EFFECT OF IMMU-21, A HERBAL FORMULATION ON GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTORS, MACROPHAGE MATURATION AND SPLENIC PLAQUE FORMING CELLS IN EXPERIMENTAL ANIMALS

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ABSTRACT

Objective: To investigate the mechanism of immunostimulating action of Immu-21.

Methods: Swiss albino mice were treated with Immu-21 at various doses (25, 50, 100 mg/kg, orally) for 15 and 30 days. Granulocyte-Macrophage Colony Stimulating Factors (GM-CSF) was estimated in the serum of the experimental animals. Numbers of esterase positive cells in the bone marrow were counted. Plaque forming cells in spleen of the experimental mice were estimated following sheep RBC challenge.

Results: Immu-21 significantly enhanced the GM-CSF activity, number of esterase positive bone marrow cells and plaque forming cells of spleen in experimental animals. The immunostimulatory effect of Immu-21 is dose-dependent.

Conclusion: The present study suggests that Immu-21 modulates macrophage maturation and function. Stimulation of plaque forming cell production from spleen by this product helps in stimulating humoral arm of immunity in experimental animals.

KEY WORDS: Immu-21, bone marrow cells, plaque forming cells

INTRODUCTION

Immu-21 is a polyherbal formulation composing of equal parts of the standardized extracts of Ocimum sanctum, Emblica officinalis, Tinospora cordifolia and Withania somnifera. The product has been reported to possess immunostimulant properties on both humoral and cellular arms of immunity in immunocompetent and immunocompromised subjects in experimental and clinical studies1-8. Present studies were conducted to elucidate the possible mechanism of immunostimulating action of Immu-21.

MATERIALS AND METHODS

Immu-21 powder was dissolved in distilled water and administered orally in Swiss albino mice (n = 6 in each group) as tabulated below. Three different sets of experiments were conducted.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>TREATMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Distilled water for 15 days</td>
</tr>
<tr>
<td>II</td>
<td>Immu-21; 25 mg/kg for 15 days</td>
</tr>
<tr>
<td>III</td>
<td>Immu-21; 50 mg/kg for 15 days</td>
</tr>
<tr>
<td>IV</td>
<td>Immu-21; 100 mg/kg for 15 days</td>
</tr>
<tr>
<td>V</td>
<td>Distilled water for 30 days</td>
</tr>
<tr>
<td>VI</td>
<td>Immu-21; 25 mg/kg for 30 days</td>
</tr>
<tr>
<td>VII</td>
<td>Immu-21; 50 mg/kg for 30 days</td>
</tr>
<tr>
<td>VIII</td>
<td>Immu-21; 100 mg/kg for 30 days</td>
</tr>
</tbody>
</table>

Effect of Immu-21 on Granulocyte-Macrophage Colony Stimulating Factors (GM-CSF): Effect of various concentrations of Immu-21 on GM-CSF of Swiss albino mice was tested using Agar colony assay method. One ml of underlayer containing 9 parts...
Table 1. Effect of Immu-21 on granulocyte-macrophages, bone marrow esterase positive cells and splenic plaque forming cells.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No of colony forming units</th>
<th>No of esterase +ve cells</th>
<th>No of plaque forming cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 days</td>
<td>30 days</td>
<td>15 days</td>
</tr>
<tr>
<td>Control</td>
<td>40 ± 1.15</td>
<td>32 ± 0.96</td>
<td>675 ± 10.38</td>
</tr>
<tr>
<td>25 mg/kg</td>
<td>120 ± 3.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>168 ± 2.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>745 ± 11.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>200 ± 5.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>250 ± 5.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>790 ± 14.89&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>250 ± 7.79&lt;sup&gt;c&lt;/sup&gt;</td>
<td>320 ± 8.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>884 ± 18.45&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

ANOVA             F 340.11 569.60 38.71 44.97 419.88 261.53
P <0.001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001
n = 6 in each group. Values are expressed as mean±SEM. Mean values in the same column with different superscripts vary significantly (P<0.05). *Mean values between 15 and 30 days treatment schedule vary significantly (P<0.05).

of Dulbecco’s modified essential medium (DMEM) plus 20% new born calf serum and 1 part 3% agar was allowed to gel in 35 mm plastic petridish. One ml of the overlay containing 9 parts DMEM plus 20% new born calf serum containing 1 part 2% Agar was added to the gelled underlayer. In the test plates 10 µl serum from Immu-21 treated mice was added while in the control plates 10 µl of serum from distilled water treated mice was added. Mononuclear cells from bone marrow of normal mice were seeded at 2 x 10<sup>5</sup>/ml in the agar overlay. The culture dishes were incubated at 37 °C in 7.5% CO<sub>2</sub> in air in a humidified atmosphere. Colonies (> 20 cells) were counted on day 15, under 2.5 X objective and 10 X eyepiece. Colonies were counted under 2.5 X objective and 10 X eye piece. Four replicates were made with each sources of serum. Ability to induce colony-forming units (GM-CFU) of granulocyte-macrophage series by serum from Immu-21 treated mice was compared to that induced by the serum of control mice.

**Effect of Immu-21 on esterase activity in mouse bone marrow cells:** Effect of various concentrations of Immu-21 on α-naphyl acetate esterase activity in bone marrow cells of Swiss albino mice was tested<sup>9</sup>. Total bone marrow cells from control and experimental mice were made into a single cell suspension. Smears were prepared and dried on clean slides. 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% NaNO<sub>2</sub>) and α-naphyl acetate (50 mg in 2.5 ml ethylene glycol monoethyl ether) for 45 min at room temperature. It was washed and counter stained with methyl green. A total of 4000 cells were counted in triplicates and numbers of cells positive for esterase were counted. Number of esterase positive cells in Immu-21 treated mice was compared to that of control mice.

**Effect of Immu-21 on plaque-forming cells of spleen:** Effect of various concentrations of Immu-21 on plaque-forming cells of spleen was assayed as described by Jerne and Nordin<sup>10</sup>. Mice of all the groups were injected with sheep RBCs (2.5 x 10<sup>6</sup> / mice, i.p.) on day 15 or 30 and were sacrificed on 5<sup>th</sup> day post immunization. Spleen cell suspension was made (8 x 10<sup>6</sup>/ml) in Hank’s Balanced Salt Solution (HBSS). To 5 ml of 5% agarose in HBSS, 50 µl of 7% SRBCs and 50 µl of spleen cell suspensions were added. These were poured over a slide. The slides were allowed to get solidified and then incubated with fresh guinea pig serum at 37°C for 1 h. Plaques were counted in a colony counter. Ability to produce plaque-forming cells by spleenocytes of mice treated with Immu-21 was compared to that of control mice.

The data generated were analysed statistically using Instat (GraphPad, USA). Statistical significance was calculated applying ANOVA followed by Tukey-Kramer multiple comparison test between various dose groups. Statistical significance between two different treatment periods (15 and 30 days) with same dose was calculated applying Student’s ’t’ test. Mean differences were considered significant at P<0.05.
RESULTS

The results of the present study are shown in Table 1. Colony stimulating factors are a group of glycoproteins that stimulate cytokine secretion from lymphocytes. Treatment of mice with Immu-21 (at all dose levels) leads to significant increase in GM-CSF in the serum of experimental animals as observed from the increase in colony forming units in bone marrow mononuclear cells. Treatment of mice with Immu-21 leads to significant increase in esterase positive cells in bone marrow of experimental animals. The staining reaction is based on enzymatic activity within the cell liberating naphthol from α-naphthyl acetate, which then binds with hexazotized pararosaniline to produce an insoluble dye at the site of activity. Positive cells stain reddish brown compared to green counter stain of negative cells. It has been found that number of plaques was significantly more in the slides that were incubated with splenocytes of Immu-21 treated mice. There were significant differences in the number of GM-CFU and plaque forming cells following Immu-21 treatment 15 and 30 days respectively.

DISCUSSION

Immu-21 has been shown to increase the number and functional activity of macrophages in experimental animals. It is known that activated macrophages secrete GM-CSF. This haemopoietic growth factor leads to an array of effects including induction of leucocytosis, prevention of cytotoxic chemotherapy induced neutropenia and induction of interleukine secretion. It has been reported that W.somnifera and T.cordifolia, two active ingredients of Immu-21, increased the number of GM-CFU, which subsequently increased the secretion of GM-CSF in the plasma of treated animals.

α-naphthyl acetate esterase activity in bone marrow cells is an indicator of maturation of stem cells to monocyte-macrophages. Immu-21 enhanced the number of α-naphthyl acetate esterase positive cells in bone marrow of mouse. The result supports the earlier findings that Immu-21 has positive influence on peritoneal macrophage count of experimental animals. Immu-21 induced increase in macrophage number may be due to stimulation of bone marrow stem cells leading to their maturation towards monocyte-macrophages.

Plaque-forming cells are otherwise antibodies producing cells. The present finding supports the earlier results where it has been reported that treatment of hosts with Immu-21 enhanced antibody titre in response to immunization. This may possibly be due to stimulation of the spleen to generate more antibody producing cells. So it may be concluded that the observed immunopotentiating effect of Immu-21 in earlier studies could be attributed to its effect on bone marrow macrophage stimulation and also stimulation of splenocytes to produce antibody producing cells.

REFERENCES