The effects of endophyte ingestion on immune function in sheep

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ABSTRACT

The ingestion of pasture with a high infection rate of endophyte may cause a variety of pathological conditions in livestock, such as ryegrass staggers, diarrhoea, hyperthermia and gangrene. This experiment was designed to ascertain the function of the immune system under such conditions.

Mixed age ewes were grazed during the summer months, on either high endophyte or low-endophyte ryegrass pastures. Lymphocytes were separated from peripheral blood samples by density gradient centrifugation, and subjected to an *in vitro* lymphocyte proliferation (lymphoblastogenesis) assay, using the mitogens phytohaemagglutinin (PHA), lipopolysaccharide (LPS), concanavalin-A (Con-A) and intestinal nematode (*Trichostrongylus colubriformis*) antigen.

There were significant differences ($P<0.05$) in the response to selected mitogens, between the group grazing high versus low endophyte pasture at 14 days (PHA) and 21 days (PHA and Con-A) after the commencement of differential grazing. The animals on high endophyte pasture showed higher stimulation indices. LPS or nematode antigen had no effect on the cultured lymphocytes.

As LPS is stimulatory for Bursal-derived (B) lymphocytes and PHA and Con-A for Thymic-derived (T) lymphocytes, it would appear as if material from the high endophyte pasture affected the latter cell population. It is unknown as to whether these effects on the immune system are important, *in vivo*.

Keywords: immune response, lymphoblastogenesis, *Acremonium*, ryegrass staggers.

INTRODUCTION

It is now established that the ingestion of Lolium endophyte (*Acremonium lolii*) is associated with the production of neurological symptoms in grazing herbivores, known as perennial ryegrass staggers (RGS) (Fletcher and Harvey, 1981; Mortimer *et al.*, 1982). This is believed to be caused by the alkaloid lolitrem B (Gallagher *et al.*, 1981) which is generated in endophyte infected ryegrass. Bioassay (Gallagher and Hawkes, 1985) methods have been used to detect lolitrem B in crude pasture preparations.

The ingestion of *A.lolii*-infected pasture has also been incriminated with other changes in grazing animals, such as diarrhoea (Fletcher and Sutherland, 1993), chronic liver damage (Piper, 1989), hyperthermia (Fletcher, 1993), changes in prolactin levels and decreases in animal production (Fletcher and Barrell, 1984). Recent research on a closely related endophyte (*Acremonium coenophialum*) found in tall fescue, indicated that the immune response of animals that had ingested these endophytes may be affected (Dew *et al.*, 1990).

In this preliminary study, the effect of ingested ryegrass infected with endophyte (*A.lolii*) on the immune function in sheep, is investigated.

MATERIALS AND METHODS

Animals:

Twelve, 16 month old Coopworth ewes, that had been treated one month previously with anthelmintic, were randomly assigned into two equal groups ($n = 6$), and grazed on pure ryegrass (*Lolium perenne*) pastures that had either high (>90%) or low (<5%) levels of endophyte infection, for 4 weeks. Herbage samples were taken from both the high and low endophyte pastures to assess the degree of infection using light microscopy and alkaloid levels (Barker *et al.*, 1993).

Lymphoblastogenesis Test (LBT):

Peripheral blood samples were taken from the jugular vein and collected in heparinised vacutainer tubes (Terumo Corporation, USA). The *in vitro* LBT was performed as described by Kambara *et al.*, (1993). Peripheral lymphocytes were separated by density - gradient centrifugation in 60% Percoll (Pharmacia, Uppsala, Sweden), and cultured in Dulbecco's Modified Eagle Media (D-MEM) containing 10% foetal calf serum with antibiotics. Cell suspensions were cultured in triplicate with mitogen or infective larvae (L_3A) antigen for 48 h, and then for a further 20 h with the addition of ^{3}H-thymidine. L_3A was prepared from the supernatant of *T.colubriformis* infective larvae that had been disrupted by crushing and sonic vibration, and was then filtered (0.2 μm), to be used at a concentration of 64 μg ml^{-1}. The mitogens used were as follows: Phytohaemagglutinin (PHA), Difco, Detroit, USA, at 12.8 μg ml^{-1}; Concanavalin A (ConA), Sigma, St Louis, USA, at 4.8 μg ml^{-1}; Lipopolysaccharide W (LPS), Difco, Detroit, USA, at 25 μg ml^{-1}. Cells were harvested onto paper filter discs using a cell harvester (Cambridge Technology, Watertown, USA) and ^{3}H-thymidine incorporation was measured in a liquid scintillation counter (Pharmacia, LKB,

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Uppsala, Sweden). The results were expressed as stimulation indices (SI): mean c.p.m. of stimulated cells divided by mean c.p.m. of cells cultured without mitogen or L_3A.

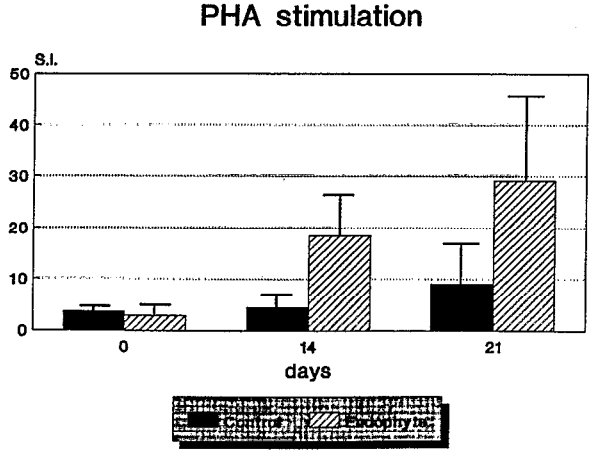
Statistical analysis: To determine statistical significance, the Student t- test using Minitab was carried out on the raw and log transformed data.

RESULTS AND DISCUSSION

In general, the stimulation indices were relatively low and there was a large variance in all data sets which is typical of this assay (Kristensen *et al.*, 1982). When the data was transformed the treatment effects were more obvious than with an analysis of the raw data.

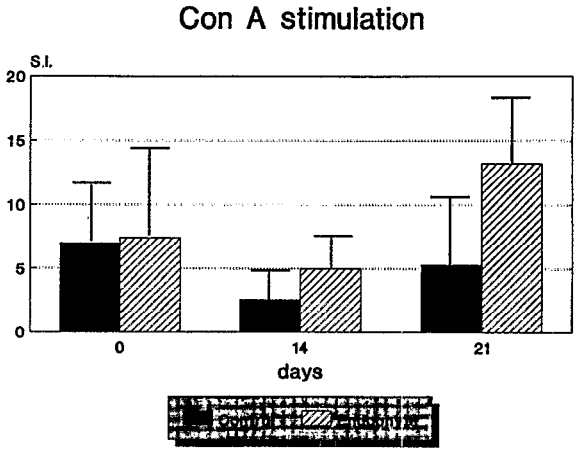
Over the course of the trial (3 weeks), the stimulation indices (SI) of the animals grazing high endophyte pasture tended to increase for both of the T cell mitogens, with mean changes from 7.4 to 13.2 and 2.9 to 29.1 for ConA and PHA respectively. The effects of stimulation by PHA were significantly different between the high versus the low endophyte treatment groups, at day 14 and 21 ($P < 0.05$), when data was transformed but were only significant at day 14 when the raw data was analysed (Fig. 1). When the lymphocytes were stimulated with ConA the effect of grazing high endophyte ryegrass was significantly different from the low group ($P < 0.05$), at day 21 (Fig. 2). In contrast, when lymphocytes were cultured with the B cell mitogen LPS or infective larvae (L_3) antigen, there was neither an increase in SI, during the trial period nor was there a difference between the two different endophyte treatment groups (data not shown). The levels of the alkaloids, ergovaline, peramine and lolitrem B were 0.95 ppm, 12 ppm and 5.5 ppm respectively, in the high endophyte pasture, but were not detectable in the low endophyte pasture. The high level of peramine and lolitrem B may have been the major contributors to the immunological findings in our current study.

FIGURE 1: Incorporation of tritiated thymidine into *in vitro* cultures of peripheral lymphocytes, following the addition of phytohaemagglutinin (PHA). Units are the mean stimulation indices ; standard deviation.



Of interest was the finding that the animals in the high endophyte treatment group were the individuals whose lymphocytes had the higher SI with the T cell mitogens. Our result is at variance with that of another study (Dew *et al.*, 1990), where a depressed immune response was found in rats and mice fed a commercial ration supplemented with tall fescue seed infected with *A.coenophialum.* This was indicated by a depressed B cell (LPS) and T cell (ConA) mitogen response, a depressed humoral response, and an increase in T suppressor cells. It is likely that ergovaline may have been a major contributor to the immunological findings of Dew *et al,* 1990, even though alkaloid analyses were not conducted in that trial. There may be other important factors that explain the differences between the two trials, such as the animal species (cattle versus mice) and lymphocyte source (peripheral blood versus spleen).

FIGURE 2: Incorporation of tritiated thymidine into *in vitro* cultures of peripheral lymphocytes, following the addition of Concanavalin A (Con A). Units are the mean stimulation indices ; standard deviation.



The increased SI may reflect proliferation of cytotoxic T lymphocytes, cytokine producing cells or non activated "bystander" cells. Clearly, it would be interesting to view these findings in parallel with a measurement of cytokines (particularly IL-2) and fluorescence activated cell sorter (FACS) analysis of lymphocyte subsets. It is not known whether these changes of the immune system are important *in vivo* and further studies are in progress to confirm and extend these findings.

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