# EFFECT OF CYTOKINES ON IL-15 RECEPTOR EXPRESSION IN HUMAN ORAL KERATINOCYTES CELL LINE

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## ABSTRACT

*Interleukin-15 (IL-15) is a cytokine produced by mononuclear phagocytes and probably many other cell types in response to viral infections, lipopoly-saccharides (LPS), and other signals that trigger innate immunity. IL -15 mRNA is expressed in non-lymphoid tissues such as heart, lungs, liver, kidneys, placenta, skeletal muscle and also in keratinocytes and dendritic cells in skin. The actions of IL-15 are mediated via its receptor (IL-15R). IL-15 is known to be expressed in a variety of tissues, including epithelial cells, beta-1 cells, and natural killer cells. It is not known whether these are present in the oral buccal mucosa. The present study was carried out at The department of Oral Pathology, Queen Mary College of Medicine and Dentistry, Barts and The London, UK, to determine whether a human oral buccal mucosal (keratino-cytes) cell line, TR 146, expressed IL-15 receptor and whether the expression of IL-15R is varied when TR146 cells were exposed to different cytokines such as IL-15, IL-8, IL-1β, or TNF-α Immunocytochemistry analysis revealed that TR146 cells expressed IL-15R when incubated with IL-15 and IL-8 and a modest increase was seen with IL-1β / TNF-α. RT-PCR indicated that the TR-146 cells constitutively expressed IL-15Rα which did not appear to be up-regulated upon exposure to the cytokines used.*

**Keywords**: *cytokines, IL-15, IL-15 mRNA, LPS, innate immunity, keratinocytes, TR146 cell line, Immunocytochemistry, RT-PCR.*

## INTRODUCTION

Cytokines are the regulatory proteins secreted by the cells of innate and adaptive immunity that mediate many of the functions of these cells. They are produced in response to microbes and other antigens, and different cytokines stimulate diverse responses of cells involved in immunity and inflammation. In the activation phase of the immune response, cytokines stimulate the growth and differentiation of lymphocytes, and in the effecter phase, they activate different effecter cells to eliminate microbes and other antigens. In clinical medicine, cytokines are important as therapeutic agents or as targets for specific antagonists in numerous immune and inflammatory diseases.[1] They are receptor specific and exert their effects by interacting with high affinity receptors present on different cells.

Amongst these cytokines, IL-15, originally identified as a T cell growth factor, has a wide range of biological effects, such as activation of natural killer cells, macrophages and T Cells.

IL-15 is a cytokine produced by mononuclear phagocytes and probably many other cell types in response to viral infections, lipopolysacchrides (LPS), and other signals that trigger innate immunity.[1] It is a recently discovered cytokine (14-15 KDa)[5] and shares many properties with IL-2 such as the ability to bind to the β (beta) and gamma chains of the IL-2 receptor (IL-2R) complex[3] and to stimulate the growth of an IL-2 dependant cell line.[16] It was originally identified as a T-cell growth factor derived from a CV-1/EBNA kidney epithelial cell line. IL-15 is a cytokine produced by mononuclear phagocytes (monocytes /macrophages)

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and probably by many other cell types in response to viral infection, lipopolysaccharide, and other signals that trigger innate immunity.[1]

IL-15 mRNA is expressed preferentially in non-lymphoid tissues such as heart, lungs, liver, kidney, placenta and skeletal muscle but is not detectable in activated T-cells, which is the primary source of IL-2.[12] IL-15 mRNA is also expressed in keratinocytes and dendritic cells in skin.[21]

IL-15 receptor is a hetero-trimeric complex which is composed of IL-2R β and gamma chains in combination with a unique alpha chain (IL-15 α). IL-15 receptors are known to be expressed in a variety of tissues, including epithelial cells,[6] β -1 cells and NK cells.[14]

Two types of IL-15 receptors have been characterized which are expressed by different types of cells. IL-15 type-1 receptors are expressed on antigen presenting cells (APCs) that consist of IL-15R- α, IL-2R- β, and IL-2R-∞(gamma) c. T-cells and NK cells express type 1 receptors. IL-15 type -2 receptors, such as IL-15R-α, are expressed by mast cells [12]

**Functions of IL-15**:

In-vitro— it induces T-cell proliferation, B-cell maturation, natural killer (NK) cell cyto-toxicity and may have pivotal role in the pathogenesis of inflammatory diseases acting upstream from TNF- α.[7] It may act as a growth factor and an antigen-dependent activator for CD8 memory T cells.[18]

IL-15 produced by apcs control IL-12R expression in dendritic cells in an autocrine manner (toshiaki et al 2001). It is a potent regulator of iNOS expression by human gingival epithelial cells (HGEC) and is involved in innate immunity in the mucosal epithelium. IL-15 is a potent inducer of iNOS mRNA expression and NO production in HGEC, which may function to confront numerous bacteria in dental plaque.[20]

In vivo—studies show contributions of IL-15 in immuno-pathogenesis of multiple sclerosis. A significant rise of IL-15 in the sera of patients with multiple sclerosis compared to normal persons is seen.[11] IL-15 is essential for IL-12 dependant IFN-∞(gamma) production by dendritic cells.[12] IL-15 and IL-2 possesses similar properties, including the ability to induce T cell proliferation. However, whereas IL-2 can promote apoptosis and limit $CD8^+$ memory T cell survival and proliferation, IL-15 helps maintain a memory $CD8^+$ T cell population and can inhibit apoptosis.[22]

Transduction of dendritic cells with IL-15 gene markedly stimulates dendritic cells function and protects them from tumor-induced apoptosis.[15] IL-15 has been shown to regulate both innate and adaptive immune cells.[10]

It is seen that IL-15 can promote NK differentiation in bone marrow cultures, as well as in fetal thymic organ cultures. It is possible that IL-15 signals provide positive homeostatic signals to mature NK cells the absence of which could compromise the survival of these cells in the periphery. These signals may be critical for several aspects of NK cell biology including development, peripheral homeostasis, and /or activation.[10] IL-15 inhibits apoptosis of lymphocytes and hepatocytes induced by cytokine deprivation, anti-Fas, anti-CD3, dexamethasone, or anti-IgM.[2] There is increasing evidence that IL-15 may play an important role in protective immune response, allograft rejection and pathogenesis of autoimmune diseases, where mononuclear cells infiltration is a hallmark feature.[13] Interleukin-15 (IL-15) has pivotal roles in the control of the life and death of lymphocytes. Interleukin-15 is dedicated to the prolonged maintenance of memory T-cell responses to invading pathogens.[24, 25] IL-2, IL-15, and IL-7 are cytokines that are critical for regulating lymphoid homeostasis.[25] Chronic alcohol consumption causes a self-unrecoverable loss of NK cells in the spleen by compromising NK cell release from the BM and enhancing splenic NK cell apoptosis that can be reversed with IL-15/IL-15R-α treatment.[26]

IL-15 activates a number of intracellular signaling molecules, including the janus kinase and members of the transcription factor family of signal transducers and activators of transcription.[8] Due to sharing of receptor subunits between IL-2 and IL-15, both of these cytokines have similar downstream effects in T cells, including the induction of bci-2, the activation of the MAP Kinase pathway, and the phosphorylation of ick and syk kinases.[10]

## MATERIALS AND METHODS

The present study was carried out at The department of Oral Pathology, Queen Mary College of Medi-

cine and Dentistry, Bart's and The London, UK. The experiments of this project were carried out in two phases. Firstly, Immunocytochemistry was performed to determine IL-15R and Secondly, Reverse transcription followed by polymerase chain reaction (RT-PCR) was carried out to determine whether the cells expressed IL-15R.

**Immunocytochemistry:**

It was carried out to determine whether human oral keratinocytes express IL-15R. Oral keratinocytes (TR146) cells were cultured in a growth medium, Dulbecco's Modified Eagle Medium (DMEM by Invitrogen, Paisley, Scotland), supplemented with 10% fetal Bovine Serum (FBS) and 0.1% Penicillin/Streptomycin (PS). Cells were maintained in a humidified atmosphere of 95% air / 5% CO2. After growth, cells from the flasks cultured in DMEM growth medium, were obtained and separated by separating solution. Immunocytochemistry was performed in 24-wells plate (Table 1) using Anti-human IL-15R- α antibody (R& D Systems, Human IL-15R-α extra-cellular domain specific goat IgG type) and Sf21-derived rhIL-15R-αextracellular domain Immunogen). The experiment was repeated 3 times.

TABLE 1:

TREATMENTS

| | | Control | IL-15 | IL-1β / TNF- α | IL-8 |
|---|---|---|---|---|---|
| PBS | → | 200µl | 200µl | 200µl | 200ul |
| IL-15R | → | 200µl | 200µl | 200µl | 200ul |

24 well plate

**2- RT-PCR (Reverse Transcription-Polymerase Chain Reaction)**

PCR is a technique for the in-vitro amplification of specific DNA sequences by the simultaneous primer extension of complimentary strands of DNA. In RT-PCR, single stranded RNA is used as a technique to template to synthesize double stranded DNA. The purpose of this experiment was to determine whether oral epithelial cells express mRNA encoding the gene for IL-15R.

Oral keratinocytes were cultured in a growth medium DMEM. Cells were separated for PCR and following steps were followed

**RNA extraction:**

Extraction of RNA from the cells. The concentration of the RNA was measured by using UV-VIS Spectrophotometer (UV-mini 1240 Spectrophotometer by Shamzadu Deutschland, GmbH) at A260 and A280 nm. And RNA concentration was calculated using the following formula:

A260 x dilution factor = µg RNA / ml

**cDNA Synthesis:**

After RNA extraction, cDNA synthesis was performed using following materials and protocol.

Dctp, Aatp, dgtp, dttp (100Mm), Promega ltd. UK.

DTT (0.1M), Oligo Dt Subscript. Primer (0.5µg / µl), RNase H (3U /µl), Superscript RT, 5 x 1st strand buffer all from Invitrogen.

RNase inhibitor (40U/µl), Helena Biosciences,

UK. RNase A (10mg/ml), Sigma, Poole, UK.

| Materials | Control | IL-15 | IL-8 | IL-1 β / TNF-α |
|---|---|---|---|---|
| Oligo Dt | 1µl | 1µl | 1µl | 1µl |
| RNA (2ug) | 5µl | 5µl | 5µl | 5µl |
| DEPC Water | 4µl | 4µl | 4µl | 4µl |
| Total volume | 10µl | 10µl | 10µl | 10µl |

**Polymerase Chain Reaction (PCR) :**

PCR reagents 10x PCR Buffer 5.0µl, Distilled water 42.8µl, dNTPs (200µM) 0.5µl, sensenprimer 0.2µl, antisense primer 0.2µl, cDNA 1.0µl, Taq polymerase 0.3µl and total of 50.0µl were set up in 0.5ml microfuge tubes and mixed gently.

DNA was substituted for water as a negative control for PCR. And then PCR reactions were put through a cycling program in a hybaid thermocycler. The program was run as

Then about 10µl of the PCR products were mixed with 2µl of loading dye and ran in a 1% agarose gel and then viewed by UV illumination and pictures taken.

| Process | Temperature | Time | Cycles |
|---|---|---|---|
| DNA denaturation | 94 c | 5 minutes | 1 cycle |
| DNA denaturation | 94 c | 1 minute | 35 cycles |
| Primer melting/annealing | 60c | I minute | 35 cycles |
| Primer extension | 72c | 2 minutes | 35 cycles |
| Primer extension | 72c | 7 minutes | 1 cycle |

## RESULTS

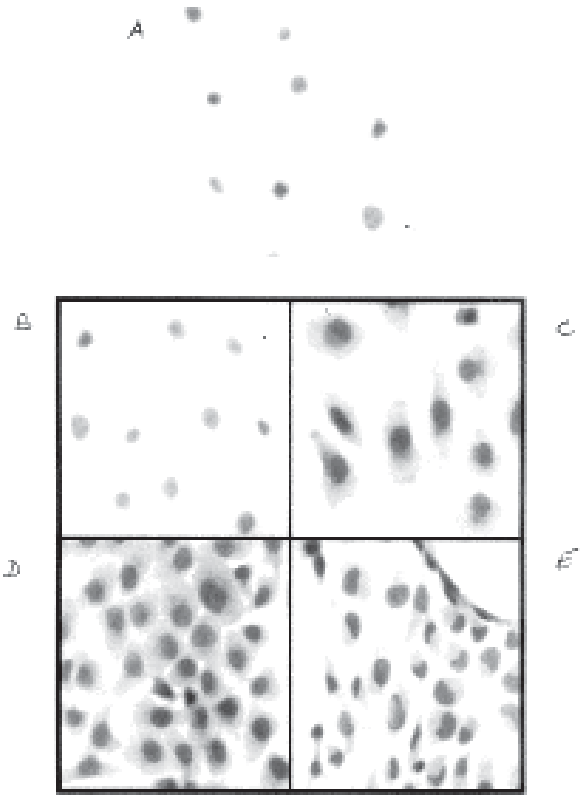


Fig 1: Immunostaining to see the expression of IL-15R-α

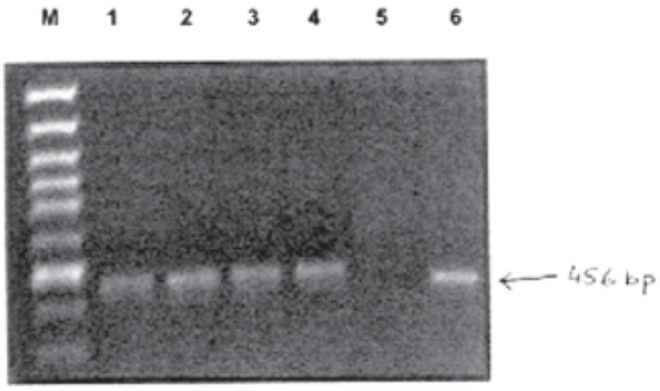


Fig 2: Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

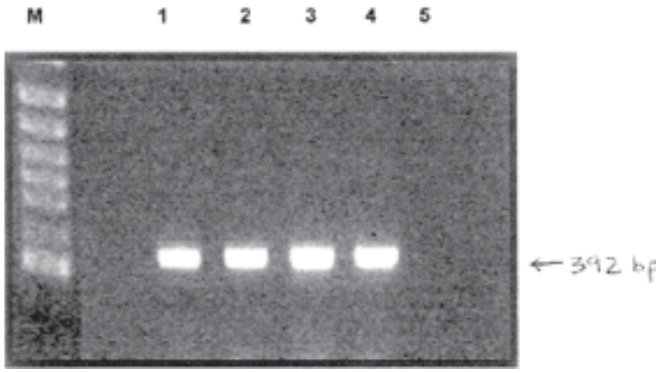


Fig 3: Detection of IL-15R α mRNA expression by RT-PCR)

Fig 1 : Immunostain showing the presence of IL-15R in TR164 cells. A no primary antibody, B no treatment, C, IL-15 (100ng/ml); D, IL-8 (100ng/ml); E, IL-1β/TNF-α (100ng/ml). antibody was used at 1:100. Cells were fixed and stained. Experiments were carried out thrice and magnification used was 200x.

Immunostaining of the treated and untreated cells was carried out to see the expression of IL-15R protein in TR146 cells. Figure 1 shows that cells with no primary antibody and cells with no treatment showed no expression of IL-15R. (fig.1A and 1B). Cells treated with IL-15 and IL-8 showed marked expression of IL-15R. (fig 1C and 1D) illustrates that the intense red color indicates increased expression of IL-15R in these cells. Fig 1E shows that cells treated with IL-1 β / TNF- α had a modest increase in IL-15R protein expression.

Fig 2: PCR was carried out for GAPDH using cDNA obtained from TR146 cells. M, DNA ladder; 1, untreated cell; 2, IL-15 (100ng/ml) treated cell; 3, IL-8 (100ng/ml) treated cells, 4, IL-1 β /TNF- α (100ng/ml) treated cells; 5, Water; 6, positive control, human endothelial cell cDNA. The size of the amplified product is indicated by the bp and arrow. Experiments were carried out 3 times.

PCR for GAPDH was done and it was expected that treated and untreated TR146 cells should show the presence of the mouse-keeping gene GAPDH. From the results obtained it can be seen that all cells expressed GAPDH gene

Fig 3 PCR was carried out for IL-15R- β using cDNA obtained from TR146 cells. M, DNA ladder; 1, untreated cell; 2, IL-15 (100ng/ml) treated cell; 3, IL-8 (100ng/ml) treated cells, 4, IL-1 β /TNF- α (100ng/ml) treated cells; 5, Water. The size of the amplified product is indicated by the bp and arrow. Experiments were carried out 3 times.

RT-PCR to see the expression of IL-15R α mRNA was carried out. From the results obtained, it can be seen that the cells with no treatment and treatments with different cytokines showed no difference in IL-15R α mRNA expression. However, the cells which were treated with IL-8 seem to show a little more IL-15R α mRNA expression than other treatments.

## DISCUSSION

Prior to the present study, it had been reported that IL-15 protein was expressed in a constitutive manner in basal epithelial keratinocytes in healthy human gingivae, and that IL-15 and IL15-R α chain mRNA could be detected in human gingival epithelial cells (HGEC) by RT-PCR analysis.[20]

In a similar fashion to Yanagita and co-workers, we found that oral keratinocytes (buccal mucosa) expressed IL-15R α mRNA in a constitutive manner (fig. 3) but not IL-15R protein (fig 1). However IL-15R protein expression was induced while the cells were exposed to IL-15, IL-8, or a combination of IL-1 β and TNF- α. Interestingly, IL-15R α mRNA levels did not alter when the cells were exposed to various cytokines used in this study (fig. 3). It means that if these cytokines are changing protein expression of IL-15R, but not gene level then some sort of effect is occurring at a post-translational level. This needs to be investigated further and could be studied using protein synthesis inhibition such as indomethacin.

The epithelial lining of the body acts as a first line defense mechanism for the organism. Epithelia are constantly being challenged by a wide range of pathogens and antigens, and have evolved to synthesize and secrete a wide range of bioactive molecules to combat invasion. It has been demonstrated that bacterial invasion induces IL-7R expression in a colonic epithelial cell line.[19] So, it might be the case that IL-15/IL-15R interactions are occurring as part of the host defense mechanism. It is known that epithelial cells secrete IL-15.[6, 17] We did not measured IL-15 release in the oral keratinocyte cell line and it would be of great interest to know if these cells did secrete IL-15.

Our present findings provide new evidence that an oral keratinocytes cell line express IL-15R. Immunostaining to see the presence of IL-15R in TR146 cells (fig 1) show that cells treated with IL-15 and other inflammatory cytokines IL-8 and IL-1 β / TNF caused an increase in IL-15R expression in these cells.

IL-15 acts via IL-15R, which shares β and Gamma subunit with IL-2R. however, unlike IL-2R, IL-15R expression is widely distributed throughout a variety of tissues and cells. It is possible that endothelial cells and fibroblasts underlying buccal epithelial cells are also influenced by IL-15 generated from these cells. A recent report has revealed that stimulation with IL-15 increases hyaluronan synthesis by endothelial cells [4] and gingival fibroblasts. Since hyaluronan is known to function as a scavenger of nitric oxide (NO) derivatives such as peroxynitrite,[9] which exerts deleterious effects on host cells, the IL-15 induced hyaluronan generation by endothelial cells and fibroblasts may contribute to the prevention of NO associated tissue damage.

In summery, our findings demonstrated that TR146 cells (buccal keratinocytes) express IL-15R.

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