Determination IL6, INF-γ Levels in Typhoid Fever Patients

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Abstract

Blood specimens were collected from typhoid fever patients, and serum levels of IFN-γ and IL-6 during the chronic and acute phase in typhoid patients group was determined according to protocol kit and calculation, results were higher levels in chronic phase (137.187±0.703.427±206.545 pg/ml respectively and in acute phase were (128.787±2.522, 137.733±23.424 pg/ml respectively) with highly significant (P≤ 0.01) than those in healthy control group.

Keywords: Salmonella typhi; Serology; IL6; INF-γ

Introduction

Typhoid fever is an enteric disease and one of the major health problems in the developing countries, fostered by many interrelated factors, including increased urbanization, inadequate supplies of clean water, antibiotic resistance, and the variable efficacies of vaccine preparation [1]. Salmonella is a member of the family Enterobacteriaceae consist of more than 2500 serovars, and infections caused by Salmonella constitute a major public health problem worldwide [2,3].

These pathogens can affect both human and animals, causing food-borne disease ranging from mild gastroenteritis to life threatening systemic infections, such as those caused by Salmonella enterica serovar typhi known as (S.typhi) [4]. Clinical studies demonstrated that S. typhi infection stimulates both an intestinal mucosal and systemic humoral and cellular immune response, which are play roles in controlling and clearing S. typhi infection [5,6], by increased levels of circulating proinflammatory and anti-inflammatory cytokines in patients with typhoid and a reduced capacity of whole blood to produce inflammatory cytokines in patients with severe disease [7,8].

Material and Methods

Clinical specimens

This study included 254 patients represented (124 males and 130 females) with age ranged from 6-60 years, and clinical suspected case of typhoid that came from Al-Kadhimiya Teaching hospital. At period from Augustto October 2017.

Blood samples for culture, and serologic analysis were collected from all patients on the same day or within 1-2 days after the first consultation.

Identification of bacterial isolates

Bacterial isolates were identified by inoculation on Salmonella Shigella agar and tetrathionate broth as selective media and by biochemical tests, then incubation at 37°C for 24hr. or by serological test and identification rapid system.

Identification of bacteria by selective media

Bacteria were cultured onto selective media include: XLD agar, Bismuth sulfate agar (BSA) and S-S agar media.

Biochemical tests

Production of H₂S and fermenting of sugars: Bacteria were inoculated into tubes containing TSI agar by streaking slant and stabbing butt. After incubation, the colony change on the slant and bottom were identified [9].

Isolation of PBMCs from blood [10]: Two ml of defibrinated or anticoagulant-treated blood was taken and equal volumes of Hank balanced salt saline solution (HBSS) (final volume 4ml) were placed into centrifuge tube. The blood and buffer were mixed by inverting the tube several times, the lymphocyte separation medium bottle inverted several times to ensure mixing. And 3ml of lymphocyte separation medium was added to the centrifuge tube, and then 4ml of diluted blood sample was added carefully to the lymphocyte separation media
solution without mixing the media solution with diluted blood sample. After that the sample was centrifuged at 400×g for 30 min. at 18-20°C. The upper layer containing plasma and platelets were drowning off using a sterile pipette, the layer of mononuclear cells was transferred to a sterile centrifuge tube using a sterile pipette, the volume of the transferred mononuclear cells was estimated. At least 5 ml of RPMI-1640 medium was added to the mononuclear cells in the centrifuge tube. The cells suspended by gently drawing them in and out of a pipette and centrifuged at 400×g for 15 min at 18-20°C. The supernatant was removed; the mononuclear cells was suspended in 3ml of RPMI-1640, and then centrifuged at 400×g (or 60 to 100×g for removal of platelets) for 10 min at 18-20°C, the supernatant was removed, and the cell pellet was re-suspended in HBSS for the application.

Calculating of PBMCNs suspended concentration: 0.1ml of lymphocyte cell suspension was mixed with 0.9ml of trypan blue 50 μl from mixture solution was taken and putting in improved Neubauer chamber slide, the visible cellswas counted in each of the four squares, the viable cell concentration/ml was counted by using the following formula:

\[ \text{Ci}=\text{t} \times \text{tb} \times 10^4 \]

Where Ci: Initial cell concentration/ml; t: The total viable cell count of four squares; tb: Correction for the trypan blue dilution; and 10^4: Conversion factor for counting chamber.

Calculating of bacterial suspended count: It was necessary to determine numbers of bacterial suspension which can be more stimulated the cultivated lymphocyte cell culture to produce of cytokines in more amounts in vitro.

2-6 colony of \( S. \) typhi were picked up, and then inoculated in 5 ml of BHIB then incubated at 37°C for 4-6 hr. until the inoculum turbidity is ≥0.1OD at 620nm or compared with McFarland standard.

To ensure the turbidity measurement compared with McFarland standard solution, 0.1ml from turbid tube was transferred and compared to McFarland standard and then inoculated the nutrient agar plates by spreading inoculum over the surface of medium by spreader, and the plates were incubated at 37°C for 24hr., the bacteria colonies were counted (in range 30-300 colony), and then the count numbers of bacterial suspension were calculated by using the following formula:

\[ \text{No. of cells/ml} = \text{No. of colonies} \times \text{Dilution factor} \times 10^6 \text{ CFU/ml} \]

The typical live numbers of bacteria which induce the PMNCs culture to produce of cytokines in vitro is 2-6 colony of \( S. \) typhi.

Stimulating of treated peripheral blood cells with live bacteria to produce cytokines [11]: Tubes containing 5 ml of growth media solution were inoculated by 50 μl of PBMCs suspend, and all tubes culture incubated at 37°C and 5% CO₂ for 72 hr., after pass 4hr. the tubes were inoculated with diluted bacteria suspension, and then culture supernatant were collected at 1-72hr. for cytokines assays.

Cytokines assay

Determining the concentration level of IL-6 in serumpatient and cell culture (according to Kit protocol):

Preparation of standard solution: Reconstitute the lyophilized recombinant protein to make a 10000 pg/ml of IL-6 solution by 1ml sample diluent buffer was added to a tube of lyophilized protein, the tube was kept at room temperature for 10min. and mixed thoroughly, 0.9 ml of the sample diluent buffer was aliquoted into tube and labeled as 10000 pg/ml protein standard, 0.1 ml of the mixed 10000 pg/ml IL-6 solution was added to tube containing 0.9 ml diluent buffer and mixed to make a 1000 pg/ml IL-6 solution, Labels 6 tubes with the protein concentration to be prepared by serial dilution: 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5pg/ml, 31.2 pg/ml and 15.6 pg/ml, 0.3 ml was aliquoted of the sample diluent buffer to the labeled tubes, 0.3 ml was transferred from the 1000 pg/ml IL-6 solution to the 500 pg/ml tube and mixed thoroughly. Then 0.3 ml transferred from each tube to another to prepare series dilute the protein standards into their respectively and store at 4°C until uses.

Loading the samples into the micro-plate (according to Kit protocol): 0.1ml of the sample diluent buffer was aliquoted into a control well to serve as the blank, 0.1 ml of the serial standard protein solutions were aliquoted into empty wells of the pre-coated well plate. the sample (serum and supernatant of cell culture) was diluted by taking 50 μl of sample and 50 μl of diluent buffer and mixed thoroughly to prepare 1:2 from sample working dilution, 0.1ml of each diluted test samples was aliquoted to empty wells, the wells plate were covered and incubated at 37°C for 90 min., During incubating, a stock biotinylated antibody working solution were prepared according to protocol kit, and the working solution used within 2 hr., the cover of the well plate was removed and plate well contents were discarded and the plate blotted onto paper towel, 0.1ml of the biotinylated 1:100 antibody working solution was added to each well and the plate was incubated at 37°C for 60 min.

During incubation period, a stock of Avidin-Biotin-Peroxidase Complex (ABC) working solution was prepared according to kit protocol, pre-warm the ABC working solution at 37°C for 30 min, before use with in1hr., the plate was washed 3 times with 0.3 ml of PBS (prepared in paragraph 2.6.6) and the washing buffer discarded and the plate blotted onto a filter paper No.1, 0.1 ml of ABC working solution was added to each well and the plate was incubated at 37°C for 30 min. During the incubation period, pre-warm TMB color at 37°C for 30 min before used, the plate was washed 5 times with 0.3 ml of PBS and the washing buffer was discarded and the plate was blotted onto a paper towel, 90 μl of TMB color developing agent was added into each well and incubated at 37°C for 11 min. as show that in the below figure (Figure 1).

In the above figure, the blue color can be seen in the wells after adding of TMB solution, the deep blue color in (A) refers to solution with the most concentration protein standard solutions wells, but the light blue color or no obvious showed to the samples wells (B) for sera and (C) for cell culture(Figure 1).

0.1 ml of TMB stop solution was added to each well to convert the color in well from blue to yellow as shown that in the figure (Figure 2).

The absorbance was read at 450 nm in a Microplate reader within...
mixed thoroughly, and then store at 4°C until use.

tubes to prepare serial dilute standards into their respectively and 
to prepare 1666.7 pg/ml and transfer 200 μl from each tube to another 
pg/ml stock standard solution to first tube of groups to prepare 5000 
diluent B for the secondly group tubes. Then transfer 0.2 μl of 15000 
A for firstly group tubes and 400 μl was transferred from (1x) of assay 
diluent: 5000 pg/ml, 1666.7 pg/ml, 555.6 pg/ml, 185.2 pg/ml, 61.7 pg/ 
minto two groups with the IFN-γ concentration to prepare serial 
centration 15.6-1000 pg/ml.

The standard curve was plotted to calculate the concentration of IFN-γ in serum and cell culture samples. The standard curve was plotted by using computer plot software, with standard concentration on the x-axis and absorbance on the y-axis. The best fit straight line was drawn through the standard points. The IFN-γ concentrations of samples were reported by multiply the interpolated standard curve by the dilution factor (sera x2) to obtain the target protein concentration in the samples. The normal range value detection was 15.6-1000 pg/ml.

Determination of the concentration level of IFN-γ in serum patients and cell culture (according to Kit protocol):  
Preparation of standard solution: The solution of vial item C was divided into two containers, for serum samples, 400 μl assay diluent A was added in first container and 400 μl of (1x) of assay diluent B for cell culture was added in second container to prepare a 50ng/ml standard and mixed thoroughly, 180 μl of IFN-γ standard from containers was added into two tubes, firstly tube containing 420 μl from assay diluent A, secondly tube containing (1x) of assay diluent B to prepare a 15000 pg/ml stock standard solution, 14 tubes were labeled and divided the minto two groups with the IFN-γ concentration to prepare serial diluent: 5000 pg/ml, 1666.7 pg/ml, 555.6 pg/ml, 185.2 pg/ml, 61.7 pg/ml, 20.6 pg/ml, and 0 pg/ml. 400 μl was transferred from assay diluent A for firstly group tubes and 400 μl was transferred from (1x) of assay diluent B for the secondly group tubes. Then transfer 0.2 μl of 15000 pg/ml stock standard solution to first tube of groups to prepare 5000 pg/ml and 200 μl was transferred from 5000 pg/ml tube to second tube to prepare 1666.7 pg/ml and transfer 200 μl from each tube to another tubes to prepare serial dilute standards into their respectively and mixed thoroughly, and then store at 4°C until uses.

Loading samples in micro-plate:100 μl of each of the serial standard solutions was added into empty wells of the pre-coated well plate, 100 μl from each samples test (serum or cell culture) was added into appropriate wells, well was covered, mixed gently and incubated at 4°C for overnight. Prepared wash buffer (1x) by dilute 20 ml of wash buffer concentrate into 400 ml D.D.W., and the solution was discarded and washed 4 times with 300 μl of (1x) wash buffer and the plate was blotted onto a filter paper No.1, 100 μl of prepared biotin antibody according to kit protocol was added to each well and incubate for 1hr. at room temperature with gentle mixing, the solution was discarded and the washing process repeated, 100 μl of prepared streptavidin solution according to kit protocol was added to each well and incubate for 45min. at room temperature with gentle mixing, the solution was discarded and the washing process repeated, 100 μl of TMB reagent was added to each well and incubated for 30min. at room temperature in the dark with gentle mixing, 50 μl of stop solution was added to each well, the absorbance was read at 450 nm immediately.

Calculation of results: The average absorbance of each set of duplicate standards was calculated, control and samples. The standard curve was plotted to calculate the concentration of IFN-γ in serum and cell culture samples. The standard curve was plotted by using computer plot software, with standard concentration on the x-axis and absorbance on the y-axis. The best fit straight line was drawn through the standard points. The IFN-γ concentrations of samples were reported by multiply the interpolated standard curve by the dilution factor (sera x2) to obtain the target protein concentration in the samples. The normal range value detection was 82-103 pg/ml to serum samples and 84-104 pg/ml to cell cultures. The minimum detectable dose of IFN-γ is typically less than 15 pg/ml.

Statistical analysis  
• Diagnostic test calculator Software program was used for statistical analysis the evaluation and comparison between diagnostic tests [12].  
• Statistical calculator software was used to statistical analysis of significance value (in 0.01value) of difference mean between two groups was assessed by Independent group’s t-test between means.  
• Statistical package of social science (t-Test) was used for statistical analysis of the results were shown as mean± standard deviation [13].  
• Person correlation coefficient was used to analyses the correlation between serum cytokines levels in typhoid fever, or in PMNCs culture.

Result and Discussion  
Identification of S. typhi by serological test  
Agglutination tests were known anti-sera and unknown culture isolate is mixed, and the clumping occurred within few minutes. So the interpretation of results were, Granular “clumps” observed in the tube were regarded as a positive result for ‘O’ antigen identification, where as a more floccules appearance observed by using a bright light against a dark background is regarded as a positive result for ‘H’ antigen identification and from cultivation method on XLD and S.S agar media show growth of bacteria with bile colonies with black center also give black at bottom of TSI medium this means bacteria was produced H2S and gas and from biochemical test the result was shown no. of isolates belong to S. typhi and number of bacteria isolates give positive results to serological method was 168, and 69 bacteria isolates were gave negative these test was performed [12].

Separation of PMNs layers  
The results were appeared four layers after separation blood the first layer was plasma and the second layer were represented lymphocytes layer which used for assaying the cytokines’ (Figure 3).

In the above figure four layers after separation by lymphocyte separation medium after centrifugation the sample at 400x g for 30 min (Figure 3).
Determining the concentration levels of cytokines in serum of typhoid patients

In the present study, the concentrated levels of IFN-γ and IL-6 were determined to investigate their role in the pathophysiology of typhoid fever in acute and chronic humans infect and investigate their role on diagnosis of typhoid patients.

Serum IFN-γ and IL-6 levels during the acute and chronic phases in typhoid patients compared to healthy control group (Table 1).

Serum IFN-γ and IL-6 level during the acute phase in typhoid patients’ group were high (128.787±2.522, 137.733±23.424 pg/ml) with highly significant (P≤0.01) than those in healthy control group. In addition, serum levels of IFN-γ and IL-6 during the chronic phase in typhoid patients’ group were higher levels (137.187±0.703, 427.20±6545 Pg/ml respectively) with highly significant (P≤0.01) than those in healthy control group.

Typhoid fever is caused by the facultative intracellular gram-negative bacillus S. typhi, the clinical features of typhoid fever confused with other febrile disease [14]. Cytokines have been shown to play principal roles in the defense against Salmonella infection; IFN-γ is one of the representatives of cytokines involved in the clearance of intracellular pathogens.

IL-6 is a Th1-inducing cytokine; IL-6 shares many of the biological properties and plays a critical role in the host defense against intracellular pathogens through T cell activation. In this study, IFN-γ and IL-6 are significantly increased in concentration levels in serum of typhoid patients compared the control, this result supports that IFN-γ and IL-6 are implicated in the pathogenesis of typhoid fever and agrees with the results reported that the levels of IFN-γ and IL-6 are elevated in typhoid patients compared to control [5,16-18].

Comparison between typhoid fever phases in inducing IFN-γ and IL-6

To determine which phase of typhoid fever (acute or chronic phase) is stimulate immune cells to produce and induce of these cytokines more than other phase in typhoid patients compared to control presented in the (Table 2).

Serum IFN-γ level was highly elevated in the chronic phase of typhoid fever compared to control with high significance (P≤0.01), and elevated in the chronic phase compared to acute phase of typhoid fever with clearly significant (P≤0.01), and serum IFN-γ level was highly elevated in the acute phase of typhoid fever compared to control with highly significant (P≤0.01).

In addition, the serum IL-6 level l was shown elevated in the chronic phase of typhoid fever compared to acute phase of typhoid fever with clearly significant (P≤0.01), the serum IL-6 level in the chronic phase showed more elevation compared to control with higher significance (P≤0.01), and serum IL-6 level was shown high elevation in the acute phase of typhoid fever compared to control with higher significance (P≤0.01) (Table 2).

The present study showed significance of correlation coefficients between the maximum levels of cytokines in each typhoid fever patient, including both the chronic and acute phase when the IFN-γ level correlated significantly with IL-6 level(P=0.014 with statistical significance p<0.05).

The present study has demonstrated the differences in the levels of cytokine responses between the acute and chronic cases of S. typhi infection by serum levels of IFN-γ and IL-6 in typhoid fever patients compared to control; these results indicated a stronger IFN-γ and IL-6 levels in the chronic typhoid patients more than acute typhoid patients compared to control; and agrees with the result of Mizunio Y., et al. (2003) who reported that the levels of IFN-γ and IL-6 were elevated in systemic form of typhoid fever more than gastro enteric form compared to control [13].

IFN-γ and IL-6 responses in the chronic typhoid fever returned to normal levels much later than those in the acute typhoid fever, it take around 6 weeks to eliminate even an attenuated virulent strain of Salmonella in mice, disseminate of S. typhi in systemic sites of chronic phase might result in prolonged survival of the bacteria and characteristic features of cytokine and cellular immune responses in the patients with systemic infection [19], that explained and supports the high levels of IFN-γ and IL-6in chronic phase more than in acute phase compared to control. The result in the present study agrees with the result of Sheikh A., et al. (2009) who that reported and observed detection of a parallel cellular response against S. typhi (PagCAg.) during human infection, including both Interferon-γ and proliferative responses, and shows that responses in convalescence were higher than during acute stage illness [18].

### Table 1: The mean levels of Cytokines (IFN-γ and IL-6) insera of healthy and typhoid Patients groups.

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Control</th>
<th>Acute Phase Patients</th>
<th>Chronic Phase Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ pg/ml</td>
<td>75.816±0.209</td>
<td>128.787±2.522</td>
<td>137.187±0.703</td>
</tr>
<tr>
<td>IL-6 pg/ml</td>
<td>10.333±1.958</td>
<td>137.733±2.432</td>
<td>427.20±6545</td>
</tr>
</tbody>
</table>

Where: * is P ≤ 0.01, SE: Standard error.

### Table 2: Comparison between two phase of typhoid fever disease in inducing IFN-γ and IL-6.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Cytokines</th>
<th>T-test</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control(1) vs. Acute(2)</td>
<td>IFN-γ pg/ml</td>
<td>0.01***</td>
<td>5.43</td>
</tr>
<tr>
<td>Chronic(3) vs. Control(1)</td>
<td>IFN-γ pg/ml</td>
<td>0.01***</td>
<td>3.44</td>
</tr>
<tr>
<td>Chronic(3) vs. Acute(2)</td>
<td>IFN-γ pg/ml</td>
<td>0.01**</td>
<td>2.20</td>
</tr>
</tbody>
</table>

Where: (1) Control healthy group; (2) Acute phase typhoid patients group; (3) Chronic phase typhoid patient group; *: Clear significant (P<0.01); **: Highly significant (P: 0.01); ***: Higher significant (P≤0.01).
The mean of concentration of serum cytokine levels (IFN-γ, IL6) in the controls, acute and chronic phases. When S. typhi is a specific human-restricted intracellular pathogen and the cause of typhoid fever; Cellular immune responses are required to control and clear Salmonella infection [20]. So, the adaptive immune response also provides positive feedback to the innate immune system through the synthesis of cytokines that either increase effector-cell numbers or activate these cells to produce an increased antibacterial response.

The protective roles of IL-18 during S. typhi infections are primarily related to its ability to induce IFN-γ, which activates the microbicidal activity of macrophages through induction of nitric oxide production. That mean an adequate T1 response is required to induce some cytokines pathway for eliminate the S. typhi such as Caspase-1 pathway to produce IL-6. But Mutate of the human genes of some crucial cytokines of this pathway, like IFN-γ, IL-12, and IL-6, greatly reduce cytokines pathway for eliminate of Salmonella infection [20].

That means an adequate Th1 response is required to induce some cytokines pathway for eliminating the S. typhi. The protective roles of IL-18 during S. typhi infections are primarily related to its ability to induce IFN-γ, which activates the microbicidal activity of macrophages through induction of nitric oxide production.

The mean levels of Cytokines (IFN-γ and IL-6) in PMNCs culture in response to S. typhi, PHA and compared to control.

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Stimulate by</th>
<th>Mean ± SE</th>
<th>T-test value</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ (pg/ml)</td>
<td>PHA(1)</td>
<td>9347.037±485.736</td>
<td>1vs2 1.889</td>
<td>0.01*</td>
</tr>
<tr>
<td></td>
<td>S. typhi(2)</td>
<td>8187.777±375.319</td>
<td>1vs3 14.592</td>
<td>0.01***</td>
</tr>
<tr>
<td></td>
<td>Control(3)</td>
<td>1713.70±194.189</td>
<td>2vs3 15.320</td>
<td>0.01***</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>PHA(1)</td>
<td>42.317±2.289</td>
<td>1vs2 8.424</td>
<td>0.01***</td>
</tr>
<tr>
<td></td>
<td>S. typhi(2)</td>
<td>66.444±4.177</td>
<td>1vs3 2.526</td>
<td>0.01***</td>
</tr>
<tr>
<td></td>
<td>Control(3)</td>
<td>28.827±4.826</td>
<td>2vs3 8.400</td>
<td>0.01***</td>
</tr>
</tbody>
</table>

Where: *P ≤ 0.01 (non-significant), **P≤ 0.01(significant), ***P≤0.01 (high significant).

**Determination of the concentration level of Cytokines in supernatant of PMNCs cultures**

To evaluate the releasing and production of IL-18 and IFN-γ by human peripheral blood mononuclear cells (PBMC) in response to S. typhi were examined in PBMCs culture stimulated to live S. typhi bacteria compared PHA and control in vitro. IFN-γ and IL-18 production levels in supernatant of PMNCs culture after stimulation to live bacteria of S. typhi compared to PHA and control (Table 3).

IFN-γ production levels in supernatant of PMNCs culture after stimulation to PHA and a live bacteria of S. typhi were higher levels (9347.037±485.736, 8187.777±375.319 pg/ml respectively), with higher significance (P≤0.01) during PBMCs culture stimulation to PHA and a live bacteria of S. typhi than those in control respectively, but non-significant of IFN-γ levels production to stimulate of PBMCs culture between PHA and S. typhi (P>0.01).

In addition, IL-6 production levels in supernatant of PMNCs culture after stimulation to a live S. typhi bacteria was higher levels (66.444±4.177 pg/ml) compared to PHA and control with higher of statistical significance (P≤0.01). And IL-6 production levels during the stimulation of PBMCs culture to PHA is significant compared to control (P≤0.01) (Table 3).

The limit evaluation of cellular responses in humans to wild-type S. typhi, with no animal model fully replicates host pathogen interactions and immunologic events that occur during this human-restricted infection, in additional evaluation in humans has largely focused on characterizing responses in recipients of attenuated vaccine strains of S. typhi. So, this study presented some of defense mechanism against Salmonella infection by ability of PMNCs culture to releasing and production of IL-6 and IFN-γ which are circuits to activate of many immune cells in typhoid fever infection.

In peripheral blood, Cellular immune responses (T-helper cells) mediated produce Th1 cytokines such as IL-6 and IFN-γ in response to S. typhi infections. The inflammatory processes trigger various types of cells, macrophages and monocytes, to release many cytokines. The released cytokines trigger other cells and initiate the cascade of cytokine release which can contribute to activating of appropriate host defenses.

IL-6 is important for the induction of IFN-γ, and these cytokine is central for successful host defense against Salmonella infection; because neutralization offIL-6leads to increased bacterial numbers in spleen and liver and decreased host survival, while IL-18 treatment decreases bacterial counts in spleen and liver and increases host survival. This shows that IL-6 plays an important role in host defense against Salmonella.

This role is effective to be mediated of IFN-γ production.

Zhou L, et al. (2010) mention IFN-γ production by PMNCs following stimulation with Salmonella was significant inhibited by anti-IL-6 monoclonal Ab (P<0.05)[8]. Consistent with the above results, the IFN-γ level correlated significant with the IL-6 level in the present study, suggesting a possible involvement of IL-18 to induce IFN-γ against human Salmonella infection in vivo. Higher levels of production of IL-6 and IFN-γ in vitro stimulated by live S. typhi than control might reflect the in vivo activation for producing of these cytokines from cells.

**References**


