IMMUNOPOTENTIATION BY IMMU 21 IN IMMUNOCOMPROMISED RATS

S. CHATTERJEE AND S.N DAS

R& D Laboratory, Indian Herbs Ltd., Saharanpur – 247 001, Uttar Pradesh

ABSTRACT: IMMU -21 a polyherbal immunomodulator effectively enhanced the immune status of animals treated with cytotoxic and immunosuppressive agents like cyclophosphamide and azathioprine. Leucopenia, lymphopenia and neutropenia in rats induced by cyclophosphamide were significantly minimized in IMMU-21 pre treated animals. IMMU-21 strengthened the host immune status against bacterial endotoxin. Cell mediated immune response was also found to be maintained at the normal level in immunocompromised mice treated with IMMU 21. The results of the experiments are of immense clinical significance IMMU -21 shows promise as a useful immunoadjuvant which may have various therapeutic applications.

INTRODUCTION

Immunomodulators of herbal origin have attracted great attention of scientific community during recent years. Medicinal plants, commonly used in herbal remedies, are known to have immunomodulatory properties. The use of herbal immunomodulators to restore and rejuvenate positive health and to maintain organic balance, has been in vogue since ancient time (1).

IMMU-21 a polyherbal formulation, (Envin Bioceuticals, A division of Indian herbs, saharanpur). Contains the extracts of different immuno- active plants viz Ocimum Sanctum, Withania somnifera, Tinospora cordifolia and Emblica officinale as major constituents in their optimum concentrations. Immunoregulatory profile of these plants are well document (2-11).

IMMU-21 was reported to have immunostimulatory effects on immunocompetant hosts (12,13). Both humoral and cellular immune response were found to be stimulated in experimental animals treated with IMMU-21 (12). IMMU – 21 was reported to be effective in stimulating the phagocytic activity of macrophage and blastogenic response of murine spleenic lymphocytes to antigenic challenge (13). Present study was designed to explore the effects of IMMU-21 in immunocompromised animals.

MATERIALS AND METHODS

Wistar albino rats (approx. 125gm) and Swiss albino mice (approx 30 gm) were used in the present experiments. The animals were acclimatized to laboratory conditions prior to experimentation. The animals were grouped into plastic cages in an airconditioned room (23 ± 1°C) with 12 hours light and dark cycle. These were maintained with pellet diet and clean tap water and libitum. IMMU-21 was dissolved in distilled water freshly before use and administered to experimental animals (30 mg/kg; p.o) at the rate of 0.5ml/100 gm in...
rats and 1 ml/100 gm in mice once daily at 10-00hr A.M).

Assessment of host resistance against myelosuppression:

Rats were used in this experiment. The animals were divided into three groups of eight animals each. The rats of group I served as healthy control. The rats of group II and III were treated with cyclophosphamide (30 mg/kg; i.p for 3 days). The rats of group III were treated with IMMU-21 from day (-7) to day (+7) of the experiment. Blood samples were collected from the retro-orbital venous plexus of each rat after 24 hour of last product administration. Total and differential leucocyte count were performed using standard procedure.

Assessment of host resistance against bacterial endotoxin

Mice were used in this experiment. The animals were divided into two groups of ten animals each. The animals were treated with cyclophosphamide (75 mg/kg; i.p) on day 0 of the experiment. The mice of group I served as control and that of group II were treated with IMMU-21 from day (10) to day (+4). All the animals were challenged with E.Coli lipopolysaccharide (0.127:B8), (Sigma) on day (=4) through intraperitoneal injection (400µg/mouse). Mortality was recorded upto 48 hours post E.Coli LPS challenge (14).

Assessment of cell medicated immunity

Mice were used in this experiment. The animals were divided into three groups on eight animals each. All the animals were sensitized with 3 mg of ovalbumin dissolved in 0.1 ml of normal saline emulsified with equal volume if freund’s complete adjuvant. They were injected (S.C) in the back. The mice of group II and III were treated with azathioprine (3 mg/kg);i.p) from the day of sanitization for 10 days (15). The animals of group III were treated with IMMU-21(30 mg/kg, orally) from the day of sensitization for 21 days. On 22nd day, all the animals were challenged with 50µg of ovalbumin (S, C) in 0.05 ml phosphate buffer (pH 7.4) in left hind foot pad. The increase in foot pad thickness was measured 24 hours after challenge with the help of a digital caliper. The degree of hypersensitivity reaction was expressed as the percent increase in foot pad thickness over the non sensitized foot (16).

RESULTS AND DISCUSSION

The experiments performed in this study indicate that IMMU-21 possesses immunostimulant properties. Total leucocyte, absolute neutrophil and absolute lymphocyte counts of cyclophosphamide treated rats were significantly reduced as compared to that of the control animals.IMMU-21 prevented the myelosuppressive effects of cyclophosphamide to a significant extent (Table -1). Overwhelming sepsis is one of the major cause of death in patients with malignancy (17), that occurs due to immunosuppression induced by the disease and cytotoxic chemotherapy. IMMU-21 effectively reduced the cyclophosphamide induced myelosuppression. Similar immunostimulatory action was also noted in Tinospora cordifolia against cyclophosphamide – induced myelosuppression. Same action was also reflected in IMMU-21.

Fig 1 shows that pretreatment of immunocompromised mice with IMMU-21 reduced their susceptibility to E.coli endotoxin. Earlier it was found that pretreatment of mice with IMMU-21 as well
as with its active ingredients (e.g. *W Somnifera* and *T. cordifolia*) significantly reduced the mortality from *E. Coli* endotoxin and *E.coli* induced peritonitis, respectively (12,18). The results indicate that IMMU 21 strengthens both immunocompetant and immunocompromised host defence mechanisms.

Fig 2. shows that cell-mediated immune response as assayed by delayed type hypersensitivity reaction was significantly depressed in azathioprine treated mice. Simultaneous treatment with IMMU-21 effectively improved the cell-mediated immune function (15). During cell-mediated immune responses, sensitized T-cells, when challenged by the antigen are converted to lymphoblasts and secrete lymphokines, attracting more scavenger cells to the site of reaction(16). Ovalbumin is a T-cell dependent antigen. So, augmentation of cell-mediated immune response to ovalbumin is suggestive of lymphokine mediated reaction (19). IMMU-21 was found to optimise the cell-mediated immune function in immunocompromised hosts. Earlier it was reported that IMMU-21 enhanced the cell-mediated immune response in immunocompetant hosts (12). Moreover, it was also reported that IMMU-21 enhanced the cell-mediated immune response in immunocompetant hosts (12). Was capable of enhancing blastogenic response of murine spleenic lymphocytes to non-specific antigenic challenge(13). The present findings leads to assume that augmentation of cell-mediated immune status of IMMU-21 treated animals may follow through favorable secretion of lymphokines.

The present study clearly demonstrates the immunostimulatory actions of IMMU-21 in immunocompromised animals. In view of its efficacy, IMMU-21 shows promise as a useful immunomodulator which may have various therapeutic applications, like therapy of infections prophylaxis of opportunistic infections of patients at risk as an adjuvant in combination with vaccines and in the therapy of malignant diseases.

| TABLE -1 : EFFECT OF IMMU-21 ON LEUCOCRAGM OF MYELOSUPPRESSED RATS. |
|-------------------------------|-----------------|-----------------|-----------------|
| Group | Treatment                      | Total WBC (X10³/µl) | Absolute Lymphocyte (X10³/µl) | Absolute neutrophil (X10³/µl) |
|-------|-------------------------------|-----------------|-----------------|-----------------|
| I     | Vehicle control               | 9.0 ± 0.81      | 7.28 ± 0.79     | 1.12 ± 0.13     |
| II    | Cyclophosphamide              | 5.6 ± 0.62*     | 4.11 ± 0.59*    | 0.84 ± 0.07*    |
| III   | Cyclophosphamide + IMMU-21    | 7.9 ± 0.78      | 5.92 ± 0.73     | 1.08 ± 0.09     |

* Significant difference (P<0.05) as compared to control.
Fig. 1

*E. coli* endotoxin-induced mortality in immunocompromised mice

Fig. 2

Delayed type hypersensitivity reaction
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