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Immunomodulatory Activities of Pyrrolizidine Alkaloids Sarracine and Neosarracine Isolated from *Senecio macedonicus*

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The immunomodulatory activity of the pyrrolizidine alkaloids mixture of sarracine and neosarracine[1:3w/w] was investigated. Mice were pretreated in vivo with 10mg/kg alkaloids and challenged with OVA. 14 days later their spleen lymphocytes were activated in vitro with the same antigen and the proliferation and antibody titer measured. It was found that the immunostimulatory effect of the alkaloids is due to activation of bone marrow precursor cells.

Key words: pyrrolizidine alkaloid, murine lymphocytes, bone marrow, immunomodulatory activity.

Introduction

The pyrrolizidine alkaloids have been shown to exert an array of biological activities these expanding from liver enzyme activity [3] to thymus morphology [4] and lung injury [5]. We have previously shown that mixture of sarracine and neosarracine, which are pyrrolizidine alkaloids isolated from *Senecio macedonicus*, activate murine spleen lymphocytes and P3U1 myeloma cell line *in vitro* [1]. The aim of the present study was to evaluate the immunomodulatory activity of these alkaloids when applied *in vivo*.

Materials and Methods

The isolation and characterisation of saracine and neosarcine was done after C h r i s t o v et al. [2].

In vivo animal treatment. Mice were treated with 10mg/kg body weight with mixture of sarracine and neosarracine [1:3 w/w], 14 days later they were injected with 30mg/animal ovalbumine [OVA] in the footpads. On day 14 after the injection the animals

were sacrificed, spleens removed and brought to cell suspensions and washed in RPMI 1640 medium. 1×10^6 cells were then activated in in vitro culture with 10mg/ml for 5 days. At day 5th the proliferation was detected by ^3H thymidine uptake and scintillation counting. Parallel cultures were tested for antibody secretion by ELISA and antibody producing cells by ELISPOT.

ELISA. ELISA plates were coated with 10mg/ml OVA in carbonate buffer for 16h at room temperature. After washing 3 times in PBS containing 3 % Triton, the culture supernatants were added in serial dilutions. An incubation for 4h followed, the plates were washed × 3 in PBS-Tween and anti-mouse Ig conjugated to peroxidase was added. The reaction was developed after 2h using 0-phenilene diamine and H_2O_2. The plates were read at 490nm and the results shown represent mean OD490±SD.

ELISPOT. ELISPOT plates were coated for overnight with 10mg/ml OVA. After washing with RPMI1640 medium they were incubated for 1h with medium containing 10% fetal calf serum. Following a washing the in vitro cultured cells were added and incubated for 4h. The cells were then removed, plates washed and anti-mouse Ig conjugated to peroxidase added. The spots were developed with H_2O_2 and observed under microscope.

Bone marrow agar cultures. Bone marrow cells were isolated from femour bone by washing the interual bone cavity with RPMA1640 medium containing 10% fetal calf serum. Cells were adjusted to 5×10^5/ml, mixed with agar to a final concentration of agat 1% and poured to 30 mm plastic Petri dishes. The mixture of sarracine and neosarracine was added in concentrations 5, 10, 20 and 40ng/ml. After 7 days of incubation at 37°C, 5%CO_2, 98% humidity the samples were dried and stained with Giemsa. The number and the type of the colonies were observed under light microscope. Supernatant from fibroblast cell line 3T3 served as a positive control.

Results and Discussion

Our previous data [2] indicated that sarracine and neosarracine might be stimulators of immune system as a whole and that was the reason to test it as a background of an immune response to a common protein antigen as OVA. We used a single alkaloid treatment with a dose of 10mg/kg body weight suggested by the in vitro cytotoxicity test not to be cytotoxic, 2 weeks later the animals were immunized with OVA and after another 2 weeks their spleen lymphocytes stimulated in in vitro culture with the same antigen. As Table 1 shows the antigen-depended proliferation of splenocytes was significantly increased in the animals pretreated with sarracine and neosarracine compared to control animals treated with the solvent only. The titer of anti-OVA antibodies was increased as shown by ELISA [Fig.1] but the number of antibody secreting cells was not altered at all (data not shown).

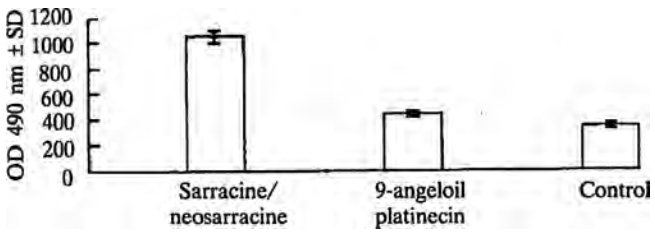

Fig. 1. Antibody titer to OVA of animals pretreated in vivo with pyrrolizidine alkaloids (mean OD 490 mn±SD from five animals)

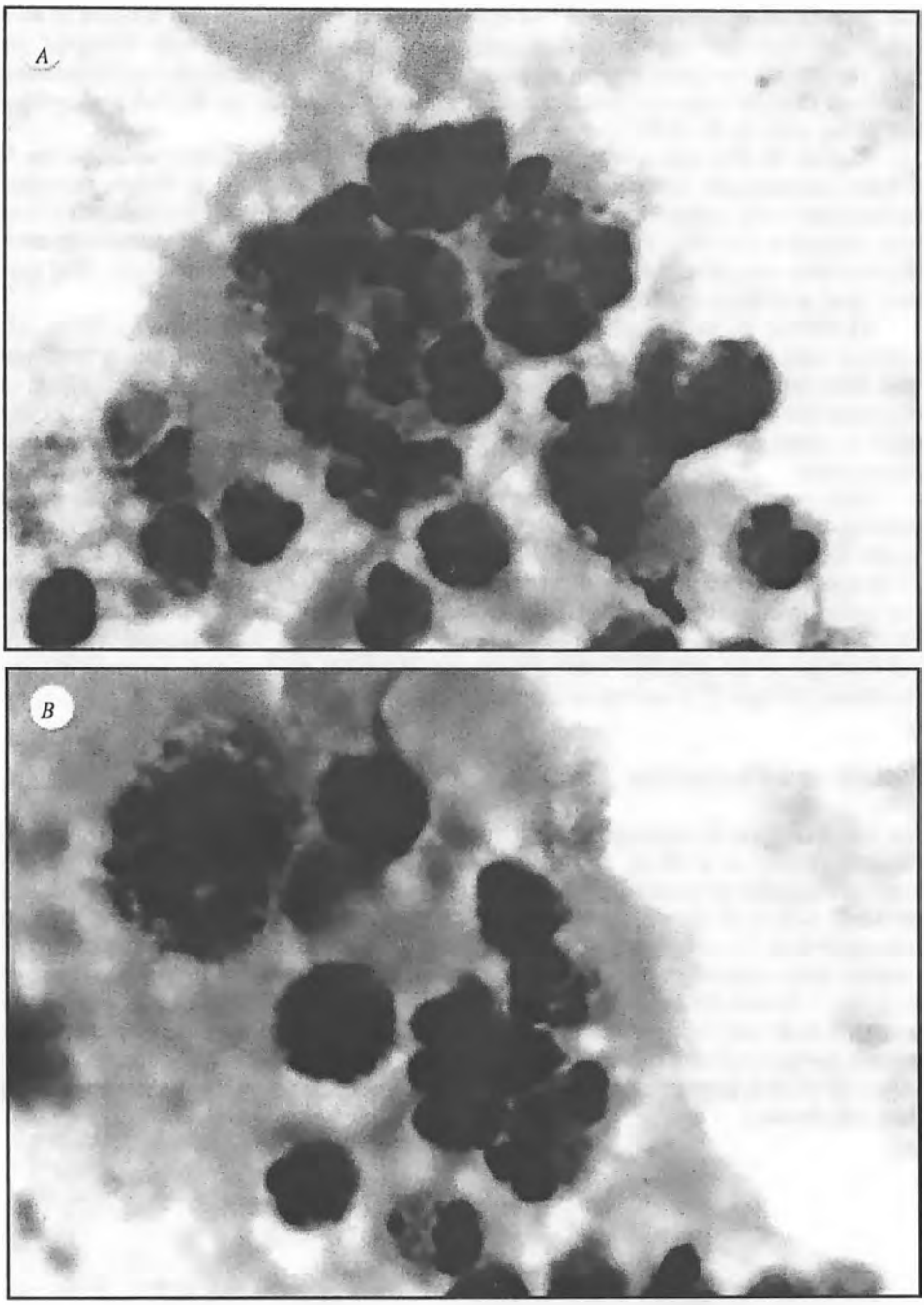

Fig. 2. Bone marrow agar cultures from mice treated in vivo with sarracine (neosarracine) (*A*) and control animals (*B*) (Microphoto × 250)

In a search to understand the mechanism of this activation of the entire immune system of the mouse by sarracine and neosarracine we tested the mixture on bone marrow murine cells. It could be seen in Fig. 2 – *A* and *B*, that 20ng/ml of the mixture of sarracine and neosarracine were enough to activate the murine bone marrow cells for division.

We could conclude that the immunostimulatory effect of the mixture sarracine and neosarracine on the mouse immune system is due to activation of the marrow precursor cells.

Table 1. *In vitro* proliferative response of spleen cells activated with OVA after *in vivo* pretreatment with sarracine/neosarracine and challenge with OVA (mean cpm+SD from five animals)

Treatment	**Sarracine/ neosarracine**	**9-Angeloil-platinecine**	**Control**
cpm±SD	**14218±611**	**8070±263**	**9301±380**

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