of eosinophils and quantitative assessment of the number of AFB was substantiated by a second pathologist.

To further challenge the hypothesis suggested by the pilot case, the observations derived from microscopic examination of the small intestine slides, the amount of AFB identified in the corresponding mesenteric or ileal lymph node slides, was included in the analysis.

RESULTS

Absence of Eosinophils within the Lamina Propria

In all six cases in which identification of eosinophils was not achieved, the amount of demonstrable AFB in the tissue slides ranged from moderate to heavy.

Presence of Eosinophils within the Lamina Propria

In six cases in which the presence of eosinophils had been noted, the amount of eosinophilia ranged from rare to occasional/frequent. The corresponding amount of AFB demonstrated also exhibited variation (Table 1). In the two cases in which the presence of eosinophils was described as moderate to heavy, the number of AFB present was termed rare to low numbers.

AFB in Small Bowel

Examination of slides of jejunum, ileum, or both demonstrated that in 7 of the 12 cases, moderate to large numbers of AFB were present. In three cases, the amount of AFB characterized by the prospector was de-

scribed as small. In two cases, with moderate to heavy lamina propria eosinophilia, the presence of acid-fast bacilli was identified as being rare.

In the six cases in which eosinophils were not described in the description of the small bowel slides, the presence of AFB was readily apparent.

Intestinal Eosinophils & AFB in Lymph Nodes

The acid-fast status of the corresponding lymph nodes is recorded in Table 1. In the cases of NO4-450 and NO4-672, the number of AFB organisms in the mesenteric and or ileum lymph nodes was low. In the remaining four cases, the amount of AFB present was described as occasional to moderate. In the six cases where eosinophilia was not commented on nor identified in subsequent review, the number of AFB present ranged from moderate to frequent.

DISCUSSION

Descriptive observations derived from microscopy tend to be more qualitative than quantitative. Sometimes in the analysis of a disease, a distinct microscopic finding becomes a grouping criterion in lieu of an explanation as to why, eg, paucibacillus vs multibacillus Johne's disease; pulmonary eosinophilic syndromes, idiopathic hypereosinophilic syndrome etc.

The study data infers that the descriptive terms, multibacillary and paucibacillary Johne's disease, may be but vertical cuts through a single horizontal disease process rather than through two distinct vertical disease processes. A reduction of AFB within diseased bovine gastrointestinal tissue in association with increased eosinophilia could have been dismissed as coincidental were it not for the hypothesis inferred from the pilot case. When the presence of the increased presence of eosinophils within diseased tissue warranted comment from a senior academic veterinary pathologist, an inverse correlation with the number of AFB and the number of eosinophils present could be shown.

Eosinophilic infiltration of tissue is the consequence of cytokine activation and chemoattraction.^{1-2, 4-5} Initial containment of Map is deemed to be the consequence of a Th1 immune response. Conversion from a Th1 to a Th2 immune response has been theorized to signal release of Map from immune containment. What has been shown in Crohn's disease is that the cytokine patterns are distinct between early and late ileal lesions.^{2,5,7} The progression within Johne's disease from a Th1 to a Th2 is counterintuitive, unless immune regulation of Map/killing of Map appears is the function of a different part of the host immune system.

The ultimate destruction of helminthic pathogens is due to an IgE dependent degranulation of eosinophils within a Th2 immune response.¹ The microscopic observations cited suggest that a comparable mechanism may exist with respect to the ultimate destruction of Map.

While the number of study observations is small, the data is nevertheless consistent with the inference derived from the pilot case. Collectively, they suggest that eosinophils are involved in the ultimate destruction of MAP.

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