Mitogenicity of Extracts from *Sarcocystis gigantea* on Human and Animal Lymphocytes

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INTRODUCTION

*Sarcocystis gigantea* (final host: cat, intermediate host: sheep) is a well known species of *Sarcosporidia* (*Sarcocystis*) belonging to the *Coccidia* group of the genus *Toxoplasma*. Extracts of *Sarcocystis gigantea* macrocysts contain a toxin which is capable of killing rabbits in a few hours [1]. We were interested in examining its interactions with lymphocytes in order to characterize the biological activity of this “Sarcotoxin”.

MATERIAL AND METHODS

Preparation of *Sarcocystis gigantea* extracts (SGE): Washed macrocysts of *Sarcocystis gigantea* obtained from the oesophagi of infected sheep were disrupted by ultrasonication. The insoluble constituents were removed by ultracentrifugation and the preparations stored at \(-20^\circ\text{C}\).

Lymphocyte Transformation Assay: Mononuclear cells of normal adults, normal newborns (cord blood), or spleen and thymus of white rats (normal and SPF) were cultivated as usual for 1 to 7 days (flat bottom microtiter plates, RPMI supplemented with 20 % human AB plasma or FCS, PWM or Con A as a positive control, 37 kBq (\(^3\)H) thymidine per well added 18 hours before harvesting).

Protein content: was determined by the method of Lowry or by measuring the \(E_{280}\) with a photometer. In both cases Bovine Serum Albumin was used as a standard.

RESULTS

Mononuclear cells (MNC) of 25 normal human adults were cultivated together with various concentrations of SGE expecting an inhibition reaction produced by Sarcotoxin. Surprisingly we saw strong stimulations of proliferation rates in the lymphocytes of all persons examined (Fig. 1). The reaction of cord blood lymphocytes obtained from 10 newborns was examined considering the possibility of immunization against *Sarcocystis* antigens caused by uncooked meat (Fig. 1). Individual responses varied from 7,154 to 29,640 cpm (adults) and 6,728 to 20,949 cpm (newborns).

Whether the SGE action on lymphocytes is antigenic or mitogenic was further studied in the rat model. As shown in Fig. 2, spleen cells obtained from normal and SPF rats displayed on addition of SGE a marked increase of (\(^3\)H) thymidine incorporation. The thymus cells of normal rats proliferate in a similar manner whereas no reaction could be obtained using thymocytes isolated from SPF animals.

Figs. 3 and 4 show the proliferative responses of normal adult and cord blood MNC after 1, and 2 to 7 days of cultivation. Values presented are the means of the individual responses to a suboptimal concentration of SGE (200 \(\mu\text{g/ml}\)). The mean responses peaked on day 2 (newborns) and day 3 (adults).
Fig. 1 Responses of cord blood and adult lymphocytes to Sarcocystis gigantea extract (SGE) and Pokeweed mitogen (PWM) Control: tissue culture medium (RPMI enriched by 20% human AB plasma) 

n = 25 adults, 10 newborns

Fig. 2 Response of animal lymphocytes (white rats, SPF and normal animals) obtained from thymus and spleen to SGE (100 μg/ml) and concanavalin A (Con A, 20 μg/ml) (background subtracted)
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**Fig. 3** Kinetics of *in vitro* responses of normal adult lymphocytes to SGE (200 μg/ml) and PWM (5 μg/ml) after 1 to 7 days of incubation

*n* = 10

**Fig. 4** Kinetics of *in vitro* response of cord blood lymphocytes to SGE (200 μg/ml) and PWM (5 μg/ml) after 2 to 7 days of incubation

*n* = 3
DISCUSSION

Extracts obtained from Sarcocystis gigantea induce proliferation of lymphocytes. The almost identical responses of adult and newborn cells indicate that the stimulation of human mononuclear cells by SGE is mitogenic and not antigenic. The same holds true for the experiments with spleen cells of normal and SPF rats.

The results show that SGE can also be mitogenic for animal cells. The non-reactivity of thymus cells obtained from SPF rats is an interesting result since these cells are immature T lymphocytes.

No toxic effects of the Sarcotoxin contained in SGE could be demonstrated on lymphocytes (data not shown). The similar kinetics of lymphocytes induced by SGE and PWM, respectively, underline the findings presented above.

REFERENCE

1) Pfeiffer H: Die Protozoen als Krankheitserreger, Gustav Fischer Verlag Jena, 1981