

Evaluation of the Role of Skin Peptides Isolated from *Rana tigrina* in Cellular and Humoral Immunity in Normal and DL-Bearing Mice

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Abstract : In the present study, the peptides secreted by the dorsal skin of *Rana tigrina* have been isolated and its effects on cellular and humoral immunity has been studied in normal and tumor bearing mice. To evaluate the role of the peptide in cellular immunity delayed type hypersensitivity (DTH) response has been assayed in normal and DL (Dalton's lymphoma) bearing mice and the effect of the skin peptides on humoral immunity has been evaluated by hemolytic plaque assay. The DTH response was assayed by the mice ear swelling test as described by Phanuphak *et. al.* (1974). The number of antibody forming cells in the spleen of SRBC (Sheep RBC) immunized mice was determined by the standard Jerne hemolytic plaque assay (Jerne, 1963) as modified by Cunningham (1968). The results show that the percentage suppression of ear swelling is significantly higher in peptide treated normal as well as DL-bearing mice. This suggests that frog peptide is a good suppressor of inflammatory response and thus a potent booster of cellular immunity. In hemolytic plaque assay a significant increase in the number of plaque forming cells (antibody producing cells) has been observed in peptide treated normal and DL-bearing mice as compared to untreated (control) mice. This suggests the potential role of peptide in antibody mediated (humoral) immune response.

Keywords: Frog skin peptide, DTH response, Dalton's lymphoma, SRBC, plaque forming cells.

I. Introduction

Historically, the immune system was separated into two branches: **humoral immunity**, for which the protective function of immunization could be found in the humor (cell-free bodily fluid or serum) and **cellular immunity**, for which the protective function of immunization was associated with cells. CD4 cells or helper T cells provide protection against different pathogens that survive within phagocytes or infect non-phagocytic cells.

Humoral immunity refers to antibody production and the accessory processes that accompany it, including: Th2 activation and cytokine production, germinal center formation and isotype switching, affinity maturation and memory cell generation. It also refers to the effector functions of antibodies, which include pathogen and toxin neutralization, classical complement activation, and opsonin promotion of phagocytosis and pathogen elimination (Janeway, 2001). Hemolytic plaque assay as modified by Cunningham (1968) is used for the detection and study of antibody-secreting cells isolated from the spleen.

Cellular immunity (CI) functions to enhance antimicrobial actions of phagocytes to eliminate microbes. Cellular immunity manifests as delayed type cellular immune responses. This T-cell-mediated activation of phagocytes depends on interferon gamma (IFN- γ); a major cytokine produced by type 1 T-helper (Th1) cells. However, anti-IFN- γ neutralizing antibodies (Abs) do not completely abrogate CI. IFN- γ or IFN- γ R knock out (KO) mice do reveal attenuated cellular immunity. These results indicate that cellular immunity cannot be solely attributed to IFN- γ – mediated Th1 responses. The identification of Th17 cells, which produce interleukins (ILs): IL-17A, IL-17F, IL-21, IL-22, granulocyte-macrophage colony-stimulating factor (GM-CSF), and many other factors, shed a light on the previously observed cellular immunity in the absence of IFN- γ . IL-17 KO mice did display attenuated delayed-type hypersensitivity (DTH) against bovine serum albumin and Bacille Calmette-Guérin (BCG) (Nakae, *et. al.*, 2002). The role of Th17 and Th1 cells in CI may vary depending on stimulants (Iwakura, *et. al.*, 2008). DTH responses in the skin have been used to assess cellular immunity *in vivo*.

Present findings suggest that frog peptides play crucial role in humoral as well as cellular immunity in normal as well as tumor bearing mice and thus have the potential to be used as a therapeutic immunomodulator.

II. Materials And Method

The DTH response was assayed by the mice ear swelling test as described by Phanuphak *et. al.* (1974), with slight modification. DTH was induced in these experiments by challenging the pinnae of previously sensitized mice with 2,4-dinitro-1-flouorobenzene (DNFB). On the first and second days of sensitization 50 μ l of DNFB was applied gradually on to the dorsal skin of mice. On day 5th after sensitization baseline pinnae

thickness was measured for the right and left pinnae. On day 6th the dorsal surface of the right pinnae was challenged with 25 µl of DNFB. Left pinnae were treated with 25 µl of vehicle (acetone: olive oil, 4:1). Thickness of right and left pinnae was measured at 24, 48, and 72 hours. The DTH response for each mouse were expressed as the percentage increase of ear thickness of the antigen challenged ear compared to the increase of the thickness of the ear challenged with physiological saline.

$$\%DTH \text{ Response} = \frac{C - E}{C} \times 100$$

Where, C = Control (Untreated)
E = Experimental (Treated)

The number of antibody forming cells in the spleen of SRBC (Sheep RBC) immunized mice was determined by the standard Jerne hemolytic plaque assay as modified by Cunningham, 1968. Mice were immunized with 2×10^8 cells/ml SRBC at 0 day and after 8 days by an intraperitoneal injection. After 13 days spleen of immunized mice were taken out and a single cell suspension (2×10^6 cells/ml) was made. SRBC stored in Alsever's solution (0.42% NaCl, 0.8% sodium citrate & 2.0% glucose in distilled water) were washed three times with phosphate buffer saline and a stock suspension of SRBC (25% v/v) was made. The incubation mixture was set up in small glass tube in the following proportion: 5 µl spleen cells, 10 µl SRBC, 10 µl normal rabbit serum and 130 µl PBS. These ingredients were gently mixed and 2 minutes later transferred in to Cunningham chambers. The chambers were then examined under microscope to enumerate the number of plaques.

III. Results

3.1 Delayed Type Hypersensitivity (DTH) Assay

The DTH response assayed by the mice ear swelling test as described above. The result is shown in Fig. 1; the percentage suppression of ear swelling in comparison to control and treated with peptides shows the significant results. The differences in thickness between DNFB-sensitized mice and vehicle treated or control mice were much less in mice treated with peptides. This result is same in tumor bearing (DL) mice also. Both the results, in control and peptide treated in normal mice and control with peptide treated in tumor bearing host at 24, 48 and 72 hours shows the significantly higher suppression in DTH response and the response was maximum at 48 hours* (P < 0.05). The data are representative of three independent experiments done in triplicate, and are represented as mean % increase in ear swelling ± SE_M.

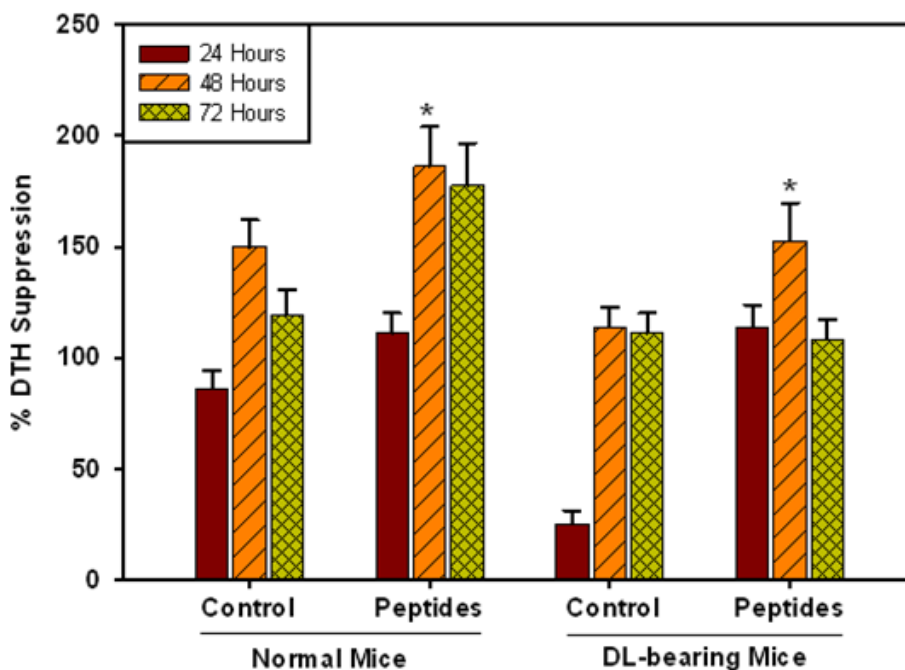


Fig. 1: Effect of skin peptide on DTH response.

3.2 Hemolytic Plaque Assay

The effects of peptides after *in vivo* administration on a humoral immune response of normal as well as tumor bearing host were investigated. Peptides were injected to normal and tumor bearing host along with sheep red blood cells (SRBC). The result is shown in Fig. 2. Splenocytes obtained from peptide administered normal and tumor bearing host mice shows an increased number of plaques compared with splenocytes sample from untreated control. Peptides administered to the early DL-bearing mice also show the enhanced number of plaque forming cells of the splenocytes preparation* ($P < 0.05$). The data are representative of three independent experiments done in triplicate, and are represented as mean number of PFCs \pm SE_M.

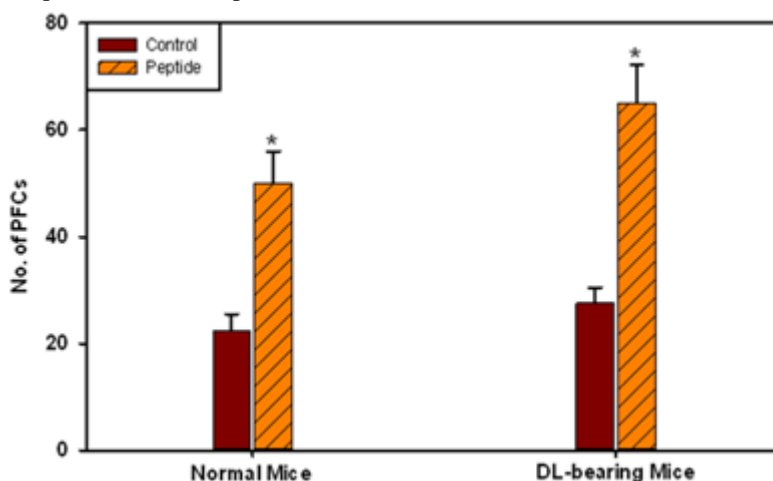


Fig. 2: Effect of the skin peptides on humoral immunity.

IV. Discussion And Conclusion

The differences in thickness between DNFB-sensitized mice and vehicle treated or control mice were much less after peptides treatment. This result is same in tumor bearing mice also. Both the results, in control and peptide treated in normal mice and control with peptide in tumor bearing host at 24, 48 and 72 hours shows the significantly higher suppression in DTH response. Similarly, significantly high increase in the number of plaque forming cells was also observed after treatment with frog skin peptides. The role of frog skin peptide in macrophage activation has already been reported by other workers (Acharya *et al.*, 2004; Prajapati *et al.*, 2012).

On the basis of these observations, it is concluded that the frog peptides have the capability to activate various immune cells directly or indirectly involved in both humoral as well as cellular immunity. The peptides derived from the skin of *Rana tigrina* are capable to restore not only the suppressed function of TAMs (Tumor Associated Macrophages), but also enhance the production of various effector molecules and proinflammatory cytokines. It also enhances DTH response and plaque forming activity in the DL-bearing mice by increasing the production of Th1 polarizing cytokines and chemokines. These entire factors together, make the immune system more compatible to suppress infections and tumor growth effectively, in the host mice and thus enhance the survival of tumor-bearing host.

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