Effect of "Rasayanas", a herbal drug preparation on immune responses and its significance in cancer treatment

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Rasayanas are considered to be immunostimulating preparations used extensively in indigenous medical practice. However there are only very few reports to substantiate this claim, and this paper gives preliminary evidence for the potentiation of immunity by Rasayanas given to mice orally. Administration of Rasayanas were found to enhance the proliferation of spleen cells significantly especially in the presence of mitogen. Similar result was also seen with bone marrow cells; however mitogenic stimulation could not be observed. Esterase activity was found to be enhanced in bone marrow cells indicating increased maturation of cells of lymphoid linkage. Rasayanas also enhanced humoral immune response as seen from the increased number of antibody forming cells and circulating antibody titre. These results indicate the usefulness of Rasayana as immunostimulating agent.

In recent years, there is an increasing interest in the search for potential drugs, especially, of plant origin, that are capable of modifying immune responses (immunomodulators) with comparatively low side effects1-5. These immunorestorative drugs may reduce myelosuppression induced by cytoreductive therapy as seen in cancer6. Eventhough several natural and synthetic compounds were developed during the past several years, clinical value of most of the immunotherapeutic regimens tested so far has not yet been proven unequivocally7. Rasayanas are a group of non-toxic herbal drug preparations which are used to improve the general health in normal and disease conditions by the stimulation of the immune system8. Our initial studies have indicated that some of the Rasayanas could increase the total leucocyte counts and percentage of neutrophils in the peripheral blood9. In addition, Rasayana treatment increased the life span of ascites tumour-bearing mice and reduced the solid tumour volume induced by Daltons lymphoma ascites (DLA) cells9. Further, Rasayanas significantly protected the mice from cyclophosphamide10 and radiation11 induced myelosuppression and subsequent leukopenia. In the present study we have evaluated the effect on T-, B- and bone-marrow cells in mice treated with Rasayanas.

Materials and Methods
Tissue culture media RPMI-1640 and DMEM were purchased from Himedia laboratories, Bombay. Phytohemagglutinin (PHA) was obtained from Difco, USA and concanavalin-A (CON A) from Sigma St.Louis, USA. Pararosaniline and alpha-naphthyl acetate were obtained from Loba Chemie, Bombay. Haematoxylin was purchased from E.Merck (India).3H-thymidine was purchased from BRIT, Babha Atomic Research Centre, Bombay. All other reagents were of Analytical Reagent quality.

Balb/c mice (4-6 weeks old) were used for all the studies. They were obtained from National Institute of Nutrition, Hyderabad and maintained in air controlled rooms. The animals were fed with normal mouse chow (Lipton, India) and water ad libitum.

Brahma Rasayana (BR), Narasimha Rasayana (NR), Ashwagandha Rasayana (AR) and Amruthaprasham (AP) were purchased from "Vaidyaratnam Oushadha sala", Thrissur. The main ingredients were described elsewhere9.

Rasayana (50/mg/animal/dose) were given orally on five alternate days before all the
experiments. This dosage has been found earlier to improve WBC counts in normal mice.

Assay of lymphocyte blastogenesis—Whole spleen cells without separating macrophages were used to measure lymphocyte blastogenesis animals were sacrificed 24 hr after the completion of the drug treatment, spleens were removed and made into single cell suspension by passing through a wire mesh. The cells from treated and untreated animals were cultured (10^6 cells/ml) in presence and absence of mitogens such as PHA (3 and 6μg/ml) and CON A (5 and 10μg/ml) in RPMI-1640 medium containing 10% FCS (final volume 3 ml) and antibiotics in a humidified atmosphere of 5% CO₂ at 37°C. After 48 hr, ^3H- thymidine was added (2μCi/vial) and incubation continued for 18hr Further DNA was precipitated using perchloric acid and incorporated radioactivity was counted using liquid scintillation counter.

Bone-marrow cell proliferation assay—Total bone marrow cells without separation were used to determine the effect of Rasayanas on bone marrow cell proliferation. The procedure employed was the same as above. Instead of spleen cells bone-marrow cells (10^6 cells/ml) from treated and non-treated animals were cultured in presence and absence of mitogens (PHA-6μg/ml and Con A 5μg/ml). The proliferation of bone-marrow cells were assayed from the amount of radioactive thymidine incorporated into the DNA.

Alpha-naphthyl acetate esterase activity in bone-marrow cells—Naphthyl acetate esterase activity in the bone marrow is an indicator of maturation of stem cells to monocytes-macrophages. Total bone marrow cells from the animals treated with and without Rasayanas were made into a single cell suspension. A smear was prepared, dried and stained according to the method of Bancroft and Cook. Briefly, the slides were incubated in a mixture (pH 6.1) containing 44.5 ml phosphate buffer (pH 7.6), hexazotized pararosaniline (1.2 ml 5% pararosaniline and 1.2 ml 4% NaNO₃ and alpha-naphthyl acetate (50mg in 2.5 ml ethylene glycol monoethyl ether) for 45 min at room temperature. It was washed and counter stained with haematoxylin. A total of 4000 cells were counted in triplicates and number of cells positive for esterase were counted.

Antibody forming cells—It was done by Jern's plaque assay using a modified slide technique. Along with the last dose of drug treatment all animals received SRBC (2.5x10^9 cells) intraperitoneally. The animals were sacrificed on various days and spleens were processed into single cell suspension (8x10^6 cells/ml) in HBSS. To 0.5 ml of 5% rabbit serum in HBSS, 50μl of 7% SRBC and 50μl of spleen cell suspension were added, mixed well and poured over a glass slide. The slides were allowed to solidify and incubated with fresh rabbit serum as a source of complement for 1h at 37°C. The plaques formed were counted using a colony counter and represented as plaque forming cells (PFCs)/million spleen cells.

Circulating antibody titre—Along with the last dose of Rasayana treatment all the animals received 0.1 ml of 20% SRBC intraperitoneally. Blood was collected before and on various days after the injection of SRBC from the tail vein. Sera samples of each group were pooled and heat inactivated at 56°C for 30 min. Two fold dilutions of sera samples were made in PBS (pH 7.2) in microtitre plates and mixed (1:1) with 1% trypsinized suspension of SRBC in PBS. Agglutination was noted after incubation for 3 hr at room temperature.

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<th>PHA 6μg/ml</th>
<th>PHA 3μg/ml</th>
<th>CON A 10μg/ml</th>
<th>CON A 5μg/ml</th>
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<td>2345±312</td>
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<td>3495±1330*</td>
<td>3458±409*</td>
<td>3171±430*</td>
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*P<0.001
Statistical analysis—Statistical analysis were done by Student's t test.

Results

Effect of Rasayanas on lymphocyte blastogenesis—The effect of various Rasayanas on the splenic lymphocyte blastogenesis is shown in Table 1. There was a 2-fold increase in the thymidine uptake by spleen cells of mice stimulated with PHA and Con A after treatment with BR and NR. AP treated mice showed a 6-fold increase in the proliferation after PHA and Con A stimulation compared to controls. The treatment with AR did not have any effect under the same conditions.

Effect of Rasayanas on bone-marrow cell proliferation—There was a significant increase in the proliferation of bone-marrow cells of mice treated with Rasayanas (Table 2). Bone-marrow cells from BR and AR treated mice proliferated between nine to ten times higher than that of bone-marrow cells from the untreated control animals indicating that administration of BR and AR increases bone marrow proliferation in vivo. As the bone marrow cells did not have any mature T cells presence of mitogens did not alter the proliferative rate of these cells. Treatment with NR and AP did not have any effect on the proliferation of the bone marrow cells of mice.

Effect of Rasayana on alpha naphthyl acetate esterase activity—Effect of Rasayanas on esterase activity which is an indicator of maturation of monocytes in bone-marrow cells are shown in Table 3. There was an increase in the number of cells with esterase activity by Rasayana treatment. Treatment with BR, AR and NR enhanced the percentage of cells positive for esterase by 20%, 33%, and 36% respectively. AP treatment had only minimal effect.

Effect of Rasayanas on antibody forming cells—There was an increase in the number of plaque forming cells by Rasayana treatment (Table 4). The number of PFCs increased gradually from third day onwards and maximum number of plaques were obtained on day 5 which was 2-3 times higher than that of controls. Among the four Rasayanas studied NR was found to be most effective (958 ± 8).

Effect of Rasayanas on the circulating antibody titre—Rasayana treatment enhanced the production of antibody in normal mice (Table 5). There was an increase of (8-12 times) in circulating antibody titre by the treatment with various Rasayanas. Among the four Rasayanas studied NR was found to be the most effective (1:512) in the enhancement of antibody production. A maximum titre was obtained on day 15 for AR and day 20 for BR, NR and AP.

Discussion

Rasayanas are non-toxic drug preparations extracted from plants with various biological
activities including immunomodulatory activity. According to the indigenous system of medicine in India (Ayurveda) Rasayana therapy arrests aging, increases intelligence and body strength and enables to prevent diseases. But no systematic studies were done yet to substantiate these claims. In the present study we demonstrate the immunopotentiating activity of some Rasayanas in normal mice.

T-cells contribute the major effector mechanisms in cell-mediated immunity. They respond to plant mitogens such as PHA and CON A by rapid blastogenesis. One of the in vitro systems to evaluate the T-cell function is its ability to get transformed and have been shown to bear correlation with in vivo parameters of cell-mediated immunity status of the individual. Rasayanas such as BR, NR and AP were found to stimulate the spleen cell proliferation in presence of mitogens as seen from the increased incorporation. We have also found an increase in the weight of spleen and thymus by Rasayana treatment (data not shown).

Bone-marrow cells from Rasayana (BR and AR) treated animals also showed an enhanced proliferation in vitro. Recently we have shown that both BR and AR could protect the mice from cyclophosphamide-and radiation-induced myelosuppression. One of the mechanism may be by the induction of proliferation of bone-marrow stem cells either directly or indirectly stimulating the release of factors that are involved in the regulation of hemopoiesis. Number of bone marrow cells positive for non-specific esterases were found to increase after Rasayana treatment which represent the maturation of cells of monocyte/macrophage lineage. Rasayanas were found to enhance the number of esterase positive cells that supports the above data. It has been reported that Withania somenifera one of the components of AR could increase the number of GM-CFU showing an increased secretion of GM-CSF in the plasma.

In addition to the activation of T-cells and bone-marrow cells, administration of Rasayanas could enhance the number of plaque forming cells in the spleen and antibody titre in the circulation which are the functions of B-cells.

Rasayanas contain several plant extracts and some of them have immunomodulatory and antioxidant activities. Moreover, BR has been shown to inhibit DMBA-induced sarcoma in mice. Recently we have shown that Rasayanas could augment the cell-mediated immune effector arms such as NK cells, K cells and macrophages in tumour-bearing animals. At present we do not know the exact material involved in the stimulation of the immune system. It may be by an additive effect of several components present in plants acting synergistically in the biological system.

Acknowledgement
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References


