



Modulation of delayed type hypersensitivity response by skin peptide from *Rana tigerina*

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Abstract

Peptide obtained from skin of *Rana tigerina* is a small, cationic peptide with antimicrobial activity and recently also reported for their anti cancerous and immunomodulatory properties. Here, we present the immunomodulatory effect of skin peptide from *R. tigerina* on the antigen presentation activity of macrophages and its correlations in induction of strong DTH response. 2, 4-dinitro-1-fluorobenzene (DNFB) sensitized tumour bearing and normal mice were injected with skin peptide of *R. tigerina* to examine appearance of DTH response. Findings of present study showed that the skin peptides from *R. tigerina* augment antigen presenting activity of macrophages and maximum DTH response occurred at 48th hrs after antigenic challenge. This study also suggest that the PKC plays major role behind the augmentation of macrophage activity and in turn DTH reaction, as observed by enhanced PKC translocation from cytosol towards membrane. Present endeavor suggests that this peptide can potentially enhance cell mediated immune response. These findings can be used to construct synthetic peptides with desirable modifications to fight cancer and other therapeutic conditions.

Keywords: Antigen Presenting Cell, DNFB, DTH, Frog Skin peptide, T-cells, *Rana tigerina*.

Introduction

Delayed type hypersensitivity (DTH) is an antigen specific cutaneous reaction mediated by Th1 cells and is accompanied by induration, swelling and monocytic infiltration into the site of the lesion within 24 to 72 hrs. DTH reaction in the skin is initiated by presentation of antigen by certain antigen presenting cells to sensitized memory T-cells. DTH presents beneficial or harmful aspects of immune function against the infection and auto immunity (Dhabhar & McEwen, 1997). DTH is a major mechanism of defense against a number of intracellular pathogens, including mycobacteria, fungi and certain parasites and may also be involved in transplant rejection and tumor immunity (Kobayashi *et al.*, 2001). T helper 1 (Th1) cells or activated CD4 T cells are responsible to drive DTH reaction. T-cells recognize antigen through T-cell receptors that form a complex with CD3 (Jyonouchi, 2009. Delayed type hypersensitivity. www.emedicine.com). Activated T-cells secrete various cytokines that act as chemoattractant for immune cells and cause early hallmarks of inflammation. Cytokines responsible for inflammation include IL-1, IL-6, TNF, IL-10 and IL-12 while IL-8, MCP-1 and MIP-1- α act as chemokine to attract and recruit various inflammatory cells (Oppenheim & Neta, 1994; Trinchieri, 1997; Black, 1999).

Amphibians have rich chemical arsenals, which form an integral part of their defense system and also assist in the regulation of dermal physiological action (Hancock & Scott, 2000; Marenah *et al.*, 2004; Pukala *et al.*, 2006; Abbasi *et al.*, 2007; King *et al.*, 2008). In response to a variety of stimuli host defense peptides are secreted from specialized glands on to the dorsal surface of the body (Brinkwort & Bowie, 2005; Maselli, 2006). These peptides have been shown to possess highly cytotoxic and antimicrobial properties (Marenah *et al.*, 2006; Zhou *et al.*, 2006). Frog skin peptides are well known for their

antimicrobial property but recent findings also favored it as a new weapon against HIV (Bradbury, 2005) and cancer (Baker *et al.*, 1993).

Rana tigerina is the predominant species of frog found throughout India. The skin of these frogs has been used traditionally by some tribal communities to heal both open and burn wounds and the antimicrobial component could possibly contribute to the wound healing process (Sai *et al.*, 2001). Previous research carried out on skin peptides of *R. tigerina* reported positive effect on activation of macrophages (Acharya & Tripathi, 2003; Acharya & Tripathi, 2004). These properties of frog skin peptides led us to find their possible action on CMI and macrophages. We have selected the most active peptide from the natural mixture of peptides that showed maximum antimicrobial activity and further investigated to study its effect on antigen presenting ability of macrophages and its correlation with the DTH response. We as well looked for any possible modulatory effect on signal transduction machinery by frog skin peptide.

Methods

Mice and tumor cell lines

Inbred strain of healthy BALB/c mice of 6- 8 weeks of age were used for experiment. Throughout the study, the animals were fed a palatable chow diet and were kept in separate plastic cages in a well ventilated room maintained at 21 \pm 21^oC with a 12 hr light-dark cycle. Sarcoma 180 was purchased from National Center for Cell Science (NCCS), Pune, India. Sarcoma 180 was maintained in ascetic fluid (1x 10⁶ cells /mice) by serial transplantation at every week in BALB/c mice.

Peptide extraction from frog skin

Peptides were extracted from the skin of *Rana tigerina* according to the method described previously (Conlon, 2010). The frogs of the species (*R. tigerina*) were skin sterilized with ethanol before collecting skin peptides and

slightly anesthetized. Then frogs were stimulated by injecting 0.5 ml of 1mM adrenaline. This causes release of peptides through adrenergic mediated granular glands present on dorsal skin surface of *R. tigerina*. The secretions were collected from the dorsal surface of *R. tigerina* by placing them in container filled with ethanol; water (3:1, v/v). Frogs were placed in this solution until the upper dorsal surface was discolored to yellow (Amiche *et al.*, 2000). After that collected peptide solution were acidified by adding 0.7M HCl, which will neutralize any peptidase present. Solution was centrifuged to 3000 X g, 4°C, 30 minutes. Solution was concentrated by allowing ethanol to evaporate.

Protein purification

The extract was dissolved in acetic acid (2M) and applied to a chromatography column (100 x 2.6 cm) packed with Sephadex G-25 equilibrated with 2M acetic acid. The column was eluted at a flow rate of 10 ml/ h and 2.5 ml fractions were collected. Partially purified skin extract were injected onto a 25 x 1 cm μ Bondapak C-18 reverse phase HPLC column equilibrated with 0.1% (v/v) trifluoroacetic acid/ water at a flow rate of 1ml/ min The concentration of acetonitrile in the eluting solvent was raised from 0 to 40% in 30 min and 40 - 100% in 5 minute using linear gradients. Absorbance was monitored at 210 nm and fractions were collected lyophilized and assessed for antimicrobial activity.

Antimicrobial activity

Table 1. Minimal inhibitory concentration (mg/ml) of peptides isolated from skin of *Rana tigerina*. The MIC is the minimal dose producing 99% inhibition of growth after incubation for 18 hours in culture medium.

| Microorganisms | Peptide fractions from HPLC showing MIC in mg/ml | | | |
|---------------------|--|-----|-----|----|
| | I | II | III | IV |
| <i>E.coli</i> | 30 | 60 | 50 | 50 |
| <i>S.cerevisiae</i> | 60 | 100 | 100 | 90 |
| <i>S.aureus</i> | 20 | 80 | 50 | 50 |
| <i>K.pneumoneae</i> | 40 | 80 | 70 | 60 |

Peptides fractions from the HPLC separation were incubated with 100 μ l of suspension of bacterial strains obtained from (Gifted by IMTECH, Chandigarh) (Table 1) from log phase culture (10⁵cells/ml) in 96 well micro titer cell culture plates for 18h at 37°C in a humidified atmosphere of air bacterial strains, maintained on different nutrient media by following standard protocol. After incubation, the absorbance at 630 nm of each well was determined using a micro titer plate reader.

Dose kinetics

Skin peptide of *R. tigerina* was dissolved in PBS to make stock solution of 10 mg/ml. This stock solution was further diluted in various doses to make concentrations of 60, 80, 100, 120, 140, 150, 160, 180 and 200 μ g/ml and injected intraperitoneally in mice. Mice were sacrificed after 24 hrs and peritoneal macrophages harvested to determine the optimum dose for frog skin peptides. The optimum dose was determined by measuring the

accumulation of nitrite (NO₂), a stable metabolite of nitric oxide in culture supernatants using the Griess reagent. Macrophage culture supernatant (100 μ l) was incubated with an equal volume of Griess reagent (one part of 1% sulfanilamide in 2.5% phosphoric acid and one part 0.1% naphthylethylene diamine dihydrochloride in distilled water) at room temperature for 10 minutes. The absorbance was measured at 550 nm on ELISA plate reader.

Antigen presentation assay

Macrophages were isolated from normal and tumor bearing mice and injected intraperitoneally with peptides (150 μ g/ml) or with PBS. The macrophage monolayer (1 x 10⁶ cells/ml) was treated with KLH (200 μ g/ml) for 6 hrs at 37°C in a CO₂ incubator. Macrophage supernatants (100 μ l) were plated on 96 well culture plate (Tarson, India) and incubated for 72 hrs in equal amount of T-lymphocytes (5 x 10⁶ cells/ml). T- Lymphocytes were isolated from popliteal lymph node of mice immunized with 20 μ l emulsion of KLH with PBS or Freund's complete adjuvant (Sigma-Aldrich, USA). Antigen presenting activity of macrophages was measured by MTT assay. Absorbance was measured at 540 nm on ELISA plate reader.

Induction of delayed type hypersensitivity (DTH)

The DTH response was assayed by the mice ear swelling test as described by Phanuphak *et al* (Phanuphak *et al.*, 1974) with slight modifications. Normal & tumor bearing mice were sensitized by epicutaneous application of 100 μ l of 1 % 2, 4- dinitro- 1- fluorebenzene (DNFB, Sigma - Aldrich, USA) on two consecutive days i.e. on day 0 and +1. On the fifth day, after the initial sensitization (day +5), the contact sensitized mice were challenged by carefully smearing with 50 μ l 0.5% 2, 4-dinitro-1-fluorobenzene (DNFB) on the right ear. The left ear was smeared with 50 μ l of the vehicle alone (acetone: olive oil, 1:4). The magnitude of DTH response in contact sensitized and vehicle-treated ears as measured with an engineer micrometer and expressed in units of 10⁻² mm, was taken as the measure of the DTH responses. The data are expressed as the percentage suppression of the DTH responses, which was calculated by using the following formula

$$\frac{At - Bt}{Ao} \times 100$$

Where, **At** is the ear thickness of the ear challenged with antigen at time t after challenge. **Bt** is the ear thickness of the ear challenged with physiological saline at time t after challenged and **Ao** is the ear thickness of the ear challenged with antigen before challenge (Strindelius *et al.*, 2002).

Protein kinase C α activity assay

The activity of PKC α was assayed by minor modifications in the method described earlier (Gay & Stilt, 1990). Macrophages collected from the peritoneum of frog skin peptide injected or PBS injected mice. Adhered



peritoneal macrophages (30×10^6) in 20 ml culture media were taken and incubate in presence and absence of LPS for 18 hrs. Cells were washed twice with cold HBSS and incubate for 7 min in 10 ml HBSS. Add 5 fold excess of ice cold buffer and the cell monolayer was washed with cold HBSS. The macrophages scrapped into a 240 μ l of extracted buffer (50mM Tris (pH 7.5) 0.15mM EGTA, 2mM EDTA, 2mM PMSF and 20mM DTT). The cell suspension was centrifuged at 100,000 x g for 60 min (4° C) and the supernatant was collected and treated as cytosol fraction. The pellet was then resuspended in 240 μ l of extraction buffer plus 0.1% Triton X 100 and incubated at 4° C for 1 h with gentle agitation. After incubation the suspension was centrifuged at 100,000 x g for 1 hr at 4° C and the supernatant was collected. This fraction was diluted prior to the assay with diluting buffer containing 25mM Tris, pH 7.2, 0.15M NaCl and 50% glycerol to reach 0.02% final concentration of Triton x100. 10 μ l of sample were incubated at 37° C for 1 hr with 15 μ l pre mixed reaction mixture (25mM Tris, pH 7.2, 0.15 M NaCl, 50 % glycerol, 2mM ATP, 10mM MgCl₂, 0.1mM CaCl₂, 0.002% Triton - X 100, 0.2 mg/ml PS, 0.02 mg/ml DG and 0.36mM of mylien basic protein labeled with a fluorescent probe). After incubation 20 μ l of the reaction were taken in ELISA plate and quantified by measuring absorbance at 570 nm.

Detection of PKC α by Western blotting.

Cytosolic and membrane extract of PKC α was quantified as described above. 40 μ g/ml of protein from each lysate was added per well of 10% SDS PAGE gel and the protein bands obtained were transferred on nitrocellulose membrane. The proteins were probed with IgG monoclonal anti PKC α enzyme (Banglore Genie, India) for 1 hr at room temperature. After washing the membranes were incubated for 1h at room temperature with polyclonal anti mouse horseradish peroxidase conjugate (Banglore Genie, India). The colour was developed by incubating with 5 bromo -4 chloro-3- indolyl phosphate/ nitroblue tetrazolium reagent for 10 min.

Statistical analysis

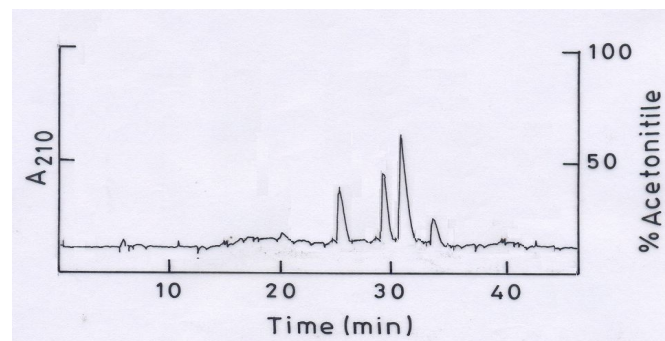
The statistical significance of difference between test groups was analyzed by two tailed student t- test. All the experiments were done in triplicate and repeated at least three times. The level of significance was expressed as $P < 0.05$.

Results

Selection of frog skin peptide

The fractions obtained after purification on sephadex G-25 further purified on μ Bondapak C18 column (Fig.1). The peaks obtained at 26.48, 29.62, 32.17, and 34.60 when tested for antimicrobial activity showed positive results. The minimal inhibitory concentration of all the four peptides is shown in Table 1. Fraction of the peak obtained at 29.62 showed maximum antimicrobial activity hence we selected this peptide fraction for further investigation.

Fig. 1. Reverse phase HPLC of skin peptides of *R.tigerina*. Partially purified skin peptide was eluted between 15 and 30% acetonitrile concn on a C18 μ Bondapak column. Absorbance was taken at 210 nm.



Estimation of optimum dose

Optimum dose for skin peptides of *Rana tigerina* was determined by quantifying nitrite, which is produced by activated macrophages and is the stable end product of nitric oxide. Doses ranging from 60 - 200 μ g/ml of extract of skin peptides were prepared and injected intraperitoneally in mice. Peritoneal macrophages were harvested after 24 hrs by sacrificing mice and plated in the concentration of (1×10^6 cells/ml). The amount of nitrite in culture supernatants was estimated by Griess reagent. Nitrite production was found increasing for doses from 60-150 μ g/ml and after that it declined (Fig.2). Thus optimum dose for eliciting effective immune response was found to be 150 μ g/ml and is used at all places in this paper.

Fig.2. Estimation of optimum dose by nitrite production in peritoneal macrophages. Various doses of skin peptides of *Rana tigerina* (60, 80, 100, 120, 140, 150, 160, 180 and 200 μ g/ml) were injected intraperitoneally in mice and peritoneal macrophages harvested after 24 hrs. Macrophages (1×10^6 cells/ml) were cultured in RPMI-1640 for 24 hrs. Quantity of nitrite in culture supernatants were estimated by Griess reagent. Absorbance was measured at 550nm on ELISA plate reader. Values are mean \pm SD from a representative experiment done in triplicate.

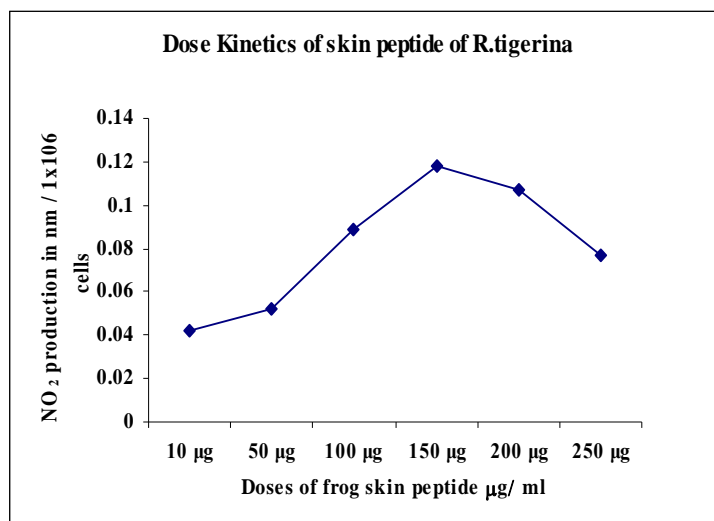
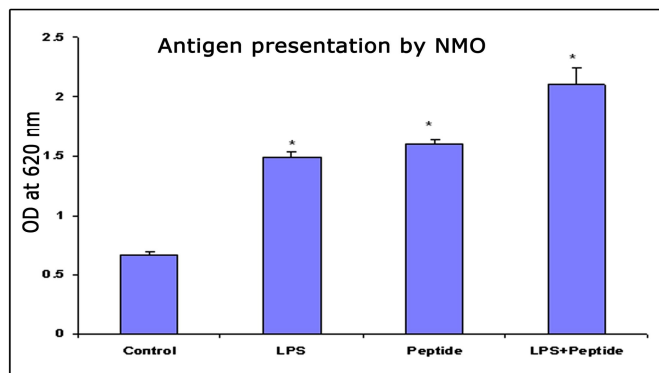


Fig 3(a). Effect of frog skin peptides on antigen presentation activity of peritoneal macrophages obtained from nontumour bearing mice. NMO (1×10^6 cells/ml) obtained from peritoneum of mice administered *in vivo* with PBS alone or containing frog skin peptides extract (150 $\mu\text{g/ml}$) were incubated *in vitro* with medium alone or with LPS (10ng/ml) for 24hrs. T cells were obtained from popliteal lymph node. Macrophage supernatants were then incubated with KLH (200 $\mu\text{g/ml}$) and mitomycin - C (1 $\mu\text{g/ml}$) treated T cells. Antigen presenting activity of macrophages to T - cells was measured by MTT assay. Values are mean \pm SD from a representative experiment done in triplicate. * $P < 0.05$ vs values of NMO obtained from PBS administered mice



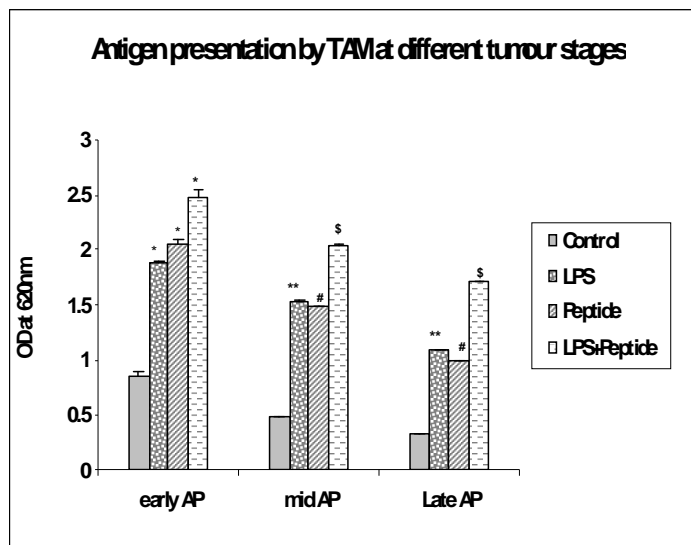
| | <i>In vivo</i> | | <i>In vitro</i> | |
|---------------|----------------|---------|-----------------|-----|
| | PBS | Peptide | Medium | LPS |
| Control | + | - | + | - |
| LPS | + | - | - | + |
| Peptide | - | + | + | - |
| LPS + Peptide | - | + | - | + |

Effect of frog skin peptide on antigen presentation activity of macrophages

Normal macrophages (NMO) and tumor associated macrophages (TAM) were obtained from the peritoneum of mice administered with frog skin peptide (150 $\mu\text{g/ml}$) or with PBS. Both NMO and TAM were plated on the plastic tissue culture plate at the concentration of (1×10^6 cells/ml) and cultured for 24 hrs in complete media with or without adding LPS. T-cells were isolated from popliteal lymph node of mice and prepared cell suspension in serum free media. Macrophages were removed from this suspension by allowing them to adhere to the plastic tissue culture flask for 2 hrs. Then cell suspension was passed over an adherence column of nylon wool to remove B-lymphocytes. NMO from frog skin peptides administered mice showed augmented antigen presenting activity to T-cells, which was studied by T-cell proliferation assay. Antigen presenting activity of peptides treated macrophages further increased on *in vitro* treatment of NMO with LPS. Furthermore, TAM isolated from frog skin peptides administered mice surprisingly enhanced the antigen presenting activity of macrophages in tumour bearing mice in comparison to frog skin peptide administered normal mice. However, the antigen

Fig. 3(b). Effect of frog skin peptides on antigen presentation activity of peritoneal macrophages obtained from tumour bearing mice. TAM (1×10^6 cells/ml) obtained from peritoneum of mice administered with PBS alone or containing frog skin peptides extract (150 $\mu\text{g/ml}$) at early, mid and late stage of tumour, were incubated with medium alone or with LPS (10ng/ml) for 24hrs. T - cells were obtained from popliteal lymph node. Macrophage supernatants were then incubated with KLH (200 $\mu\text{g/ml}$) and mitomycin - C (1 $\mu\text{g/ml}$) treated T cells. Antigen presenting activity of macrophages to T - cells was measured by MTT assay. Values are mean \pm SD from a representative experiment done in triplicate.

* $P < 0.05$ vs values of TAM obtained from mice having early stage of tumour and administered with PBS.
 \$ $P < 0.05$ vs values of TAM obtained from mice having mid stage of tumour and administered with PBS.
 # $P < 0.05$ vs values of TAM obtained from mice having late stage of tumour and administered with PBS.



| | <i>In vivo</i> | | <i>In vitro</i> | |
|---------------|----------------|---------|-----------------|-----|
| | PBS | Peptide | Medium | LPS |
| Control | + | - | + | - |
| LPS | + | - | - | + |
| Peptide | - | + | + | - |
| LPS + Peptide | - | + | - | + |

presenting activity of TAM at the early stage of tumour was much higher than mid and late tumour stage. Results are shown in Fig.3 (a) & (b).

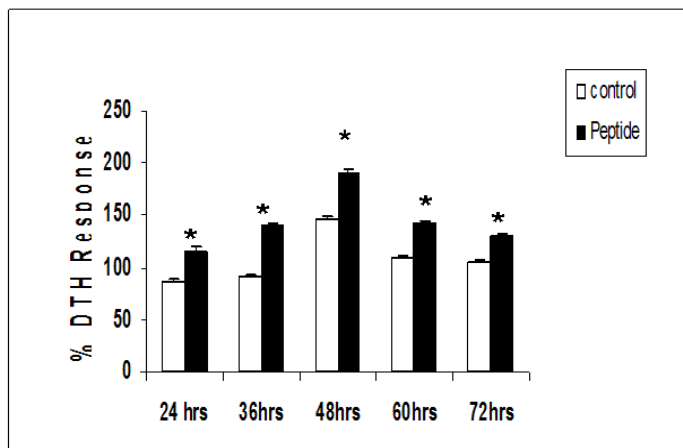
Elicitation of DTH response in non-tumour bearing mice

After DNFB sensitization on the dorsal surface of mice for two consecutive days, frog skin peptide (150 $\mu\text{g/ml}$) was injected intra-dermal at 5th day and control mice were injected with saline (PBS) only. The DTH response was recorded by measuring the thickness of the ear at 24, 36, 48, 60, 72 hrs. Percentage DTH response in frog skin peptide injected mice is much magnitude higher than the PBS injected control mice. The maximum Percentage DTH response was detected 48 hrs after challenge of mice (Fig.4).

Fig.4. Elicitation of DTH response in non tumour bearing mice. DNFB Sensitized mice were injected with frog skin peptides (150 µg/ml) or with PBS and percentage DTH response of skin peptide injected mice was recorded by measuring thickness of pinnae at 24, 36, 48, 60 & 72 hrs and compared with percentage DTH response measured in PBS injected control mice at corresponding time period.

Values are mean ± SD from a representative experiment done in triplicate.

*P < 0.05 vs values for corresponding DTH response recorded in PBS injected control mice at 24, 36, 48, 60 and 72 hrs duration.



Elicitation of DTH Response in tumour bearing mice

Sarcoma 180 was injected intra-peritoneal in mice and DTH response was recorded at early, mid and late phase of tumour growth. In all the stages of tumour growth (early mid and late) the maximum DTH response was observed at 48th hours of initial sensitization with DNFB. The percent DTH response was increased from 24th hours of second sensitization to the 48th hour and after that declined. At the early stage of tumour, percentage DTH response was found a maximum in comparison to mid and late stages of tumour. Frog skin peptide injected mice showed more percentage DTH response in comparison to PBS injected mice at all stages of tumour growth. However, DTH response recorded in tumour bearing mice was found suppressed with respect to normal tumor bearing mice (Fig.5).

Effect of skin peptide of R. tigrina on activation of PKC in macrophages

The macrophage monolayer was incubated with or without LPS. After incubating in the assay buffer cytosolic and membrane fraction was collected separately and PKC activity was determined by western blotting. Treatment of macrophages, obtained from peptide injected or PBS injected mice, with LPS significantly enhanced PKC activity in the membrane fraction with a concomitant decrease in the cytosol fraction (Fig 6).

Discussion

The skin of a frog may have 10-20 antimicrobial peptides of differing sizes, sequences, charges, hydrophobicity, tridimensional structures and spectrum of

Fig. 5. Elicitation of DTH response in tumour bearing mice: DNFB sensitized S-180 bearing mice were injected with frog skin peptide (150 µg/ml) or with PBS and percentage DTH response was recorded by measuring thickness of pinnae at 24, 36, 48, 60 and 72 hrs.

Values are mean ± SD from a representative experiment done in triplicate.

P < 0.05 vs values for corresponding DTH response recorded in PBS injected control mice at 24, 36, 48, 60 and 72 hrs duration.

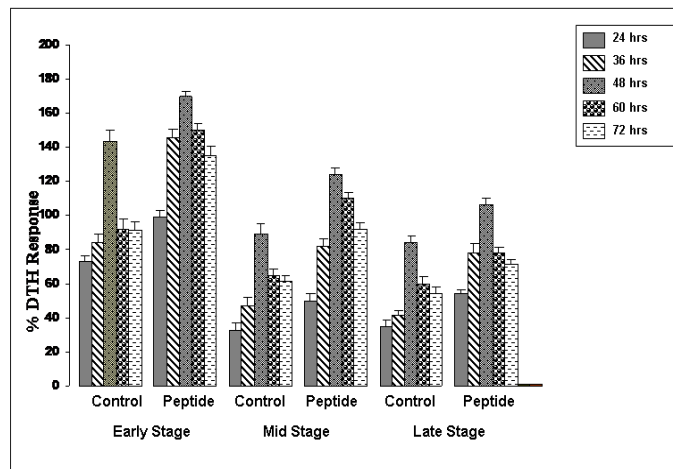
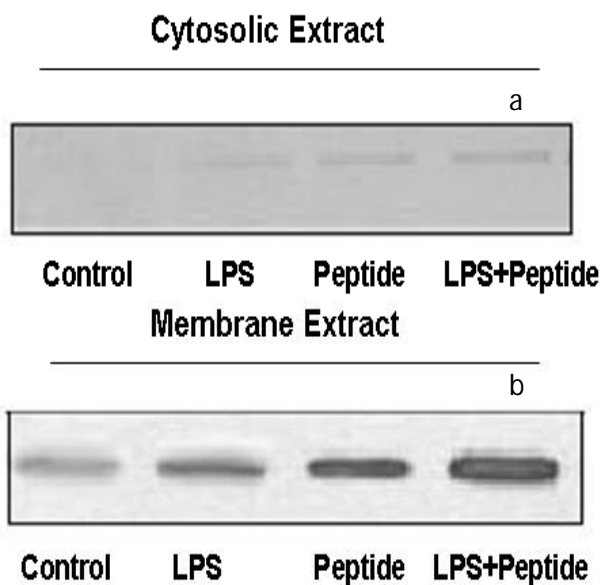


Fig. 6. Effect of Frog Skin peptide on cytosolic to membrane migration of PKC. Frog skin peptide administered or PBS administered macrophages later incubated with medium or medium containing LPS 10ng/ml. Cytosolic and membrane fractions of PKC α (40 µg/ml) obtained from macrophages were transferred in the wells of 10% SDS PAGE. Bands obtained were probed with IgG monoclonal anti PKC α enzyme for 1 hr. a) Western blot analysis in cytosolic extract. b) Western blot analysis in membrane extract



action and this armament differ between frogs belonging to different families, genera, species or even subspecies. Recently, research has also been made towards the use of frog skin peptides as an anti cancerous agent and our observation further strengthened this fact. Mostly, frog skin peptides are cationic peptides and their anti cancerous activity is attributed to this cationicity of frog skin peptides. These peptides can actively differentiate between normal and cancer cells because of presence of up to eight fold more anionic phospholipids on cancer cells membrane surface than normal cells (Rozek *et al.*, 2000; Vanhoye *et al.*, 2003). The growing interest behind search of new anticancer peptides is because present anticancer drugs are becoming resistant to cancer cells. It has been found that some of the cationic AMP's which are toxic to bacteria but not to normal mammalian cells exhibit a broad spectrum of cytotoxic activity against cancer cells (Hoskin & Ramamoorthy, 2008).

The DTH reaction has been used widely from long time as marker for various pathological conditions such as disease progression, viral infection and graft rejection as well as in tumour. Cutaneous DTH reactions are initiated when CD4 memory T-cells are activated by some antigen presenting cells such as macrophages, Langerhans cells or dendritic cells. However CD1+ Langerhans cells are found in the dermal infiltrate only after 48 hrs, while macrophages are found to start accumulating around dermal vessels at 12 hrs and their number increase up to 72 hrs (Tsicopoulos *et al.*, 1991). This shows that the macrophages began working much earlier than Langerhans cells. Furthermore, in mice depletion of epidermal Langerhans cells found no alteration in the DTH response (Kaplan *et al.*, 1987). These findings assigned major role of antigen presentation in DTH response to macrophages. Skin peptide isolated from *Rana tigerina* in the present investigation is reported to have enhanced antigen presentation activity in macrophages (both TAM and NMO) and this enhanced antigen presenting activity of macrophages might be the reason behind enhanced DTH response recorded in present investigation. However, data obtained for antigen presentation at early stage of tumor did not coincide with this statement. Despite of greater antigen presentation activity of TAM at early stage of tumor than NMO, we did not measured greater DTH response in tumor bearing mice with respect to non tumor bearing mice as expected. The reason behind this could be the suppressive strategies adopted by tumor cells against T lymphocytes, indeed exact mechanism cannot be given presently. Moreover, greater antigen presentation activity of TAM than NMO is possible because of complex and increased activities of macrophages in tumor bearing host. Tumor macrophage relationship varies so much from tumor to tumor (Mitra *et al.*, 2003). Also suppressive behavior is usually more prevalent during later stages of tumor. Our results also shown that at mid and late stages of tumor, antigen

presentation activity is significantly suppressed. Here we would like to emphasize that skin peptide of *R. tigerina* treated mice showed enhanced antigen presentation activity as well as DTH response than control mice. This clearly indicate that the skin peptide of *R. tigerina* have both immunomodulatory as well as antitumorcidal property. Next we focused this endeavor to find out the signaling machinery involved behind observed augmented activity of immune cells. We suggest here that skin peptide of *R. tigerina* modulate PKC activity in macrophages. In the present investigation we observed that skin peptide of *R. tigerina* injected mice promote translocation of PKC to the membrane. PKC has been shown to play a central role in the signal transduction processes that regulate the response of the macrophages to the external stimuli.

Scientists from all over the world have reported time to time that the frog skin peptides are a more effective and new candidate in the field of cancer, AIDS and microbiological research, but the major problem in practical working with that is to obtain them in the large amount. One solution for this problem could be the synthetic peptide and an advantage of using synthetic peptides is that we can do desirable changes in their sequence to make them more effective. Recently, Conlon *et al.* (2007, 2008) reported increase in cation promoted the antimicrobial activity, whereas an increase in hydrophobicity, halicity and amphipathicity promoted hemolytic activity and loss of selectivity for microorganisms. Similar changes can be made in synthetic peptides and also study can extend to observe effects on tumors and immunomodulation. Present investigation immensely supported the potential of frog skin peptide in enhancing cell mediated immune response and anti tumor response. Moreover, it also suggests its pharmacological, biochemical and therapeutic potentiality against infection and cancer cells.

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