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IFNγ AND IL-10 PRODUCTION BY HEPATIC LYMPH NODE AND PERIPHERAL BLOOD LYMPHOCYTES IN *FASCIOLA HEPATICA* INFECTED SHEEP

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Summary :

The present study was designed to determine whether IFNγ and IL-10 were secreted by peripheral blood mononuclear cells (PBMC) and hepatic lymph node mononuclear cells (HLNMC) from 17 *Fasciola hepatica* infected sheep after *in vitro* stimulation by *Fasciola hepatica* excretory-secretory products (FhESP) during the first six weeks of two different infections. The IFNγ concentration in mononuclear cells (MC) culture supernatants was determined by sandwich ELISA. The presence of IL-10 in MC culture supernatants was determined by the capacity of these to inhibit, with or without monoclonal antibodies to IL-10, the NO production of recombinant bovine IFNγ (rBoIFNγ) activated monocytes. FhESP-stimulated PBMC and HLNMC produced IFNγ in culture supernatants from DPI 7 (day post-infestation 7) to DPI 14. Culture supernatants of PBMC stimulated with FhESP first induced an increase in the NO production of rBoIFNγ activated monocytes. After 2 or 3 weeks of infection, MC culture supernatant reduced the NO production of rBoIFNγ activated monocytes. From DPI 35, anti-IL-10 antibodies addition restored or enhanced NO production. During sheep fasciolosis, IFNγ production increased only until the first two weeks post infection. IL-10 could be secreted throughout the first six weeks of infection and could inhibit monocytes from DPI 35.

KEY WORDS : *Fasciola hepatica*, lymphocyte, IL-10, IFNγ, hepatic lymph node.

Résumé : PRODUCTION D'IFNγ ET D'IL-10 PAR LES LYMPHOCYTES DES NŒUDS LYMPHATIQUES HÉPATIQUES ET DU SANG PÉRIPHÉRIQUE DE MOUTONS INFESTÉS PAR *FASCIOLA HEPATICA*

Cette étude a été réalisée afin de déterminer si les cellules mononucléées du sang périphérique (CMSP) et des nœuds lymphatiques hépatiques (CMNLH) de 17 moutons infestés par Fasciola hepatica, *stimulées avec des produits d'excretion-sécrétion de* F. hepatica *(PESFh), sécrétent de l'IFNγ et de l'IL-10, et ce durant les six premières semaines de deux infestations. La concentration d'IFNγ dans les surnageants de culture des cellules mononuclées (CM) a été mesurée par une technique d'ELISA sandwich. La présence d'IL-10 dans les surnageants de culture des CM a été déterminée par leur capacité à inhiber, avec ou sans anticorps monoclonaux anti-IL-10, la production de NO par des monocytes activés avec de l'IFNγ recombinant de bovin (IFNγrBo). Les CMSP et les CMNLH stimulées par les PESFh produisent de l'IFNγ entre le 7e et le 14e jour après infestation (entre JPI 7 et JPI 14). Les surnageants de culture des CMSP activées avec des PESFh induisent dans un premier temps une augmentation de la production de NO par les monocytes activées par l'IFNγrBo. Après deux à trois semaines post infestation, les surnageants de culture des CM entrainent une réduction de la production de NO. Après JPI 35, l'addition d'anticorps anti-IL-10 entraîne une restauration ou une augmentation de la production de NO. Durant la fasciolose chez le Mouton, la production d'IFNγ augmente uniquement durant les deux premières semaines d'infestation. L'IL-10 pourrait être sécrétée tout au long des six premières semaines d'infestation et pourrait inhiber les monocytes après la 5e semaine.*

MOTS CLÉS : Fasciola hepatica, *lymphocyte, IL-10, IFNγ, nœud lymphatique hépatique.*

INTRODUCTION

Fasciolosis is a parasitic disease that causes considerable economic loss in ruminants. Chemotherapy is commonly used to prevent this disease, with several disadvantages (residues in animal products, drug resistance). Therefore, medical prophylaxis could be used to avoid these disadvantages. Several vaccination studies have been carried out by infecting animals with *Fasciola hepatica* irradiated metacercariae (Campbell *et al.*, 1978) or by immunizing them with parasitic substances, like excretory-secretory products (Rajasekariah *et al.*, 1979), T1 antigen (Hanna *et al.*, 1988), glutathione S transferase (Sexton *et al.*, 1990), cathepsin-L protease (Wijffels *et al.*, 1994), haemoglobin (Dalton *et al.*, 1996). These have not given complete protection against *F. hepatica*. At the present time, in order to improve the efficacy of vaccination, we must better understand the immune mechanisms during fasciolosis (effector mechanisms developed by the host, evasion mechanisms developed by *F. hepatica*) to define precisely the targets for the vaccine.

Experimentally, most steps of the effector mechanisms of antibody-dependent-cellular-cytotoxicity (ADCC)

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seem to take place during fasciolosis : recruitment and adherence of eosinophils around flukes (Rushton & Murray, 1977; Keegan & Trudgett, 1992; Chauvin *et al.*, 1995), degranulation and liberation of substances toxic for the parasite (Eosinophil Peroxydase EPO, Major Basic Protein MBP) inducing vacuole formation in the fluke tegument and breaking its continuity (Duffus *et al.*, 1980; Davies & Goose, 1981). But these mechanisms do not seem to be efficient in preventing the development of flukes. Indeed, it has been demonstrated that *F. hepatica* shows permanent turnover of its surface antigens, inducing the formation of immune complex precipitates on the juvenile fluke surface (Hanna, 1980; Sandeman & Howell, 1980; Glauert *et al.*, 1985; Tkalevic *et al.*, 1996) which prevents effector cells adhering to juvenile flukes. *F. hepatica* excretory-secretory products (FhESP) contain proteolytic enzymes such as cysteine proteases (cathepsin L-like) (Chapman & Mitchell, 1982; Smith *et al.*, 1993a; Smith *et al.*, 1993b) which cleave *in vitro* immunoglobulins and inhibit the *in vitro* adherence of eosinophils to juvenile flukes in the presence of immune serum (Carmona *et al.*, 1993). It has also been observed that flukes migrating in the hepatic parenchyma are covered by IgM (Chauvin & Boulard, 1996), when eosinophils do not possess a Fcμ-receptor.

During murine schistosomosis (*Schistosoma mansoni*) another effector mechanism mediated by gamma interferon (IFNγ) activated macrophages has been demonstrated (Sher *et al.*, 1992). But the parasite can evade this mechanism by inducing the secretion of IL-10 which inhibits macrophage effector functions (Sher *et al.*, 1992). Thus, during murine schistosomosis, an early protective mechanism regulated by Th1 cytokines is controlled by a second mechanism regulated by Th2 cytokines which favours chronic infection by the parasite.

The present study was designed to test the hypothesis that in ovine fasciolosis, an effector mechanism mediated by IFNγ-activated macrophages was inhibited by IL-10 secretion. So, we determined the kinetics of the cytokines IFNγ and IL-10 production by blood and hepatic lymph node sheep lymphocytes stimulated by FhESP during the first six weeks of *F. hepatica* infection.

MATERIALS AND METHODS

Animals infection

Belle-Ilois sheep approximatively 18 months old were used throughout. The animals were born and maintained in sheep-fold. Food and water were available *ad libitum*.

Twelve to 18 month old sheep were selected for the ability of their lymphocytes to respond to Concanavalin A, as described previously by Zimmerman *et al.* (1983), in order to limit the dispersion of the cellular response. They were infected *per os* with a homogeneous batch of 250 *F. hepatica* metacercariae. The success of the infection was estimated by evaluation of the anti-FhESP antibody response by ELISA, as described by Chauvin *et al.* (1997), by examination of faeces for *F. hepatica* eggs from the 8th week and by counting the flukes present in the liver at necropsy.

In protocol A, five sheep were infected, and five others served as a control group. Every week during the first six weeks of infection, the proliferation of peripheral blood mononuclear cells (PBMC) induced by FhESP and cytokine (IFNγ and IL-10) secretion by these *in vitro* stimulated PBMC was measured.

In protocol B, 20 sheep were randomized in four groups: one control group, three infected groups. The control group was composed of eight sheep. The infected groups were each composed of four animals infected with 250 *F. hepatica* metacercariae. Animals in the first infected group were necropsied ten days post-infection (DPI 10), those in the second infected group at DPI 20 and those in the third infected group at DPI 42. Two animals in the control group were necropsied at DPI 10, DPI 20 and DPI 42. Proliferation of PBMC to FhESP was measured at DPI 10, DPI 20, DPI 30 and DPI 42 as well as proliferation of mononuclear cells of hepatic lymph nodes (HLNMC) to FhESP at DPI 10, DPI 20 and DPI 42. Cytokine (IFNγ and IL-10) secretion by these lymphocytes was also characterized.

Mononuclear cell purification

Peripheral blood mononuclear cells

Blood samples (20 ml) were collected from a jugular vein into 10 ml sterile heparinized vaccuum collecting tubes (Venoject, France). The mononuclear cells were separated by density gradient centrifugation, as previously described by Chauvin *et al.* (1995). After centrifugation at 700*g* for 20 min, the buffy-coat was removed, diluted in 6 ml of RPMI 1640 (Eurobio, France) and layered over 6 ml of Ficoll-hypaque (d = 1.077, Eurobio, France). After centrifugation at 700 *g* for 40 min, the mononuclear cells were collected and washed three times with RPMI 1640. The cells were resuspended in culture medium (complete RPMI: RPMI 1640, 10 % Fetal Calf Serum, 50 μg/ml gentamicin, 2.5 μg/ml amphotericin B, 2 mM L glutamine, 1 mM pyruvate, 50 μM βmercaptoethanol). The cell count was determined and cells diluted to a concentration of 2×10^6 cells/ml in complete RPMI.

Hepatic lymph node mononuclear cells

Hepatic lymph nodes (HLN) were washed in Ca^{2+} and Mg^{2+} free medium HBSS (Hank's balanced salts

medium, Eurobio, France) and placed in a Petri dish. Thirty milliliters of HBSS medium were injected with a syringe into the HLN. Then the HLN was pressed and the collected liquid containing the HLN cells was centrifuged at 300 *g* for 10 min. The cells were then diluted in 7 ml of RPMI 1640 and layered over 6 ml of Ficoll-hypaque. The remaining steps were as for PBMC purification.

MONONUCLEAR CELLS CULTURE

Mononuclear cells (2×10^5 cells/well) from each sheep were cultivated in triplicate in the presence of either FhESP at 1.25 or 5 µg/well (FhESP stimulated culture) or in the absence of FhESP (six wells) (control culture). After five days of culture at 37 °C in a humidified atmosphere with 6 % CO_2, 100 µl of the mononuclear cell (MC) culture supernatant was collected in each well and frozen at – 80 °C for cytokine quantification. Lymphoproliferation was estimated by tritiated thymidine incorporation.

For each sheep, stimulation indices (S.I) were calculated:

$$\text{S.I.} = \frac{\text{mean CPM of triplicate FhESP stimulated cultures}}{\text{mean CPM of triplicate control cultures}}$$

The activation of lymphocytes from infected sheep was expressed as a percentage of the activation from the control group:

$$\% \text{ activation} = \frac{\text{mean of infected sheep S.I.}}{\text{mean of control sheep S.I.}} \times 100$$

The infected group was compared with the control group by the non-parametric Mann-Whitney test at each study time point.

IFNγ ASSAY

IFNγ secreted by lymphocytes from infected sheep stimulated by FhESP (5 µg/well) in culture supernatants was measured with a *Mycobacterium paratuberculosis* IFNγ test kit (IDEXX Laboratories, USA). This is a sandwich ELISA for the detection of a cellular immune response to *M. paratuberculosis* in infected cattle. One hundred microliters of MC culture supernatant was added to a 96- well plate coated with an anti-BoIFNγ. After incubation for one hour at room temperature, a peroxidase-conjugated anti-BoIFNγ immunoglobulin was added at the plates and incubated for 30 min. The peroxidase substrate TMB (1, 1'-trimethylene-bis (4-formylpyridinium bromide) dioxime) was then added. The absorbance was read at 405 nm. The IFNγ concentration of the sample was calculated with reference to a standard curve using recombinant bovine IFNγ (rBoIFNγ), produced and kindly donated by Ciba-Geigy (Switzerland), at concentrations ranging from 0.325 to 10 ng/ml in complete RPMI.

For each infected sheep, IFNγ concentrations at each timepoint post-infection were compared by the non-parametric Wilcoxon test.

IL-10 ASSAY

IL-10 activity of MC culture supernatants was estimated by the evaluation of the capacity of MC culture supernatants to inhibit the production of NO by rBoIFNγ activated monocytes. The role of IL-10 in this inhibitory activity was confirmed by blocking this effect with anti-IL-10 monoclonal antibodies provided by G. Barcham of the University of Melbourne, which were used at a dilution of 1/400.

All FhESP-stimulated MC culture supernatants of infected sheep were tested at each date. Some supernatants of the control group were also tested to study the actions of FhESP and substances produced by the lymphocytes from non-infected sheep on monocytes.

Monocyte purification

After PBMC isolation from a healthy sheep, the monocyte population was enriched by allowing the cells to adhere to sterile plastic Petri dishes for one hour at 37 °C (6 % CO_2) in complete RPMI. Non-adherent cells were removed by washing them four times with warm HBSS. "Monocytes" were removed by gently scraping the Petri dishes, and washed once in HBSS. At this stage the cellular suspension contained 70 to 80 % of monocytes (estimation by fluorescence with macrophage surface ovine marker OM1 (Pépin *et al.*, 1992)).

Monocyte culture

The monocytes (10^5/well) were cultured in microplates. Two controls were included:

- cell controls in which monocytes were cultivated only in the presence of complete RPMI defined previously,
- stimulation controls in which monocytes were cultivated in the presence rBoIFNγ at 0.1 ng/well.

The activity of IL-10 from each MC culture supernatant on the rBoIFNγ activation of monocytes was tested in the presence or absence of anti-IL-10 antibodies. Reagents were added in the following order: 25 µl of rBoIFNγ (0.1 ng/well), 10 µl of anti-IL-10 (final dilution 1/400) or 10 µl of complete RPMI, 50 µl of culture supernatant, 15 µl of monocytes (10^5 cells/well). Plates were incubated at 37 °C (6 % of CO_2) for 48 hours.

Measurement of monocyte nitrite production

Monocyte culture supernatants were assessed for evidence of NO production by detection of NO_2, the stable oxidized form of NO using the Griess reaction. Fifty microliters of samples in duplicate were added to a 96-well plate followed by 50 µl of a mixture at 1:1 of 1 % sulfanilamide in 2.5 % H_3PO_4 and 0.1 %

naphtylenediamine dihydrochloride in 2,5 % H_3PO_4. After ten min reaction, the absorbance was read at 550 nm. The NO_2 concentration of the sample was determined with reference to a standard curve using sodium nitrite at concentration ranging from 1,5 to 200 µM in complete RPMI.

Two indices of stimulation were calculated :

- S.I'.: the capacity of MC culture supernatant to inhibit or co-stimulate rBoIFNγ activated monocyte NO production.
- S.I'.IL-10: the capacity of anti-IL-10 to restore rBoIFNγ activated monocyte NO production inhibited by MC culture supernatant. In this case, inhibition of NO production was probably due to IL-10 secreted by FhESP stimulated MC.

$$S.I.' = \frac{\text{mean of duplicate [NO] produced by monocytes in presence of rBoIFN}\gamma\text{ and supernatants}}{\text{mean of duplicate [NO] produced by monocytes in presence of rBoIFN}\gamma}$$

$$S.I.'IL\text{-}10 = \frac{\text{mean of duplicate [NO] produced by monocytes in presence of rBoIFN}\gamma\text{, supernatants and anti-IL-10}}{\text{mean of duplicate [NO] produced by monocytes in presence of rBoIFN}\gamma}$$

For each infected sheep, the S.I.' at each timepoint post-infection was compared using the non parametric Wilcoxon test. For each studied DPI, infected sheep S.I.' and S.I.'.IL-10 were compared by the non parametric Mann-Whitney test.

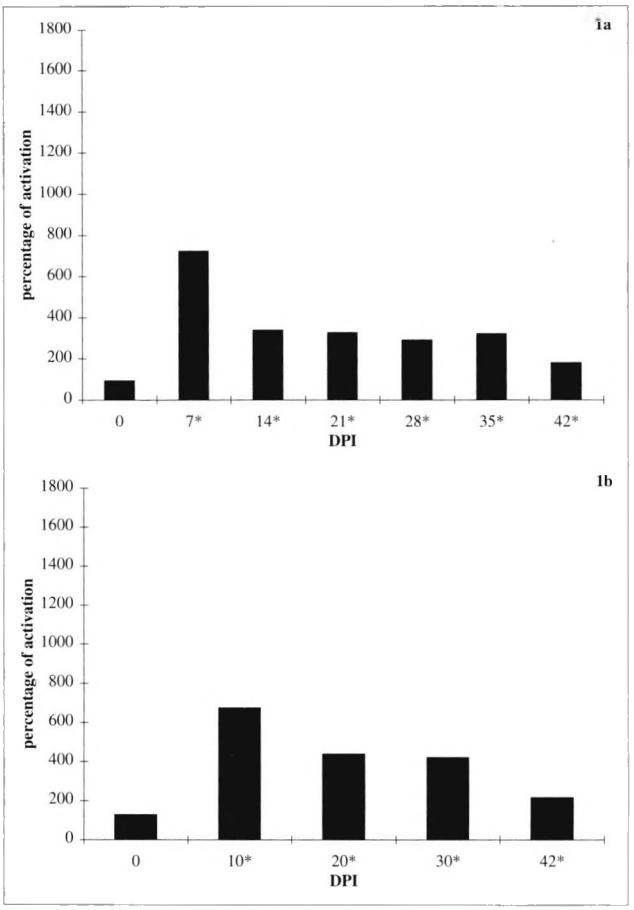


Fig. 1. – Proliferative responses of peripheral blood mononuclear cells induced by 5 µg/well of FhESP in *F. hepatica*-infected sheep for protocols A (1a) and B (1b) during the six first weeks of infection. * indicates significant differences between the infected and the corresponding control groups ($p < 0.05$) by Mann-Withney test.

RESULTS

Development of infection

The sheep in experiment A were successfully infected: between 24 and 58 flukes were found in their liver at 14 weeks post-infection.

In the livers of experiment B infected sheep, numerous macroscopic, tortuous migratory tracts were observed. These chronic hepatitis lesions were typical of *F. hepatica* infection. Anti-FhESP-antibody responses estimated by ELISA developed in a classical manner. No flukes were recovered in the liver because in this experiment B sheep were necropsied from DPI 10 to DPI 42 (adult flukes reach the bile ducts from 56-60 DPI).

Lymphoproliferation induced by FhESP

Peripheral blood mononuclear cells

Lymphoproliferation in response to FhESP increased in a significant manner ($p < 0.05$) from DPI 7-DPI 10 (Fig. 1). The response then decreased gradually and at DPI 42, the values of % activation were near those observed at DPI 0. A dose-dependant effect was observed, the intensity of the response being lower with a 1.25 µg dose of FhESP (data not shown).

However at DPI 7, the intensity and kinetic of the lymphoproliferation in the presence of FhESP varied between infected individuals. Stimulation indices varied from 1.78 to 115.14 for protocol A.

Hepatic lymph node mononuclear cells

Proliferation of HLNMC in infected sheep in response to FhESP increases throughout the course of infection (Fig. 2). This increase was not statistically significant, perhaps due to the stimulation of control sheep HLNMC by FhESP (S.I. >2) at 5 µg/well, to the individual heterogeneity (in each infected group, HLNMC of one sheep did not proliferate in presence of FhESP) or to the low number of sheep used at each date (four infected, two control).

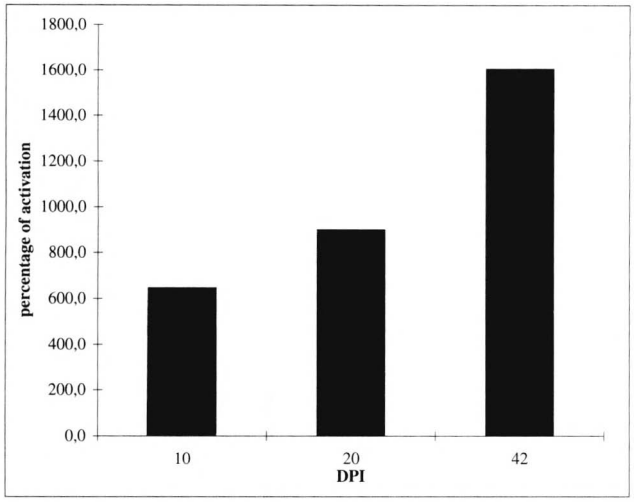


Fig. 2. – Proliferative responses of hepatic lymph node mononuclear cells induced by 5 µg/well of FhESP in *F. hepatica* infected sheep for protocol B during the six first weeks of infection. Significant differences between the infected and the corresponding control group were not observed.

Sheep	S.I.'
2A	0.61
4A	0.84
6A	1.78
7A	0.72
10A	0.61
9B	0.74
10B	1.05
11B	0.79
12B	0.97
EB	0.13
FB	2.04
GB	2.63
HB	0.13

Table 1. – NO production (stimulation indices SI') of rBoIFNγ-activated monocytes incubated with FhESP-stimulated PBMC culture supernatant collected on DPI 0 in protocol A and protocol B infected sheep (2A, 4A, 6A, 7A, 8A, 10A, 9B, 10B, 11B, 12B) and in protocol B control group (EB, FB, GB, HB).

IFNγ PRODUCTION

IFNg production of PBMC control sheep was low (0.159 ng/ml ± 0.016 (confident value 1%)). IFNγ production of PBMC infected sheep stimulated by FhESP increased early and transiently in the course of fasciolosis infection (Fig. 3). This increase was statistically significant ($p < 0.05$) from DPI 7-DPI 10 and reached a peak at DPI 7 or DPI 14. For two sheep in protocol B, the production of IFNγ was very low during the infection (data not shown). From DPI 21-28, IFNγ concentration declined to baseline. No correlation between the amount of IFNγ produced and the lymphoproliferative response induced by FhESP or between the amount of produced IFNγ and the parasitic intensity was observed in either experiment.

There was also an early, transient increase in IFNγ production by infected sheep HLNMC (Fig. 4). This was seen especially in three out of four sheep at DPI 10 (IFNγ concentration higher at 6 ng/ml for two sheep) then fell at DPI 20 and DPI 42 (concentrations below 1.5 ng/ml in DPI 20 and 0.5 ng/ml in DPI 42).

EFFECTS OF MONONUCLEAR CELLS CULTURE SUPERNATANTS ON RBOIFNγ-ACTIVATED MONOCYTE NO PRODUCTION

Culture supernatants of FhESP-stimulated PBMC collected on DPI 0 were tested. Considerable individual variation was observed (Table 1): eight out of 13 supernatants tested decreased rBoIFNγ-activated monocyte NO production, two had no effect and three increased rBoIFNγ-activated monocyte NO production.

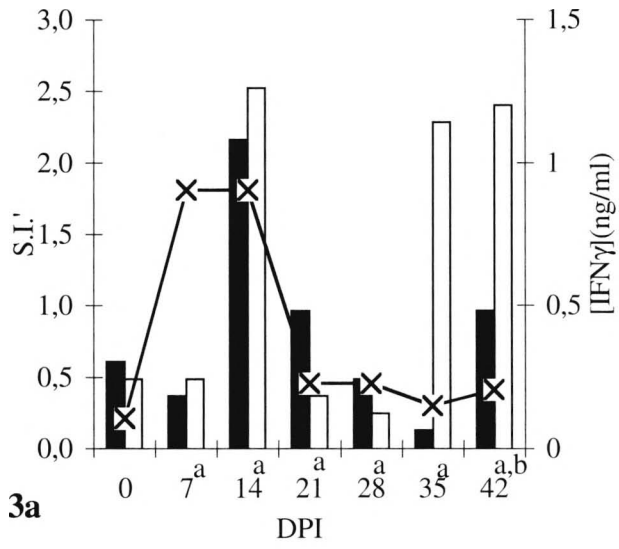
The effect of PBMC culture supernatants from experiment A sheep on rBoIFNγ-activated monocytes NO production is shown in figure 3. The same results were observed during protocol B (data not shown). The results varied among individuals. For the majority of sheep (six out of nine tested sheep)(Fig. 3a, b, c), PBMC culture supernatants enhanced then decreased NO secretion of activated monocytes. The increase-decrease delay was 20 days and started between DPI 7 and DPI 21. For two sheep of protocol A, the effect of PBMC culture supernatants activity was different: there was an inhibition on monocyte activation throughout the course of infection in one case (Fig. 3d) and two periods of activation/inhibition in another case (Fig. 3e).

At DPI 10, no effect of HLNMC culture supernatant was observed, except for one sheep (B4) whose supernatants induced an inhibition of rBoIFNγ-activated monocyte NO production (Fig. 4). After DPI 10, HLNMC culture supernatants enhanced monocyte NO production for five sheep, had no effect for two sheep and inhibited monocyte NO production for one sheep.

IL-10 PRODUCTION

The anti-IL-10 effect on PBMC culture supernatants is shown in figure 3. In the majority of timepoint throughout the course of infection, when a decrease of monocyte NO production induced by PBMC culture supernatants was observed, anti-IL-10 antibodies restored NO production. From DPI 35 anti-IL-10 antibodies addition restored or enhanced NO production with a significant difference between S.I.' and S.I.'IL-10 at DPI 42 ($p < 0.05$).

Thus for four sheep (Fig. 3a and 3b), when PBMC culture supernatants inhibited monocyte NO production



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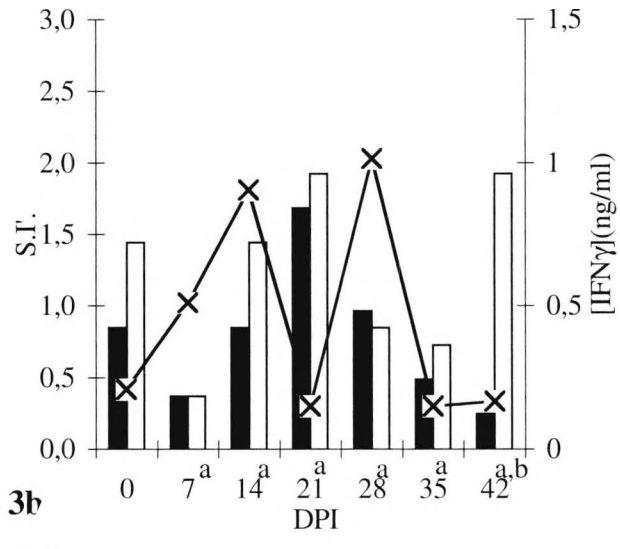

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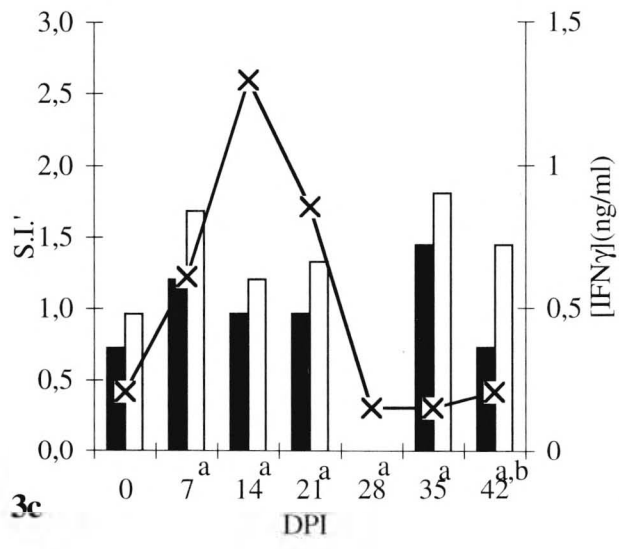

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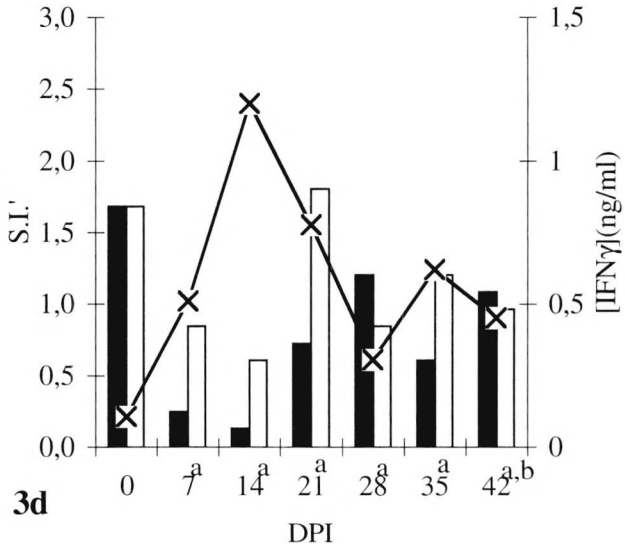

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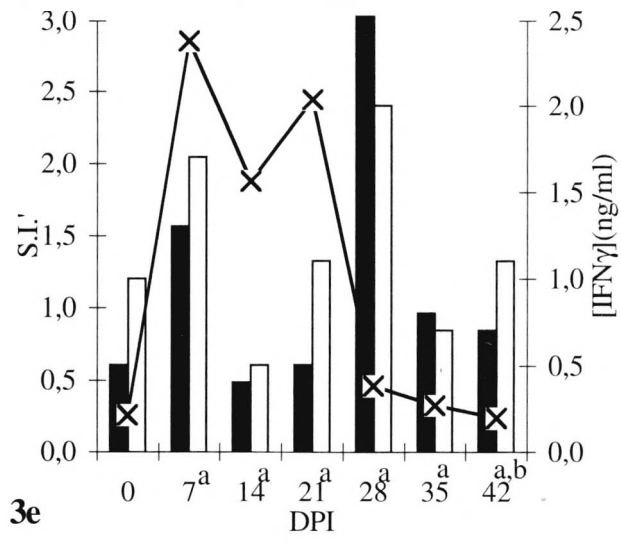

3e

Fig. 3. – Production of IFNγ by FhESP stimulated PBMC in protocol A sheep and NO production of (×) rBoIFNγ-activated monocytes incubated with FhESP-stimulated PBMC culture supernatants without (S.I.') (■) or with (S.I'.IL-10) (❒) anti-IL-10 antibodies. (a) indicates significant differences between IFNγ production between DPI 0 and DPIn by Wilcoxon test ($p < 0.05$). (b) indicates statistical differences between S.I'. and S.I'.IL-10 by Mann Withney test ($p < 0.05$).

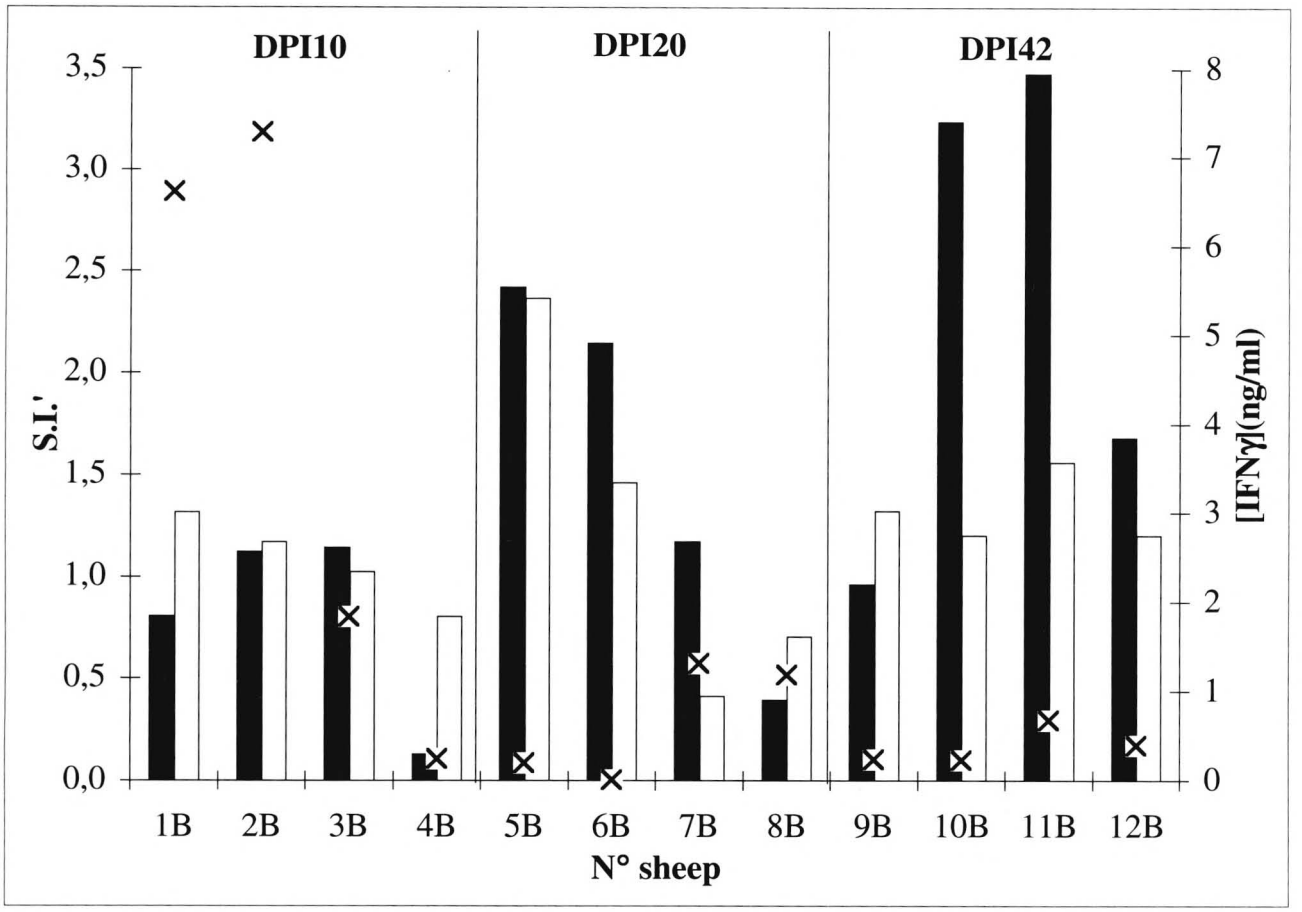

Fig. 4. – Production of IFNγ by FhESP stimulated PBMC in protocol A sheep and NO production of (×) rBoIFNγ-activated monocytes incubated with FhESP-stimulated PBMC culture supernatants without (S.I.') (■) or with (S.I'.IL-10) (❐) anti-IL-10 antibodies.

between DPI 20 and DPI 42, anti-IL-10 antibodies restored or enhanced monocyte NO production. For three sheep (Fig. 3c and 3e), when the PBMC culture supernatants induced a slight increase of monocyte NO production (DPI 30 or DPI 42), anti-IL-10 antibodies strongly increased NO production. For one sheep (Fig. 3d) whose monocyte activation was inhibited by PBMC culture supernatants all along the infection, it was restored by anti-IL-10 antibodies.

When HLNMC culture supernatants had no effect or inhibited NO liberation by rBoIFNγ-activated monocytes, anti-IL-10 antibodies restored or increased this activation (Fig. 4). But when an additive or synergistic effect between HLNMC culture supernatants and IFNγ was observed, anti-IL-10 antibodies diminished this effect.

DISCUSSION

Proliferation of PBMC from *F. hepatica* infected sheep in response to FhESP was observed precociously and transiently, between DPI 10-14 and DPI 35, as previously described in sheep (Chauvin *et al.*, 1995), in rats and cattle (Oldham, 1985; Oldham & Williams, 1985; Poitou *et al.*, 1992). At DPI 7, the stimulation was at times very high (S.I. = 115 for one sheep of protocol A), with a great heterogeneity among sheep. This phenomenon did not seem to be linked to individual parasitic burden. The kinetics of the local response of HNLMC was different from the general cellular response. It was precocious and increased throughout the course of infection. HLNMC proliferation in the presence of FhESP was stronger than PBMC proliferation. This was probably due to the fact that HLN were efferent to the liver and therefore drained the lymphocytes that had been directly in contact with flukes.

During fasciolosis, PBMC and HNLMC stimulated by FhESP secreted IFNγ only in the first two weeks of infection. From DPI 21-28, no production of IFNγ could be observed, as described by Clery *et al.* (1996) in chronically infected cattle. Culture supernatants of mononuclear cells stimulated by FhESP induced a precocious increase in NO production of rBoIFNγ-activated

monocytes. But this monocyte activation was not associated with IFNγ secreted by FhESP-activated lymphocytes as no correlation could be observed between the supernatant activity on monocytes and the measured IFNγ secretion peak for mononuclear cells in each sheep. Furthermore, HLNMC secreted high amounts of IFNγ on DPI 10 but lymphocyte culture supernatants did not induce monocyte activation. Various other lymphocyte mediators could be produced and act as activators substances of monocytes, like TNFα. The stage of development of the flukes may be important in the modulation of cytokine liberation. The secreted IFNγ could also be inactivated by the presence of inhibitory substances or by structure modification. Further studies are necessary to investigate the other secreted interleukins and the activity of IFNγ secreted by lymphocytes in infected animals.

Results of the present study suggested the IL 10 secretion by FhESP-stimulated mononuclear cell which could decreased rBoIFNγ-activated monocyte activation. The decrease of NO production could be due to several substances but from DPI 35, anti-IL-10 antibodies restored or increased NO production suggesting that this decrease was due to IL 10 and so, that FhESP-activated mononuclear cells secreted IL-10. But in some cases, the results suggests that supernatants contained IL-10 throughout the course of infection. The activatory or inhibitory effects of FhESP-activated lymphocyte culture supernatants on rBoIFNγ activated monocyte were probably due to an imbalance of activator and inhibitor substance production. In mice, IL-10 is a product of Th0 or Th2 lymphocyte (Mosmann *et al.*, 1986). Brown *et al.*, (1994) described, in chronically infected cattle, T cell clones which responded to total extracts of *F. hepatica* expressed Th0 or Th2 cytokine profiles (IL-2, IL-4, IFNγ).

IFNγ-activated macrophages are thought to be important cells of protective immunity against *schistosoma* in mice (Sher *et al.*, 1992). Macrophages activated by IFNγ treatment are potent killers of *schistosoma* larvae *in vitro* (James & Sher, 1990). But IL 10 is secreted during schistosoma infection and influenced the killing activity of IFNγ-activated macrophages against schistosomula (Sher *et al.*, 1991). This cytokine is known to affect IFNγ effects on macrophages including TNFα, IL 1 and IL 6 production, the microbicidal activities by decreasing the release of reactive oxygen intermediates and NO and the antigen presentating function by reducing class II MHC molecule expression (Bogdan *et al.*, 1991; Fiorentino *et al.*, 1991a; Fiorentino *et al.*, 1991b; Gazinelli *et al.*, 1992; De Waals *et al.*, 1991).

During fasciolosis, as during murine schistosomosis, after a short period of activation by IFNγ and other substances, the monocyte/macrophage system could be inhibited from DPI 35 by IL-10 secretion. The induction of IL-10 secretion during fasciolosis should be confirmed since it could be considered to be an evasion mechanism of the efficient host inflammatory and immune responses.

Spithill & Dalton (1998) suggested that in cattle vaccinated with fluke cathepsin L protease 2 and liver fluke haemoglobin, protective immune responses may be of the Th1 type involving IFNγ-activated macrophages and cytotoxic T cells. As in schistosomosis, the evolution of a protective response regulated by Th1 cytokines, to an unprotective response regulated by Th2 cytokines could be involved in fasciolosis. The role of the early Th1 cytokine (and specially IFNγ) equilibriums in the host effector mechanisms and in parasitic evasion mechanisms remains to be determined.

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