The Immunoenhancing properties of living Rhizobium meliloti in experimental animals

Al-joofy K. Ikbal*

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ABSTRACT

This study was designed to shed some light on the influence of living Rhizobium meliloti (non pathogenic bacteria) on the immune system of mature mice. Thirty male mice, 6-8 weeks old, 19-22gm weight divided into two groups, test group (n.=20), orally administrated with 100μl (4x106 CFU/ml normal saline) for 12 successive days, at the same time and rout control group (n.=10) received 100 μl of sterile normal saline. Tests for immunomodulation effects of this bacteria were applied after immunization of both groups with 0.2ml, (10% sheep red blood cells) interaperitonialy on day 4 and 8 of scheduale. Arthus reaction, delayed type hypersensitivity, E-rosette forming test, serum antibody level, phagocytosis index and nitro blue tetrazolium reduction were assessed on day 11 and 12 of program, mean while effect of the bacteria on the body weight of each group were studied. Interestingly, this non-pathogenic living bacteria showed immunopotentiating activities in experimental animals reflected by these in vivo & in vitro assays.

INTRODUCTION :

Nowadays focusing on herbs, fruits, vegetables and microorganisms such as bacteria, fungi and yeast that can boost, direct or restores the immune system and acts as adjuvant after and/or with other modalities of treatment,(1,2,3) Rhizobium meliloti is non- pathogenic, Gram negative bacteria, found in the root nodules of Melilotus indicus (herbaceous plant) as polymorphic forms (bacteroids), normally involved in fixing atmospheric nitrogen into combined form (ammonia) utilizable to the host plant. (4) In many reports different preparations of Rhizobium polysaccharide are found to posses immunomodulating activities in both in vivo & in vitro assays. (5,6)

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MATERIALS AND METHODS:

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Immunological tests:
Arthus and delayed type hypersensitivity (DTH):
These skin reaction were assessed by observing local reaction on day 11 and 12 after immunization.
Arthus reaction was observed as an increase in footpad swelling 4 hours following challenge with sheep RBCs, while DTH reaction peaks at 18-24 hours.
In this test each mouse was challenged by injecting the left hind footpad subcutaneously (s.c.) with 50 μl of 10% sheep RBCs, the vehicle (50μl normal saline) was injected s.c. into the right hind footpad as a control. Footpad swelling measured as reported in Triolo, et al., (1989) (7).

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After injection of 5ml normal saline containing 50U/ml heparin in the peritoneal cavity, gentle massage of the abdomen, then peritoneal fluid (rich with macrophages) was pooled with pasteur pipette, the cells washed twice with normal saline, then heat killed yeast (Saccharomyces cerviciae) was added at macrophage yeast ratio of 1:4 in PRMI1640 medium, incubated for one hour at 37°C in 5%CO2 atmosphere, washed with normal saline, smears of these cells were dried, fixed by methanol & stained with Giemsa stain for 20 minutes, washed with tap water, dried & examined under oil immersion, the phagocytic index was calculated by the following equation (8):

\[ \text{Phagocytic index} = \frac{\text{No. of phagocytic cells}}{\text{Total No. of macrophages}} \times 100 \]

Nitroblue titrazolium reduction test(NBT):
This test was done as following:
One a half ml of NBT stain was added to peritoned macrophages in a test tube, mixed gently, incubated at 37°C for 25minute, after that a drop of cell suspension was added on slid, dried, fixed with methanol, stained with Giemsa stain, then calculate the percentage of positive cells that contain dark blue granules of formazane (9).
Active E-rosette assay:
Mice were scarified after blood collection, the spleen were removed and minced by mincer. Single cell suspensions were obtained as in Brown, et al., (1978) and counted by dye exclusion method and adjusted to the desired number. Mixture of equal volume of splenic suspended cells (200μl) and 5% sheep RBCs incubated at 4°C for on hour, after incubation, smears were made gently, fixed, stained with Giemsa stain, examined under 40x & countes the percentage of rosette forming cell (cell with three or more sheep RBCs adhered to it(10).

Antibody titer determination:
Samples of blood were obtained from the studied groups on day 12 from ret orbital sinus, serum samples were titrated against sheep RBCs by direct hemagglutination and complement fixation test(10).

Statistical analysis:
The data was calculated by means of the students t-test.

RESULTS:
The weight of animals before and after administrated with living bacteria is shown in table 1. Significant increase in mean body weight of test group as compared to control group (P<0.005).
Index level of Arthus and DTH to sheep RBCs on day 11 and 12 of program is demonstrated in table 2, highly significant elevation (P<0.0005) of the mean index of both Arthus and DTH reactions in test group as compared to control group.
Highly significant increase (P<0.0005) in the means of antibody titer in the test group in comparison to control group, by using direct hemoagglutination & complement fixation tests as clearly shown in table (2).
The result of E-rosette test in table 2 showed that the mean value of T lymphocyte which isolated from the spleen of mice treated with living bacteria was significantly higher (P<0.0005) than the value of control group.
Statistical analysis of the result of nitroblue tetrazolium reduction test denoted highly significant increase (P<0.0005) in the percentage of formazan forming cells in test group as compared to control group, table (2).
Results of phagocytic activity showed highly significant elevation (P<0.0005) in phagocytic index in test group in comparison with control group as well presented in table 2.

Table 1 . Effect of orally administration of living R. meliloti on mice body weight

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Sig. = P<0.005
Table 2. Immunomodulating effects of R. meliloti in immunized mice

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DISCUSSION:

The present results of experimental study with living R. meliloti indicate that, this non-pathogenic bacteria have immunoenhancing activities for murine immune system. It has a marked stimulating actions on growth, function of lymphocytes and macrophages, these actions are especially shown in DTH, Arthus reaction and antibody responses that controlled by the soluble mediators of the immune system like IL-1 and IL-2. Mechanisms seem to be involved in the action, stimulation of lymphocytes could be caused by an alteration of NADPH metabolism or by a change in the redox potential on the lymphocyte membrane. In antigen stimulated lymphocytes this lead to an increased release of IL-1, IL-2 and interferons which induce greater cell proliferation and antibody production.

It is not worthy that living R. Meliloti is considered as a T-cell and B-cell mitogen as demonstrated in this study. Mitogenicity by this bacteria is related to stimulation of macrophage, B lymphocyte and T lymphocytes, this activity may be due to stimulation of macrophages which play important role in antigen processing and monokine production, studies have suggested that many immunopotentiating agents are both mitogen and adjuvants because of their large polymorphic nature so that multiple receptors on lymphoid cell surfaces could be simultaneously bound, activating membrane enzymes and cyclic nucleotides, which in turn would alter cellular metabolism resulting in altered immune responsiveness.

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