

Immunological mechanisms involved in the antitumoral effect produced by Oligoelements Zn, Se and Mn plus *Lachesis Muta* venom (O-LM).

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We have previously reported the antitumoral effect of the combination of oligoelements Se, Zn and Mn (4ug/ml each) plus *Lachesis muta* (4ng/ml), O-LM, in experimental mammary carcinomas in rats and pancreatic carcinomas in nude mice (Int J Cancer S13:193, 2002). Interestingly, tumor bearing nude mice daily treated with O-LM present a great number of lymphocytes between the tumor edge and the surrounding capillaries as well as lymphocytes separating the atypical cells from medium size vascular structures (EARC 2004, pg. 168). The aim of the present work was to investigate the immunological response induced by O-LM treatment.

As the systematic study of potential alterations in lymphoid infiltrates during tumor growth is extremely limited in humans, we investigated the surrounding lymphocytic infiltrate observed in tumor bearing nude mice treated with O-LM. The presence of B and T lymphocytes as well as macrophages was investigated using specific fluorescein-conjugated antibodies. To further analyze the possibility of immune system modulation by O-LM, BALB/c immunocompetent mice were injected during 15, 30 and 60 days with O-LM and *in vitro* responses to T and B selective mitogens was studied.

Results showed B220 positive while CD3 and CD19 negative cells in the infiltrates. As B220 antibody labels B, activated T and NK cells these results strongly suggest the presence of NK cells. Also an important number of CD11 bearing cells were found within the infiltrates thus indicating the participation of cells from macrophagic origin.

In immunocompetent mice T selective mitogen concanavalin A (Con A) but not B selective mitogens such as lipopolysaccharide (LPS) or PWM responses, were enhanced after 30 days, with a maximum after 60 days of *in vivo* treatment with O-LM. Also cultures from lymphoid cells from 15-days treated mice displayed an important increase of histiocytic like cells. Furthermore an increment in IFN γ release was observed in cell-free supernatants from lymph node cultures stimulated with Con A. When lymphoid cells were obtained from 60-days O-LM treated mice, 5 to 7 fold greater values were found in O-LM vs. Control.

We conclude that *in vivo* treatment with O-LM induced in nude mice an important up-regulation of innate immunity while in immunocompetent mice an enhancement of T-cell mediated immunity was observed. These results contribute to elucidate the mechanism involved in the widely described therapeutic effect of O-LM in cancer patients.