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Lymphocyte Activation by IL28B Protein in Mice

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A B S T R A C T

Interleukin 28B (IL28B) which is also called as Interferon lambda3 (IFNλ3) has been analyzed for its immunomodulatory activity in mice. Bovine IL28B protein was administered to mice intramuscularly and then the spleenocytes were harvested from mice on 7th day post injection. The collected spleenocytes were analyzed for the activation of B and T lymphocytes using Fluorescent Activated Cell Sorting (FACS) analysis. Helper T cell activation using CD3-FITC, CD4-PE/Cy7 and CD69-PE; cytotoxic T cell activation using CD3-FITC, CD8-PE/Cy7 and CD69-PE and B cell activation using CD19-PE and CD22-FITC was studied. The results show that the IL28B protein activates both B and T lymphocytes. These immunomodulatory activities will have an added value in addition to its antiviral activity to elicit better immune response.

Introduction

Interferon lambdas (IFNλs) are the most recently discovered IFNs which have important immunomodulatory activity in addition to its antiviral activity. Type III IFNs include IFNλ1 (IL29), IFNλ2 (IL28A), IFNλ3 (IL28B) and the very recently described IFNλ4. Like the type I IFN, binding of the IFNλ receptor results in the activation of JAK/ STAT signaling and further expression of interferon-stimulated genes (ISGs) which induces antiviral state. However, unlike type I IFN receptors, which is expressed on virtually all cell types, IFNλ receptor is only expressed on specific tissues such as epithelia (Odendall and Kagan, 2015). So, In contrast to the type I and type II IFNs, type III IFNs is apparently acting in specific areas of the body to activate resident immune cells and induces a local immunity (Lasfar *et al*., 2016).

Zheng *et al.*, (2013) reported that IL28B can influence dendritic cells (DCs) by which it can regulate the function of T cells and it can also directly affect T cells through inhibition of the T helper 2 cell (Th2) responses. Dolganiuc *et al*., (2013) identified that IFNλ enables generation of DC populations with regulatory capacity, which facilitates expansion of regulatory T cells.

Role of IFNs in innate and adaptive immunity that complement their antiviral functions is

yet to be well characterized. In this present study, the immunomodulatory activity of bovine IL28B was studied by Fluorescent Activated Cell Sorting (FACS) using specific CD markers.

Materials and Methods

Mice

Mice used in our study were approved by Institutional Animal Ethics Committee, Bangalore, India. Male mice (Balb/c) of about 6 weeks old were obtained from experimental animal facility in Indian Institute of Science, Bangalore. They were provided with *ad libitum* food and water. All mice were kept under observation seven days before treatment. Mice were observed daily during the treatment and there was no adverse events occurred.

IL28B protein

Bovine IL28B protein expressed and purified from *Pichia pastoris* was used for the study. IL28B protein coated with Poly (lactic-co-glycolic acid) (PLG) microsphere which was prepared by double emulsion-solvent evaporation technique (Feczko *et al.*, 2011) was used for the study.

Experimental conditions

Mice were grouped in to two (group I and II) each containing three animals. Group I (control group) was given 1XPBS and group II were given 10µg of PLG micosphere/IFNλ3 protein on day 0. The route of inoculation of IL28B protein was intra-muscular on left leg. The animals were sacrificed on day7 and preceded for analysis.

Spleenocyte preparation

Mice were sacrificed by exsanguination through cutting cervical artery under pentobarbital sodium anesthesia and the abdomen was opened, spleen was located and separated from rest of the viscera. The collected spleen was washed 3 times in sterile 1X Phosphate Buffered Saline (PBS). Spleenocytes were harvested by ballooning method using 1XPBS and filtered using a 70µM cell strainer. Spleenocytes were collected by centrifugation at 1500 rpm for 5 min at 25ºC. The cell pellet was made free of red blood cells (RBC) by treating with RBC lysis buffer and washed once with 1X cell staining buffer. The spleenocytes were resuspended in 5ml of staining buffer and preceded with the staining procedure.

FACS analysis

CD molecules conjugated with different fluorescent tags used for FACS analysis: B cell activation markers CD19-PE and CD22-FITC, cytotoxic T cell markers CD3-FITC, CD8-PE/Cy7 and CD69-PE and helper T cell markers CD3-FITC, CD4-PE/Cy7 and CD69-PE were used for the study. Spleenocytes about one million cells per 100µl of cell staining solution was used for the study. Spleenocytes with antibodies in each reaction tubes were incubated at 4ºC for 20 min. After incubation about 400 µl of cell staining buffer was added and cells were pelleted at 4000 rpm for 6 min. Cell pellet was washed using 500 µl of 1XPBS to remove the unbound antibodies. Cells were again pelleted at 4000 rpm for 6 min and resuspended in 500µl of cell suspension buffer and analysed by FACS. Before analysis of B and T cell activation, colour compensation was done using pre-stained beads.

Results and Discussion

B cell activation

Spleenocytes harvested from mice were analyzed for activation of B lymphocytes using the markers CD19-PE and CD22-FITC

where CD19 is B cell marker and CD22 is B cell activation marker in mice. FACS analysis had shown the activated B cell population of 20.3% in protein treatment group compared to 6.7% in control group which is statistically significant (Figure 1A and B).

Cytotoxic T cell activation

Spleenocytes were analyzed for activation of cytotoxic T cells in mice spleen by FACS using the molecules CD3-FITC, CD8-PE/Cy7 and CD69-PE, where CD69 is T cell activation marker in mice. There was an about 14.1% increase in cytotoxic T cell activation in IL28B protein treatment group compared to control group (Figure 2).

Helper T cell activation

Spleenocytes isolated from mice were analyzed for activation of helper T lymphocytes using CD3-FITC, CD4-PE/Cy7 and CD69-PE, where CD69 is T cell activation marker in mice.

Fig.1 B lymphocyte activation in mice spleen. (A): Control group. (B): IL28B protein group showed 13.6% more increase in activated B lymphocytes compared to control

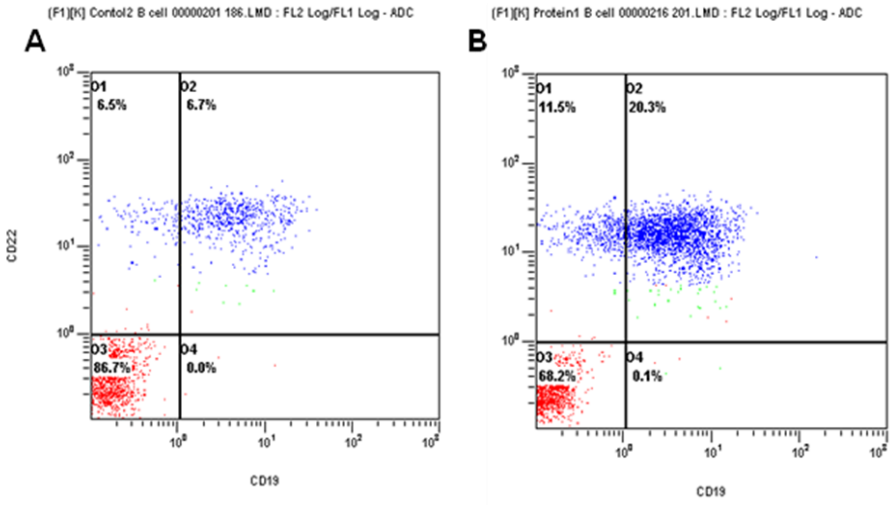


Fig.2 Cytotoxic T cell activation in mice. (A): Control group. (B) IL28B protein group showing 14.1% more increase in cytotoxic T cells compared to control

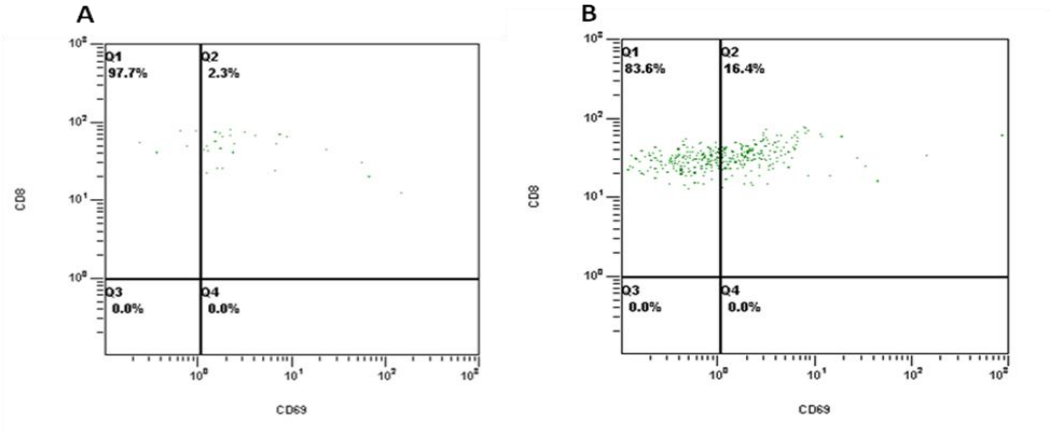
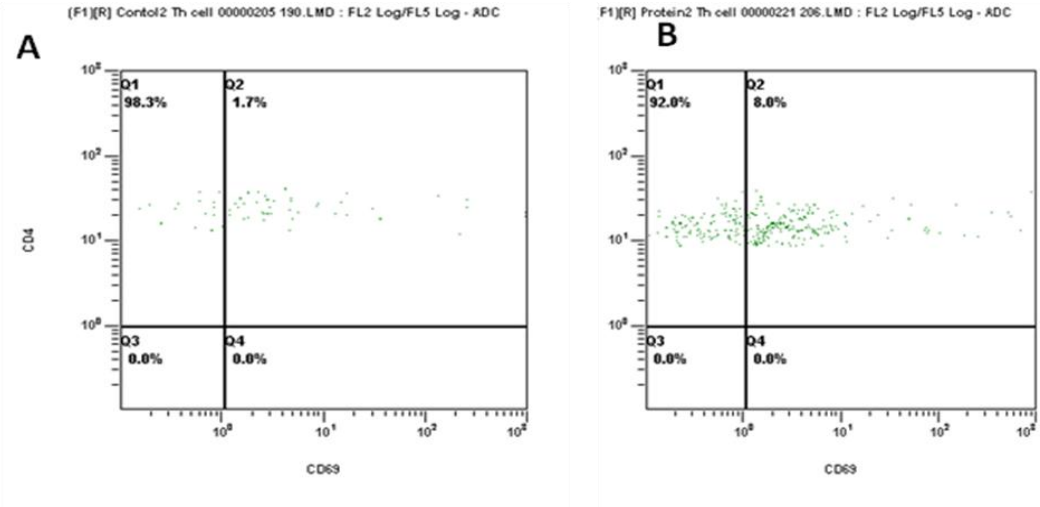


Fig.3 T-helper cell activation. (A): control group. (B): IL28B protein group showing 6.3% more increase in activated B lymphocytes compared to control



There was increase in activation of helper T cell population in mice spleenocytes with protein treatment. IL28B protein group has shown 8% increase when compared to 1.1% in the control group (Figure 3).

Type III Interferons are being tested for its antiviral activity in many disease conditions. Egli *et al*., (2014) reported that IFNλ3 is a key regulator of B and T cell vaccine responses against Influenza. As they have Immunomodulatory activities in addition to their antiviral activity, the study were done by analyzing the B and T lymphocyte activation in mice spleenocytes. Analysis of cells surface molecules of B and T lymphocytes by FACS analysis will provide an indication of immunodulatory activity of IL28B protein.

Mice were administered IL28B protein and spleenocytes after one week were analyzed for the presence of activated B and T lymphocytes by FACS analysis using specific CD markers. The present study shows that there was increase in activated B lymphocytes (about 13.6%) in mice spleenocytes in comparison to control groups. Lasfar *et al.*, (2011) reported that IFNλ1 activates B lymphocytes and modulates of Th_2 cytokines. There was no specific report available on IL28B protein.

IL28B found to activate both cytotoxic and helper T cells in mice spleenocytes but there was also a significant activation of cytotoxic T cells in comparison to Helper T cells. Cytotoxic T cell activation was 14% high and helper T cells activation was 6% high in comparison to control animals. Mennechet and Uze (2006) have shown that the treatment of monocyte-derived DCs with IL-29, member of type III IFNs led to induction of the proliferation of regulatory T cells.

Lymphocyte activation by IL28B protein will provide an added advantage to host immune system to overcome the viral diseases. As there is no demonstrated data available on lymphocyte activation by bovine IL28B protein, these findings will improve it and also help the host to clear viral infection effectively.

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