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Effect of MSC on the immune response of mice

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Abstract

The supposed immunostimulatory actions of MSC, a new fermented wheat germ extract standardized to its benzoquinone composition (trade name: AVEMAR) were studied examining blastic transformation of peripheral blood lymphocytes of mice treated with MSC. It was found that MSC significantly increased the degree of blastic transformation caused by Concanavalin A. Using the $B_{10}LP$ to $C_{57}Bl$ skin graft system, MSC (0.03 and 3.0 g kg^{-1} applied orally) acted in favour of restoring the immune function. On the other hand, 2,6-dimethoxy-*p*-benzoquinone (DMBQ), applied in equivalent doses (0.012 and 1.2 mg kg^{-1}), did not shorten the rejection time of skin grafts. The immune restoring effect, as well as the blastic transformation enhancing potential of MSC may be exploited in various cases of decreased immune response.

Keywords: Fermented wheat germ extract; Benzoquinones; AVEMAR; Lymphocytes; Blastic transformation; Skin graft; Immune reconstruction

1. Introduction

According to Pethig et al. (1983), growth inhibition of Ehrlich ascites tumor can be achieved by treatment of the tumor-bearing mice with a mixture of 2,6-dimethoxy-*p*-benzoquinone (DMBQ) and ascorbic acid. This mixture produces long-lived semiquinone and ascorbic free radicals. Vitamin C is present in many plants, while DMBQ in wheat germ. It has been shown that quenching of quinone and ascorbic radicals depends on an NAD(P)H-dependent SH group containing enzyme (Pethig et al., 1984). The cytotoxicity of the radical mixture was supposed to be associated with the decreased NAD(P) reducing capacity of tumor cells (Pethig et al., 1985). During the fermentation of wheat germ with yeast, quinones are released by the glycosidase enzyme of the yeast fungus. The original perception of Szent-Györgyi (1982) was that by means of the biological activity of these released quinones, the fermented wheat germ may possess immunostimulatory effect.

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Based on the studies of Szent-Györgyi, a dried, standardized extract of wheat germ fermented by *Saccharomyces cerevisiae* was produced. The fermentation process had previously been optimized to yield 0.4 mg g^{-1} (on dry matter basis) concentration of DMBQ in the extract. The dried extract, named MSC (trade name: AVEMAR) was administered to mice. Taking into consideration (a) the DMBQ content of MSC, (b) the DMBQ dose applied by Pethig et al. (1983) resulting in complete retraction of Ehrlich ascites tumor in mice, (c) solubility and/or suspendability of MSC in water, and (d) the size of the volume which can be injected into a mice via gastric tube, 3 g kg^{-1} was chosen as the daily intake dose. The aim of our experiments was to examine the effect of this agent on blastic transformation of peripheral blood lymphocytes in the treated animals, and also to try to influence the rejection period of skin grafts.

2. Material and methods

2.1. Preparation of MSC

Seventy kilograms *S. cerevisiae*, obtained from a local grain distillers company, was suspended into a 3 m^3 isothermic (30 ± 1°C) fermentor containing 2 m^3 of tap water. After mixing, 210 kg of freshly ground wheat germ, obtained from a local grain milling company, was added to the yeast suspension. The mixture was then fermented, filtered, concentrated and spray-dried. The resulted pulver was homogenized and kept in sealed containers.

2.2. Treatment of mice for lymphocyte proliferation assay

$C_{57}B1/6$ mice (8 weeks old 20 ± 2 g weighing males) were treated orally, via gastric tube, as aliquots of 0.1 ml, five times a week for 6 weeks as follows:

1. Group 1: untreated control
2. Group 2: control, given 0.1 ml of tap water
3. Group 3: treated with 3 g kg^{-1} MSC.

Cells obtained from perfused spleen of $C_{57}B1/6$ mice were suspended in RPMI tissue culture medium (Sigma) supplemented with 10% heat inactivated fetal calf serum (Sigma), 2 nM L-glutamine (Gibco) and 100 μg ml^{-1} gentamicin (Sigma). A total of 5×10^4 spleen cells in 200 μl medium were placed in each well of a flat-bottomed 96-well microplate (Costar) using five parallel samples. Concanavalin A (Con A, Pharmacia) (1 μg ml^{-1}) was added to the cell suspensions. Control cultures without lectin were also included. The plates were incubated at 37°C in a humidified atmosphere with 5% CO_2 for 72 h. The cultures were pulse labelled with 0.4 μCi ^{3}H-thymidine (Sigma) 24 h before termination. The cells were harvested on filter paper disks by an automated sample harvester (Skarton). Isotope determinations were made in a liquid scintillation counter (Beckman). The results were expressed in cycles per minute using the arithmetic mean of five replicate values.

2.3. Animals for skin graft

Eight weeks old (20 ± 2 g) inbred $C_{57}B1_{10}$ female and male mice were used as recipients in the skin graft experiments. The donors were 8 weeks old (20 ± 2 g) inbred $B_{10}LP$ female and male mice. The animals were kept in plastic cages (five per cage), fed with standard rodent food pellets (LATI, Gödöllõ, Hungary) and given tap water ad libitum. The room temperature was 22 ± 2°C, the relative humidity was 50 ± 5%.

2.4. Thymectomy

Suction thymectomy was performed at the age of 8 weeks, as described by Kopper and Steel (1975).

2.5. Anaesthesia

All interventions were performed under Nembutal (Jenapharm, Germany) anaesthesia (70 mg kg^{-1} i.p.).

2.6. Skin graft

Ear skin of $B_{10}LP$ mice was grafted onto the dorsal skin of $C_{57}Bl_{10}$ mice, 7 weeks after thymectomy. The grafts were fixed with plaster of Paris bandage. The bandage was removed 7 days after grafting.

Table 1
The effect of MSC on the lymphoblastic transformation of mouse spleen cells (^{3}H-thymidine incorporation)

Treatment	0 μg ml^{-1} Con A		1 μg ml^{-1} Con A	
	Mean (cpm)	SEM	Mean (cpm)	SEM
Control—untreated	194.9	36.0	3760.6	583.3
Control—water	397.1	52.0	5910.9	677.1
MSC	443.4	101.9	8041.8	957.1

2.7. Follow-up of rejection

The mice were inspected daily after removing the bandage. Animals losing the graft with the removal of the plaster of Paris were considered as technical failures. The day of rejection was defined as the day when the first crustation of the graft occurred and when this initial reaction was intensified on the following days.

The time of skin graft rejection of each animal was registered, up to 63 days after the removal of the bandage.

2.8. Experimental groups: (n = 10 / group)

2.8.1. Experiment I

1. Control females (given 0.1 ml of tap water via gastric tube, five times a week)
2. Control males (given 0.1 ml of tap water via gastric tube, five times a week)
3. Thymectomized females (given 0.1 ml of tap water via gastric tube, five times a week)
4. Thymectomized males (given 0.1 ml of tap water via gastric tube, five times a week)
5. Thymectomized females treated with a 100-fold dilution of 3 g kg^{-1} MSC, given as aliquots of 0.1 ml, via gastric tube, five times a week.
6. Thymectomized males treated with 0.03 g kg^{-1} MSC, given as aliquots of 0.1 ml, via gastric tube, five times a week.

2.8.2. Experiment II

1. Thymectomized males (given 0.1 ml of tap water via gastric tube, five times a week)
2. Thymectomized males treated with 3.0 g kg^{-1} MSC given as aliquots of 0.1 ml, via gastric tube, five times a week
3. Thymectomized males treated with 0.03 g kg^{-1} MSC given as aliquots of 0.1 ml, via gastric tube, five times a week
4. Thymectomized males treated with 1.2 mg kg^{-1} DMBQ (equivalent to 3 g kg^{-1} MSC), in aliquots of 0.1 ml, via gastric tube, five times a week
5. Thymectomized males treated with 0.012 mg kg^{-1} DMBQ (equivalent to 0.03 g kg^{-1} MSC), in aliquots of 0.1 ml, via gastric tube, five times a week.

3. Results

3.1. Effect of MSC on the blastic transformation of lymphocytes

The results are shown in Table 1. Con A treatment of the cell cultures caused significant increase in ^{3}H-thymidine incorporation into the splenic cells. The increase was highest in the cultures obtained from the MSC-treated mice.

3.2. Effect of MSC on skin graft survival

Table 2 shows the survival times of skin grafts in the various groups of Experiment I. Untreated (only saline) non-thymectomized $C_{57}B1_{10}$ mice rejected the skin of $B_{10}LP$ mice significantly earlier [males 21.0 (SEM: 3.1), females 28.7 (SEM: 4.5) days] than thymectomized $C_{57}B1_{10}$ mice [males 52.4 (SEM: 5.0), females 41.6 (SEM: 5.5) days]. When thymectomized mice were treated with MSC, the graft survival times were significantly shorter than in the case of the untreated thymectomized mice [males 28.8 (SEM: 8.6), females 32.6 (SEM: 4.5) days], but

Table 2
Effect of MSC on skin graft rejection in mice (Experiment I)

Treatment	Male		Female	
	Mean (days)	SEM	Mean (days)	SEM
Control[a]	21.0	3.1	28.7	4.5
Control	52.4	5.0	41.6	5.5
MSC (0.03 g kg^{-1})	28.8**	8.6	32.6***	4.5

[a] Without thymectomy.
** $0.001 < p < 0.01$ paired t-test.
*** $0.01 < p < 0.05$ paired t-test.

Table 3
Effect of MSC and DMBQ on skin graft rejection in male mice (Experiment II)

Treatment	Mean (days)	SEM
Control	48.0	3.2
MSC (3 g kg^{-1})	26.4*	4.2
MSC (0.03 g kg^{-1})	27.8*	4.8
DMBQ (1.2 mg kg^{-1})	58.5	5.0
DMBQ (0.012 mg kg^{-1})	49.1	4.5

* $0.001 < p < 0.01$ paired t-test vs. control.

did not reach the values of non-thymectomized untreated mice.

Table 3 shows the results of Experiment II. MSC, both in 3.0 g kg^{-1} and 0.03 g kg^{-1} doses, considerably shortened the survival time of skin grafts, as compared to the control. The low dose of DMBQ (0.012 mg kg^{-1} day^{-1}) did not exert any effect on the skin grafts, but the higher dose (1.2 mg kg^{-1}) elongated the graft survival. Moreover, the 1.2 mg kg^{-1} day^{-1} oral DMBQ treatment proved to be toxic, as five animals died in the course of the experiment.

4. Discussion

Our in vitro results show that MSC (AVEMAR) pre-treatment of mice increases the blastic transformation inducing effect of Con A on peripheral T lymphocytes. As the stimulatory effect on lymphocytes was exerted by in vivo treatment, the relatively moderate augmentation of lymphoblast transformation may be considered as important.

The inbred mouse strain $B_{10}LP$ is a co-isogenic substrain of $C_{57}B1$ mice. A so-called weak histocompatibility exists between these two strains. This means that rejection of the skin grafts occurs 16–25 days after transplantation (Taylor and Lehrfeld, 1955; Snell and Bunker, 1964; Snell et al., 1967).

In accordance with our earlier results, the present experimental data show that thymectomy itself increased the period of survival of skin grafts. On the other hand, MSC treatment resulted in a decrease of this period. Interestingly, this effect appeared at the daily dose of 0.03 g kg^{-1} and remained the same even when the daily dose was increased to 3.0 g kg^{-1}. On the other hand, DMBQ treatment proved ineffective at a dose equivalent to 0.03 g kg^{-1} MSC, showing high toxicity and a moderate immune-depressive effect at a dose equivalent to 3.0 g kg^{-1} MSC. Considering these results, DMBQ alone cannot be responsible for the immunostimulatory properties of MSC. It may be supposed that other substances present in the fermentation product of wheat germ exert the effect observed in our experiments.

From therapeutic point of view, the immunostimulatory and immune function reconstructing effects, as well as blastic transformation enhancing potential of MSC (AVEMAR), may be exploited in various cases of decreased immune response.

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