

understand the genetic mechanisms of susceptibility to ivermectin and acquired refractiveness.

2. *M. martini* is normally susceptible to suramin, levamisole and albendazole.

3. Eight drugs provided by WHO have been tested : CGP 20376, CGP 6140, CGI 18041, Artemether, Trichloromethylthiadiazole, Trifluoromethylguanidinopyrimidine, and the two benzimidazoles, UMF 078 and UMF 289. Four principal data were shown :

a. The macrofilaricidal activity is first demonstrated by the abnormalities of the embryogenesis in the uteri of the female worms ; divided eggs are the most fragile stages ; uterine microfilariae persist (CGP 6140) or are destroyed (UMF 078 and 289, etc.).

b. A migration of the adult worm is generally observed. *M. martini* filariae are normally in the lymphatic vessels of the intestine wall. When treated, they are no more able to maintain themselves *in situ* and they are drawn passively towards the pulmonary blood system *via* the thoracic duct and right heart ventricle. This migration induces lymphangitis and sometimes thrombosis followed by infarction (albendazole, CGI 18041). Similar events may occur in all lymphatic filariae (*Brugia*, *Wuchereria*, and ? non nodular *Onchocerca volvulus*).

c. When a macrofilaricide is also microfilaricide, it may induce a Mazzotti reaction, as with DEC : microfilariae are driven from the lymphatic vessels, in which they normally inhabit, and they are destroyed in the perivascular connective tissue by a multitude of synchronous inflammatory reactions (albendazole, UMF 078, UMF 289).

d. A proportion of microfilariae and adult filariae do not migrate and are destroyed *in situ* in the lymphatic vessels, inducing lymphangitis.

On the basis of two trials performed, UMF 289 seems to link high macrofilaricide efficacy and more rapid recovery of the side-effects than the two other benzimidazoles.

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PARENTERAL ADMINISTRATION OF CYTOKINES AND DRUGS IN RODENT FILARIASIS VIA OSMOTIC PUMPS

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KEYWORDS : Interferon- γ , osmotic pump, *Lemniscomys striatus*, *Monanema martini*.

SUMMARY

Changes in the cytokine production profile are supposed to be responsible for the different courses of parasitic infections. Therefore, a big effort is made to understand the roles of cytokines produced by different cells during parasitic infections. In the following text we describe first experiments with Interferon- γ done in a natural model for filariasis, the filariae *Monanema martini* in *Lemniscomys striatus*. For the application of this cytokine to the animals we implanted osmotic pumps, which deliver IFN- γ continuously.

INTRODUCTION

Cytokines play an important role in the regulation of the immune response caused by parasitic infections. Two sets of cytokines are responsible for the activation of different effector mechanisms (Mosmann *et al.*, 1989). Interleukin-2 (IL-2) and interferon- γ (IFN- γ), produced by Th1-lymphocytes, are important for the activation of macrophages, cytotoxic T-lymphocytes and natural killer cells, whereas IL-4 and IL-5, produced by Th2-lymphocytes, induce an Ig E response and lead to the generation of eosinophilia. The type of cytokines produced depends on the host, the parasite, the site and the phase of infection. While Th1 responses are usually directed against intracellular parasites, the responses of Th2 often act against intestinal helminth infections. These patterns, however, are not uniform. Filarial infections often cause Th2 responses, but filariae possess different evasion strategies, especially the downregulation of the immune defense resulting in chronic infections (Behnke, 1987). Immunological hyperreactivity is associated with severe pathology, i.e. sowda in the case of onchocerciasis.

The dichotomy of the Th1 and Th2 cell population is based on multiple, subtle and dynamic equilibria. For studies on these mechanisms a suitable experimental filarial model is needed as well as new techniques for the constant and continuous application of cytokines and other immunological substances into the host. Methods to monitor changes in cytokine profiles and cell reactivity are also required.

Monanema martini in the striped mouse, *Lemniscomys striatus*, is at present the best natural rodent model for

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onchocerciasis due to its skin dwelling microfilariae and a pathology similar to onchocerciasis (Vuong *et al.*, 1991). The need for continuous cytokine application could be fulfilled by the use of osmotic pumps. These pumps, filled prior to implantation, deliver different diluted substances at constant rates for several days.

Various murine cytokines specific for *Mus musculus* are commercially available. However, their suitability for *L. striatus* has to be demonstrated. First *in vitro* experiments carried out with recombinant murine IFN- γ showed a significant decrease in proliferation of mitogen or antigen stimulated spleen cells and peritoneal macrophages from naive and infected *L. striatus* (Figure 1). But other experiments *in vitro* showed that IFN- γ causes enhanced killing of microfilariae (Table I).

To investigate whether this interferon- γ is effective in *L. striatus* infected with *M. martini* we designed two experiments, one where IFN- γ was administered to striped mice prior to and during inoculation and another when it was given during full patency.

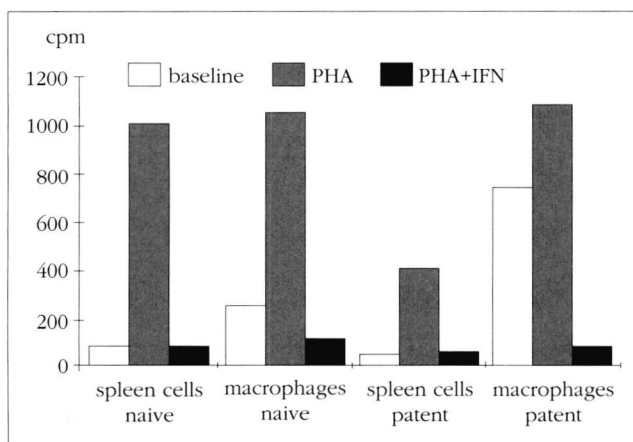


Fig. 1. – Proliferation of spleen cells and peritoneal macrophages from naive and infected striped mice measured after a 48 h incubation in RPMI 1640 supplemented partially with phytohemagglutinin (PHA) and PHA and interferon- γ (IFN).

		24 hours	48 hours	72 hours	96 hours
spleen cells	naive	++	++	++	++
	patent	++	++	++	++
spleen cells + macrophages	naive	+	+	±	-
	patent	+	±	-	--
macrophages	naive	++	++	++	++
	patent	++	++	++	++
spleen cells + IFN	naive	+	+	±	-
	patent	±	--	--	--
spleen + macrophages + IFN	naive	±	--	--	--
	patent	-	--	--	--
macrophages + IFN	naive	+	±	--	--
	patent	±	--	--	--

Table I. – Microfilariae motility assay – microfilariae were incubated in RPMI 1640 with different combination of spleen cells and peritoneal macrophages from naive and infected striped mice and interferon- γ . Motility was determined every 24 h.

++ good motility
— no motility.

Similar to other cytokines, IFN- γ has a short biological half-life, whereas immunological effects, such as macrophage activation, are induced when a continuous stable level of this cytokine is present (Murray, 1990). Repeated daily injections are necessary in order to provide an adequate level of IFN- γ in the host. This could be achieved more efficiently by intraperitoneal or subcutaneous implantation of osmotic pumps (Alzet), which deliver solutions regularly over a period of several days. This method also can reduce stress placed on animals by repeated (daily) handling.

MATERIAL AND METHODS

Three to four months old striped mice were infected subcutaneously with 30 infective larvae (L3) of *M. martini*. At different time points blood samples were taken from the retroorbital vein plexus for blood smears, leucocyte counts and isolation of sera for serological tests. Additionally, microfilarial densities in the skin were determined by incubating skin biopsies from the ear lobes in cell culture medium with collagenase. After 18 hours the emerged microfilariae were counted.

A. : In a first experiment *L. striatus* were treated 40 weeks post infection with recombinant murine IFN- γ , to investigate whether IFN- γ mediates an effect on the parasites, at the peak of microfilariodermis. Each animal received 10^6 U IFN- γ over a period of 14 days and then all the animals were dissected.

B. : In the second experiment the same dose of IFN- γ was given between one week before to one week post infection to verify if IFN- γ treatment is able to influence the course of infection.

Osmotic pumps were implanted subcutaneously on the back of the animals slightly posterior to the scapulae. First the skin was shaved, disinfected and a short incision was made. Forceps were inserted to create a pocket of sufficient size, the pumps were implanted and the incision was closed with sutures.

Both experiments were carried out with osmotic pumps (Alzet model 2002) delivering a total dose of 10^6 U r-murine IFN- γ over a period of 14 days (= 3×10^6 U/h). Animals with pumps filled with PBS and untreated animals served as controls. Since there was no difference between the two control groups we put them together to a single control group.

RESULTS

Although we were using recombinant IFN- γ derived from murine origin, clear effects in *L. striatus* could be demonstrated. In the animals dissected 14 days after implantation of the pump (exp. A) the size of the spleen was significantly enlarged. About eight times more cells were found in spleens of these animals than in untreated controls. However, these spleen cells were not able to proliferate in response to mitogen or antigen stimulation.

Two weeks after implantation of osmotic pumps with IFN- γ a decrease in the leucocyte counts in the peripheral blood of both treated groups was observed (significant in exp. B).

In experiment B, where the pumps were removed, the leucocyte counts increased again.

These animals, treated with interferon- γ prior to infection, showed higher immunoglobulin titers (IgG and IgM) in the early phase of infection. After one week of treatment, at the day of infection, the neutrophil counts were significantly increased, whereas the eosinophils were reduced during the first 14 days. Up to week 20 p.i. microfilariodermis reached titers which were twice as high as in the control group, but this difference was not significant. Four of the treated and two of the control animals were dissected 35 weeks post infection in order to carry out lymphocyte transformation tests. No differences were seen between the treated and the untreated control group.

Macrophages (M Φ) isolated from the peritoneum of patent animals showed a high baseline proliferation, whereas cocultivation of M Φ 's with spleen cells resulted in a lower proliferation. In both cases proliferation could be increased by the addition of filarial antigen or Phytohemagglutinin (PHA). However, the presence of IFN- γ reduced the proliferation to baseline or lower levels. Despite this proliferation inhibiting effect, IFN- γ enables macrophages to kill microfilariae *in vitro* within one to three days. The production of nitric oxide by IFN- γ activated M Φ 's could be shown.

DISCUSSION AND CONCLUSION

Implantation of osmotic pumps is a suitable method for the application of short lived substances like IFN- γ or other cytokines. Drugs can be delivered satisfactorily as well. Drugs and cytokines administered in this way had the same or an even better efficacy than injected ones (Murray, 1990, own observation). Other implantation sites in the animal can be used, if there is sufficient space for the pump. The pocket should be large enough and about 1 cm longer than the pump. This prevents the pump from resting directly on the wound. In addition, the pump should be inserted with its delivery portal first to minimize interaction between the delivered compound and the healing of the wound. Normally the incision heals within three days, and the animals show no sign of discomfort. Therefore, it is a useful alternative to repeated injections especially for rodents which are sensitive to frequent manipulation and are not well adapted to laboratory maintenance.

This investigation also shows that recombinant murine IFN- γ is effective in *L. striatus*. The increasing cell counts in the spleen of IFN- γ treated animals do not correspond with *in vitro* assays carried out in parallel, where the proliferation of spleen cells isolated from infected and naive animals was suppressed in the presence of IFN- γ , even when stimulated with filarial antigen.

Although there was no great effect on the course of the filarial infection, some of the results suggest that cytokine work in this model should be continued. Encouraging results include the reduced leucocyte counts, the changes between the different types of leucocytes and the raised microfilariodermis. These results also support the hypothesis that IFN- γ , a Th1-cytokine, counteracts a Th2 response, which is supposed to be involved in protection.

The different effects of IFN- γ to lymphocytes *in vivo* and *in vitro* show that this cytokine has different features, depen-

ding on different factors like other cytokines and immune cells. In an organism cytokines as well as immune cells belong to a highly crosslinked immunological network. More significant results could perhaps be obtained by using combinations of cytokines or with depletion of different cytokines with the help of antibodies against them.

For further investigations, in addition to the administration of cytokines, it is necessary to monitor the changes of cytokine profiles during the course of filarial infection. Tests based on PCR and ELISA are being established.

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THE EFFICACY OF UMF 078 ON *ACANTHO-CHEILONEMA VITAE* AND *LITOMOSOIDES SIGMODONTIS* IN *MERIONES UNGUICULATUS*

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KEYWORDS : UMF 078. *Acanthocheilonema viteae*. *Litomosoides sigmodontis*.

SUMMARY

UMF 078, a new benzimidazole analogue of Flubendazole, was tested on jirds infected with the filarial parasites *Acanthocheilonema viteae* and *Litomosoides sigmodontis* respectively. A total of 19 jirds, eight subcutaneously inoculated with infective larvae of *A. viteae* and eleven with *L. sigmodontis* infective larvae, were used in this study. UMF 078 had both microfilaricidal and macrofilaricidal activities against both parasites. Blood microfilariae were completely eliminated in the two treated groups by day 56 post-treatment. Very few adult worms were recovered, and in both groups all the worms recovered were abnormal. In *L. sigmodontis* however, microfilariae were detected in various organs/fluids (peritoneal fluid, pleural cavity fluid, lungs) on autopsy of the jirds.

INTRODUCTION

A. viteae in jirds is one of the accepted filarial models for the screening of drugs targeted against *Onchocerca volvulus*. Adult *O. volvulus* has proved a big challenge to researchers, because of its host specificity and the absence of an effective macrofilaricide. There is still an urgent need for a non-toxic drug with a macrofilaricidal or a permanent sterilizing effect on the adult *O. volvulus*. UMF 078 (develo-

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