

Influence of Interferon- γ on Salmonella Typhi Induced Macrophage Apoptosis

Fidan I, Yesilyurt E, Kalkan A, Erdal B, Gurelik FC and Imir T

Gazi University, Faculty of Medicine, Department of Medical Microbiology, Turkey

ABSTRACT

Introduction: *Salmonella Typhi* (*S. Typhi*), which causes typhoid fever, is a widespread pathogen in developing countries. Interferon-gamma (IFN- γ) is a critical cytokine in host defense against *Salmonella* infection. IFN- γ provides protection against *Salmonella* infection by inducing macrophage activation. This study was designed to determine the effect of recombinant IFN- γ (rIFN- γ) on *S. Typhi* induced macrophage apoptosis and to examine the effect of rIFN- γ on caspase-1 expression during apoptosis. **Materials and Methods:** After isolation of macrophages, apoptotic cells were analyzed using both annexin V-FITC detection kit by flow cytometry and TUNEL technique. Caspase-1 expression was determined by RT-PCR. **Results:** The rIFN- γ concentrations of 100 IU/ml ve 1000 IU/ml decreased macrophage apoptosis caused by *S. Typhi*, 13.1 % and 6.3 % respectively. **Conclusion:** Consequently, we observed that rIFN- γ decreased *Salmonella*-induced apoptosis and inhibited caspase-1 expression during apoptosis. It is considered that the modulatory effect of IFN- γ on macrophage apoptosis may impact a protective effect during *Salmonella* infection and this may help to abort invasive *S. Typhi* infections.

KEYWORDS: *S. typhi*, macrophages, apoptosis

INTRODUCTION

Salmonella enterica serovar Typhi (*S. Typhi*) is a facultative, intracellular pathogen that is a common cause of typhoid fever in human. Typhoid fever is a serious and widespread disease in developing countries.¹

Phagocytic leukocytes such as macrophages and neutrophils are essential for the innate immune response of the host against invading pathogens.² Macrophages serve a central role in host defense against pathogenic microbes by their ability to rapidly recognize bacterial components, phagocytose pathogens, and activate an arsenal of antimicrobial effectors to contain and eliminate the microbe.³ *Salmonella* can survive within mononuclear phagocytic cells and tissue macrophages transport *Salmonella* from the intestine to the systemic circulation.⁴⁻⁶

Apoptosis is a programmed cell death process triggered by various stimuli such as bacterial and viral infections.⁷ Apoptosis of host cells has been identified to be involved in the regulation of immune response. Depending on the pathogen, apoptosis of host cells may be harmful or beneficial to the host.⁸

Typhoid fever disease is an example of severe sepsis, because it presents enormous amount of cellular death besides severe toxemia.⁹ Cell death induction by *Salmonella* often occurs by apoptotic cell death. *Salmonella* directly activates pro-apoptotic signaling pathways to initiate apoptosis.¹⁰ In some studies, it has been shown that *Salmonella* triggers apoptosis in macrophages as a virulence strategy.^{2,7,8,11} In this way, *Salmonella* eludes innate immune responses and successfully colonize the host. Besides, *Salmonella*-induced cell death may trigger an inflammatory response due to release of proinflammatory cytokines, thereby contributing to the pathogenesis of diarrheal disease.¹²

Interferon gamma (IFN- γ) plays an important role at the early bacterial growth stage by restricting the rate of multiplication of the bacteria in macrophages.¹³ IFN- γ is produced primarily by natural killer (NK) and T cells and mediates the upregulation of nitric oxide synthase (iNOS)-dependent macrophage antibacterial mechanisms. IFN- γ enhances both earlier oxidative killing and later nitrosative killing and control of *Salmonella* infection. Tissue culture studies have demonstrated that IFN- γ primes murine macrophages for efficient killing of *Salmonella*. With the use different experimental models, IFN- γ has been shown to play a central role in both early limitation of replication and later clearance of *Salmonella*.¹⁴

Apoptosis of the cells may be regulated by the presence of growth factors and cytokines. The type II cytokine IFN- γ is capable of eliciting apoptotic effects in immune and tumour cells. On the other hand,

Corresponding Author:

Isil Fidan, MD

Gazi University, Faculty of Medicine,

Department of Medical Microbiology

Ankara 06500, TURKEY

e-mail: isilfidan@yahoo.com

it is also involved in prosurvival signals in normal and leukemic cells. Hence, it is suggested that depending on the target cells, IFN γ can induce either death or protection against apoptosis.^{15,16}

In this study, we examined the changes in *S. typhi* induced macrophage apoptosis in human monocyte-derived macrophages primed with recombinant IFN- γ (*r*IFN- γ). We measured the effect of *r*IFN- γ on human macrophage apoptosis following in vitro infection with *S. typhi*. We also studied the mechanism of IFN γ action in modulating *S. typhi*-induced macrophage apoptosis using monocyte-derived macrophage cells. For this reason, caspase-1 expression on the monocyte-derived macrophages was also examined.

MATERIALS AND METHODS

Bacterial strain

S. Typhi strain used in this study was isolated from stool. After isolation the organism was inoculated into brain heart infusion broth (BHIB) (Oxoid, UK) and incubated for 18 h at 37°C. At the end of the incubation, bacteria were washed and resuspended in PBS. The bacterial suspension was adjusted to a concentration of 108 CFU/ml (colony forming unit) in antibiotic-free RPMI medium with 10% FCS.

Preparation of human monocyte-derived macrophages (MDM)

Heparinized blood was collected from healthy human volunteers. Approval of the Local Ethical Committee was obtained for the collection of heparinized blood samples from healthy blood donor. Peripheral blood mononuclear cells (PBMC) were separated from blood by Ficoll-hypaque density gradient centrifugation (Sigma, UK). After centrifugation, buffy coats were collected and washed three times in phosphate buffered saline (PBS, Gibco, Germany) and resuspended at a concentration of 10⁷ cells/ml in complete RPMI 1640 medium containing 2mM L-glutamine, 100 U/ml penicillin, 100 Ug/ml streptomycin, 0.05 mM 2-Mercapto-ethanol and supplemented with 10% fetal calf serum (FCS, Gibco, Germany). After 2 h adherence, the medium was replaced with RPMI supplemented with 10% FCS and 1-ml aliquots were cultured at 37°C in 5% CO₂ in 24-well tissue culture trays (Nunc, United Kingdom). Cultures were kept at 37°C in 5% CO₂. The medium was changed every 48-72 h and fresh media were added. Human MDM cultures were tested following 5-7 days incubation.^{14,17}

Priming of MDM with IFN- γ

On day 8 of MDM culture, the MDMs were primed with the recombinant human IFN- γ at two different concentrations (1000 IU/ml and 100 IU/ml) (Biosource, USA) in RPMI medium with 10% FCS, reincubated at 37°C in 5% CO₂ for 72 hours. Control wells contained only MDM or RPMI but not IFN- γ .¹⁴

Infection of MDM with *S. Typhi*

Bacterial suspensions were prepared freshly for the experiment. On day 11 the MDM cultures, were inoculated with *S. Typhi* (108 CFU/ml) and incubated for 2 hours at 37°C in 5% CO₂. Negative control wells did not receive *S. Typhi*. After stimulation with bacteria, contents of wells were transferred into tubes and centrifuged. The pelleted cells were analyzed by flow cytometry and fluorescence microscopy for determining the apoptotic cells. Each assay was performed in triplicate.

Macrophage apoptosis

Analysis of macrophages undergoing apoptosis was done using two different methods. In the first method, annexin V-FITC detection kit was used. While TUNEL technique was applied for the second.

Annexin V-FITC detection kit

Analysis of macrophages undergoing apoptosis was performed with the annexin V-FITC detection kit according to the manufacturer's instructions (Beckman Coulter, USA). 5 \times 10⁵ cells uninfected or infected with *S. Typhi* as described above, were incubated with 1 μ l of annexin V-FITC and 5 μ l of Propidium iodide for 15 min in the dark. After the incubation, 400 μ l ice-cold 1X binding buffer was added and the cells were analyzed by flow cytometry. Flow cytometric analysis was done by using a Coulter FC500 flow cytometer (Coulter, USA).

TUNEL technique for determination of apoptosis

TUNEL technique was applied using In situ Cell Death Detection Kit, Fluorescein (Roche, Germany). The TUNEL reaction preferentially labels DNA strand breaks generated during apoptosis. Cleavage of genomic DNA during apoptosis may produce double-stranded, low molecular weight DNA fragments. These DNA strand breaks can be determined by labelling free 3'-OH termini in an enzymatic reaction. DNA fragments in cells by fluorescence microscopy was determined by In situ Cell Death Detection Kit according to the manufacturer's instructions. Uninfected or infected 2 \times 10⁷ cells/ml were with *S. Typhi* were fixed with a freshly prepared fixation solution (4% Paraformaldehyde in PBS) for 1 hour at 15-25°C. Then cells were incubated in permeabilisation solution for 2 minutes on ice. At the end of the incubation, TUNEL reaction mixture containing terminal deoxynucleotidyl transferase (TdT) (enzyme solution) and nucleotide mixture (labeling solution) was added and incubated for 1 hour at room temperature. TdT incorporates labeled nucleotides into DNA strand breaks. Fixed and permeabilized cells in Label solution were used as negative control. Fixed and permeabilized cells with DNase I, grade I (Roche, Germany) were used as positive control. After incubation, cells were evaluated under a fluorescent microscope (Olympus, USA). The apoptotic index (AI=percentage of apoptotic cells) was determined.¹⁸

Apoptotic index (AI)= (number of TUNEL reactive nuclei / total number of cells counted) x 100.

Detection of caspase-1 expression by RT-PCR

Caspase-1 expression was determined at the mRNA level using reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was extracted from cultured cells by RNA isolation kit according to the manufacturer's instructions (Biological Industries, India). Briefly, total RNA was used as a template to synthesize cDNA. Reverse transcription was carried out at 95°C for 1 min, 50°C for 1 h, 4°C for 5 min. The integrity of the cDNAs was confirmed by PCR amplification of β -actin. The caspase-1 cDNA was then amplified using primers listed in Table 1.19 The primers were synthesized by Alpha DNA, Canada. PCR was conducted by adding approximately 10 ng cDNA, to a 0.5 ml microfuge tube containing 20 pmol oligonucleotide primer, 250 μ M each of dATP, dTTP, dCTP and dGTP, 3 mM MgCl₂, 2.5 U Taq DNA polymerase and 10X buffer in a final volume of 25 μ l (all of the chemicals were obtained from Biochron, Germany). The amplification procedure was performed with an initial denaturation at 95°C for 1 min, followed by 35 cycles of, 45 s at 95°C, 60°C for 45 s for recommended annealing temperatures of each primer pair and 60s at 72°C, with a final extension at 72°C for 5 min in a thermal cycler (Eppendorf, Canada). The amplified products were separated in 2% (w/v) agarose gels containing 0.5 μ g ethidium bromide ml⁻¹ and viewed on a UV transilluminator.

Table 1. Primer sets used for amplifying the human caspase-1 and β -actin coding genes

	Primer sets
Caspase-1	5'-TCGGCAGAGATTTATCCAATAATG-3'
Caspase-1	5'-ATCTGGCTGCTCAAATGAAAATCG-3'
β - α uv	5'-GTGGGGCGCCCCAGGCACCA-3'
β - α uv	5'-CTCCTTAATGTCACGCACGATTTC-3'

Statistical methods

The results were analysed using the one-way analysis of variance (ANOVA). The Bonferroni test was used as Post Hoc analysis. P<0.05 was considered to be significant.

RESULTS

S. Typhi-induced macrophage apoptosis

In our study which focused on the effect of IFN- γ on S. Typhi-induced macrophage apoptosis, we observed that there was 35.9% apoptosis in macrophages stimulated by S. Typhi while this rate was only 2.6% in macrophages without S. Typhi. There was a significant difference in apoptosis levels between stimulated and non-stimulated cells (p<0.001). So, it is clear that apoptosis was increased remarkably in macrophages infected by S. Typhi (Figure 1).

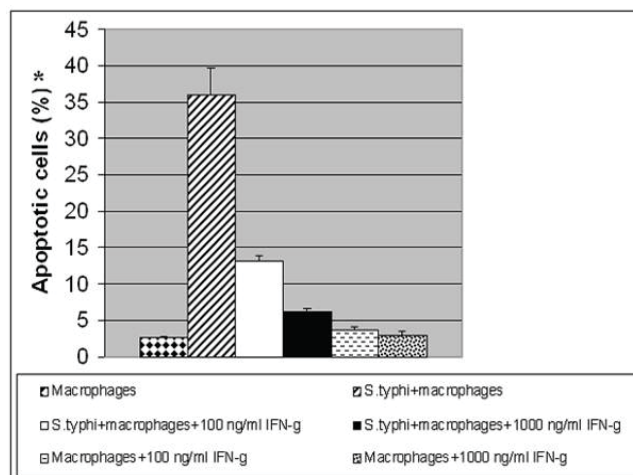


Figure 1. The effect of rIFN- γ on S.Typhi-induced macrophage apoptosis using the Annexin V FITC method. *Values represent the mean \pm SEM.

Effect of rIFN- γ on S.typhi-induced macrophage apoptosis

Annexin V-FITC detection kit

In this study, it was observed that rIFN- γ at 100 and 1000 IU/ml concentrations decreased apoptosis from 35.9% to 13.1% and 6.3% respectively. Thus, the addition of rIFN- γ to the macrophages infected by S. typhi caused a statistically significant decrease in the cells undergoing apoptosis (p <0.001). The addition of IFN- γ in high concentrations caused a more remarkable decrease in S.Typhi induced macrophage apoptosis than the low concentrations did, but no statistically significant difference was observed between these two concentrations (p=0.186) (Figure 1). Especially high concentrations of IFN- γ had almost the same decreasing effect on S.Typhi induced macrophage apoptosis as the one observed in the apoptosis non-stimulated by S.Typhi. Thus, it was observed that the 1000 ng/ml concentration of rIFN- γ had a more remarkable apoptosis decreasing effect compared to the low concentrations and the apoptosis decreasing effect was dose-dependent.

Fluorescence microscopic analysis of TUNEL technique

In TUNEL technique, it was observed that rIFN- γ of 100 and 1000 IU/ml concentrations decreased apoptosis from 46% to 20% and 4% respectively. In the TUNEL technique, 1000 IU/ml concentrations of rIFN- γ caused a statistically significant decrease in S.typhi induced macrophage apoptosis as composed to the 100 IU/ml concentrations (p >0.001). Moreover, no statistically significant difference was observed between the results of Annexin V-FITC detection kit and TUNEL technique and they were consistent with each other (p>0.05) (Figure 2).

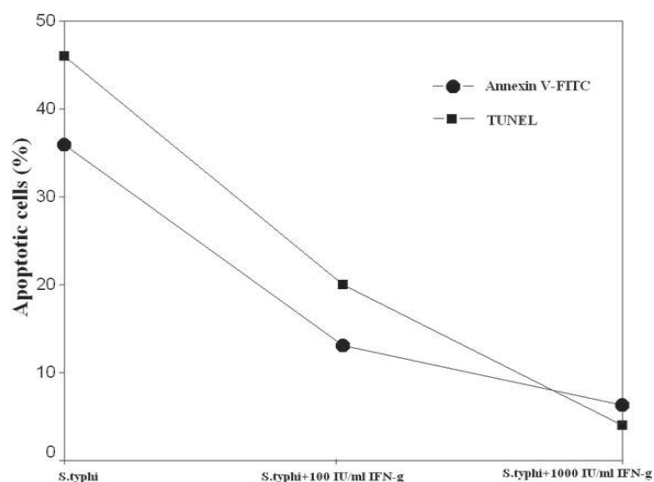


Figure 2. Comparison of the effect of *rIFN-γ* on *S.Typhi*-induced macrophage apoptosis by the use of two different techniques: TUNEL and Annexin V- FITC

Caspase-1 expression

In our study caspase-1 mRNA expression was determined in the macrophages infected by *S.Typhi* using RT-PCR method. However, no caspase-1 mRNA expression was observed in the macrophage cultures infected by *S.Typhi* when *rIFN-γ* was added at 100 and 1000 IU/ml concentrations (Figure 3).

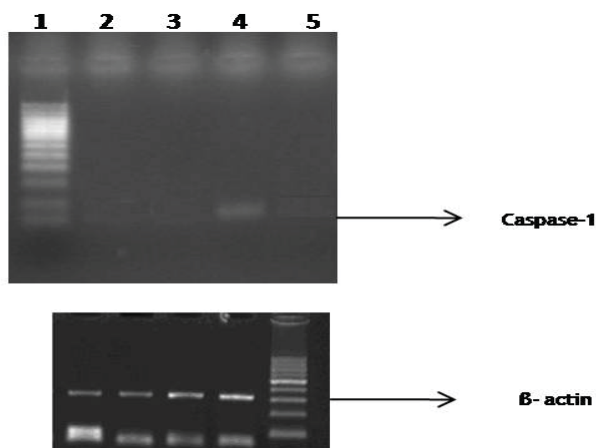


Figure 3. Isolation of caspase-1 cDNA by RT-PCR. Lane 1: The molecular marker; Lane 2: *S.Typhi*+macrophages+100 IU/ml *IFN-γ*; Lane 3: *S.Typhi*+macrophages+1000 IU/ml *IFN-γ*; Lane 4: *S.Typhi*+macrophages; Lane 5: Uninfected macrophages

DISCUSSION

As it was shown in many other studies, at the first stage of our study *S.Typhi* was observed to cause apoptosis in macrophages.^{2,20,21} Salmonella-induced cell death requires caspase-1 activity. Caspase-1-dependent apoptosis might facilitate bacterial spread.^{22,23} *S.Typhi* induced macrophage apoptosis is an important factor in the development of infections.¹² It is known that the ability of pathogens to induce apoptosis may play a role in the initiation of the infection, survival

of the pathogens, and escape from the host immune response.²⁴ Thus, the determination of the factors that could prevent *S.Typhi* induced apoptosis will be helpful for the control of the infection. Cytokines are important factors that regulate apoptosis and inhibition of cytokine mediated apoptosis is one pathway for halting salmonella infection.

In some studies it has been reported that the proinflammatory cytokine *IFN-γ* is essential component of the host response for inhibiting systemic infection by *S.Typhimurium* in the early phase of disease, before specific immunity has been stimulated.^{14,25,26} The protective efficacy of *IFN-γ* against infection is due to its effects on activating the macrophages, and protecting them from apoptosis.¹⁵ In the light of these findings, we carried out our study to determine whether *IFN-γ* had any efficacy against in vitro salmonella infection of macrophages by inhibiting apoptosis. We also studied the effect of *rIFN-γ* on caspase-1 expression, a necessary event for inducing apoptosis due to salmonella infection.

It is known that that *IFN-γ* can induce either cell death or protection against apoptosis of normal and malignant cells. This double effect depends on the target cells and the differences in the expression of *IFN-γ*'s receptor chains.^{15,27} Although Schroder et al, found that *IFN-γ* was able to provide protection against pathogen-induced apoptosis, they noticed that levels of interferon regulatory factor-1 (IRF-1) may be a decisive factor in whether *IFN-γ* induces or protects from apoptosis.²⁸ Bernabei et al, observed that after *IFN-γ* stimulation, high levels of IRF-1 triggered the apoptotic program, whereas lower levels induced proliferation of peripheral blood mononuclear cells.²⁹

In our study, we found out that *rIFN-g* had a significant inhibitory effect on apoptosis occurring in the macrophages infected by *S.Typhi* using TUNEL technology and flow cytometry with Annexin V-FITC detection kit ($p < 0.001$). Moreover, high concentrations of *rIFN-γ* decreased *S.Typhi* induced apoptosis almost to the previous intimation levels. That is to say, *rIFN-γ* effected apoptosis inhibition in a dose-dependent manner.

Xaus et al, also observed that incubation of macrophages with 250 U/ml *IFN-γ* suppressed the fragmentation of the DNA during the induction of apoptosis.¹⁵ Several authors indicated that *rIFN-γ* has an inhibitory effect on apoptosis caused by the microorganisms other than salmonella.^{30,31} Perfettini et al reported that *IFN-γ* could contribute to pathogenesis of persistent chlamydia infections in vivo by preventing apoptosis of infected cells.³⁰ In our study, the inhibition of *salmonella*-induced apoptosis by *rIFN-γ* may presumably contribute to the intracellular persistence of *Salmonella*.³²

Several different mechanisms have been considered in the preventive effect of *rIFN-γ* on apoptosis. For this reason, we examined caspase-1 expression to

understand the mechanisms that play a role in the inhibition of apoptosis. The suppression of caspase-1 expression was considered to be an important factor in IFN- γ inhibition of Salmonella-induced apoptosis. Actually, in our study, it was observed that caspase-1 expression was inhibited after the administration of *r*IFN- γ . Perfettini et al, have also reported that cells infected with *C. trachomatis* in the presence of IFN- γ resisted apoptosis due to external ligands, via inhibition of caspase activation.³⁰ Moreover, inhibition of caspase activation was shown in the absence of IFN- γ .³³ Contrary to our findings, Xaus et al, reported that IFN- γ prevented macrophages from undergoing apoptosis by blocking the cell cycle via the induction of p21^{Waf1} and made the macrophages unresponsive to apoptotic stimuli.¹⁵ They suggested that the protection of IFN- γ against apoptosis could be related to the effect of p21^{Waf1}. As can be seen, we think that there is not only one mechanism responsible for the antiapoptotic effect of *r*IFN- γ but different factors play a role in the process.

In other words, our data were consistent with the reports indicating that IFN-gamma was essential for the host defense against Salmonella infection and involved in enhancing the antibacterial activities of the macrophages.²⁶ However, there are some other findings showing that IFN- γ leads to apoptosis via caspase-1 dependent induction.¹⁶ But, these findings are obtained from the studies generally carried out to test the efficacy of IFN- γ on the inhibition of growth of tumor cells. As we have already indicated, cell type has an important role in the dual effect of IFN- γ on apoptosis. Our observation about the inhibitory effect of IFN- γ on pathogen-induced macrophage apoptosis, but not in tumor cells, may have resulted from differences in these cell types.

Conclusion, previous studies have generally focused on the killing effect of *r*IFN- γ on *Salmonella* species, but our study gains its importance from the fact that it is one of the first studies that were done to determine the efficacy of the inhibitory effect of *r*IFN- γ on *Salmonella* induced macrophage apoptosis and to understand the mechanisms playing a role in this inhibition.

We have demonstrated that priming of human macrophages with IFN- γ had significant effects on macrophage apoptosis induced by *S.Typhi*. It may be misleading to consider that *r*IFN- γ prevention of salmonella infections solely due to its inhibitory effect on apoptosis, but it is certain that this is an important mechanism that should not be ignored. Planned future studies will focus on other mechanisms through which *r*IFN- γ prevents apoptosis. Still, our findings may stimulate further studies about the immunopathological mechanisms of invasive or recurrent *S.Typhi* infection and studies on the therapeutic use of IFN- γ as an immunoadjuvant therapy in patients susceptible to recurrent or persistent salmonellosis.

REFERENCES

1. Mastroeni P, Ménager N. Development of acquired immunity to Salmonella. *J Med Microbiol* 2003; 52:453-9
2. Chanana V, Majumdar S, Rishi P. Involvement of caspase-3, lipid peroxidation and TNF-alpha in causing apoptosis of macrophages by coordinately expressed Salmonella phenotype under stress conditions. *Mol Immunol* 2007; 44:1551-8
3. Rosenberger CM, Finlay BB. Macrophages Inhibit Salmonella typhimurium Replication through MEK/ERK Kinase and Phagocyte NADPH Oxidase Activities. *J Biol Chem* 2002; 277:18753-62
4. Vazquez-Torres A, Jones-Carson J, Bäumlér AJ, et al. Extraintestinal dissemination of Salmonella by CD18-expressing phagocytes. *Nature* 1999; 401:804-8
5. Knodler LA, Finlay BB. Salmonella and apoptosis: to live or let die? *Microb Infect* 2001; 3:1321-6
6. Okamura M, Lillehoj HS, Raybourne RB, et al. Differential responses of macrophages to Salmonella enterica serovars Enteritidis and Typhimurium. *Vet Immunol Immunopathol* 2005; 107:327-35
7. Hueffer K, Galán JE. Salmonella-induced macrophage death: multiple mechanisms, different outcomes. *Cell Microbiol* 2004; 6:1019-25
8. Chander H, Majumdar S, Sapru S, Rishi P. Macrophage cell death due to Salmonella enterica Serovar Typhi and its acid stress protein Has features of apoptosis. *Microbiol Immunol* 2005; 49:323-30
9. de Andrade DR, de Andrade Junior DR. Typhoid fever as cellular microbiological model. *Rev Inst Med Trop Sao Paulo* 2003; 45:185-91
10. Grassme H, Jendrossek V, Gulbins E. Molecular mechanisms of bacteria induced apoptosis. *Apoptosis* 2001; 6:441-5
11. Boise LH, Collins CM. Salmonella-induced cell death: apoptosis, necrosis or programmed cell death? *Trends Microbiol* 2001; 9:64-7
12. Chanana V, Majumdar S, Rishi P. Tumour - necrosis factor α mediated apoptosis in murine macrophages Salmonella enterica serovar Typhi under oxidative stress. *FEMS Immunol Med Microbiol* 2006; 47:278-86
13. Muotiala A, Makela PH. Role of gamma interferon in late stages of murine Salmonellosis. *Infect Immun* 1993; 61:4248-53
14. Gordon MA, Jack DL, Dockrell DH, Lee ME, and Read RC. Gamma Interferon Enhances Internalization and Early Nonoxidative Killing of Salmonella enterica Serovar Typhimurium by Human Macrophages and Modifies Cytokine Responses. *Infect Immun* 2005; 73:3445-52
15. Xaus J, Cardó M, Valledor AF, Soler C,

16. Lloberas J, Celada A. Interferon γ Induces the Expression of p21waf-1 and Arrests Macrophage Cell Cycle, Preventing Induction of Apoptosis. *Immunity* 1999; 11:103-13
17. Detjen KM, Farwig K, Welzel M, Rosewicz S. Interferon γ inhibits growth of human pancreatic carcinoma cells via caspase-1 dependent induction of apoptosis. *Gut* 2001; 49:251-62
18. Keidar S, Strizevsky A, Raz A, Gamliel-Lazarovich A. ACE2 activity is increased in monocyte-derived macrophages from prehypertensive subjects. *Nephrol Dial Transplant* 2007; 22:597-601
19. Bhosle SM, Huilgol NG, Mishra KP. Apoptotic index as predictive marker for radiosensitivity of cervical carcinoma: Evaluation of membrane fluidity, biochemical parameters and apoptosis after the first dose of fractionated radiotherapy to patients. *Cancer Detect Prev* 2005; 29:369-75
20. Li S-H, Lam S, Cheng AL, Li X-J. Intranuclear huntingtin increases the expression of caspase-1 and induces apoptosis. *Hum Mol Gen* 2000; 9:2859-67
21. Monack DM, Raupach B, Hromockyj AE, Falkow S. Salmonella typhimurium invasion induces apoptosis in infected macrophages. *Proc Natl Acad Sci* 1996; 93:9833-8
22. Valle E, Guiney DG. Characterization of Salmonella-induced cell death in human macrophage-like THP-1 cells. *Infect Immun* 2005; 73:2836-40
23. Van det Velden AWM, Velasquez M, Starnbach MN. Salmonella rapidly kill dendritic cells via a Caspase-1- dependent mechanism. *J Immunol* 2003; 171:6742-9
24. Jarvelainen HA, Galmiche A, Zychlinsky A. Caspase-1 activation by Salmonella. *TRENDS Cell Biol* 2003; 13:204-9
25. Ojcius DM, Souque P, Dautry-Varsat A. Apoptosis of epithelial cells and macrophages due to infection with the obligate intracellular pathogen Chlamydia psittaci. *J Immunol* 1998; 61:4220-6
26. Kagaya K, Watanabe K, Fukazawa Y. Capacity of recombinant gamma interferon to activate macrophages for Salmonella-killing activity. *Infect Immun* 1989; 57(2):609-15
27. Gulig PA, Doyle TJ, Clare-Salzler MJ, Maiese RL, Matsui H. Systemic infection of mice by wild-type but not Spv- Salmonella typhimurium is enhanced by neutralization of gamma interferon and Tumor Necrosis Factor Alpha. *Infect Immun* 1997; 65:5191-7
28. Allione A, Bernabei P, Bosticardo M, Ariotti S, Forni G, Novelli F. Nitric oxide suppresses human T lymphocyte proliferation through IFN- γ -independent induction of apoptosis. *J Immunol* 1999; 163:4182-91
29. Schroder K, Hertzog PJ, Ravasi T, Hume DA. Interferon- γ : an overview of signals, mechanisms and functions. *J Leuko Biol* 2004; 75:163-189
30. Bernabei P, Coccia EM, Rigamonti L, et al. Interferon- γ receptor 2 expression as the deciding factor in human T, B, and myeloid cell proliferation or death. *J Leuko Biol* 2001; 70:950-60
31. Perfettini JL, Darville T, Dautry-Varsat A, Rank RG, Ojcius DM. Inhibition of Apoptosis by Gamma Interferon in Cells and Mice Infected with Chlamydia muridarum (the Mouse Pneumonitis Strain of Chlamydia trachomatis). *Infect Immun* 2002; 70:2559-65
32. Gold JA, Hoshino Y, Hoshino S, Jones MB, Nolan A, Weiden MD. Exogenous Gamma and Alpha/Beta Interferon Rescues Human Macrophages from Cell Death Induced by Bacillus anthracis. *Infect Immun* 2004; 72:1291-7
33. Freeman AF, Holland SM. Persistent bacterial infections and primary immune disorders. *Curr Opin Microbiol* 2007; 10:70-5
34. Dean D, Powers VC. Persistent Chlamydia trachomatis Infections Resist Apoptotic Stimuli. *Infect Immun* 2001; 69:2442-7