British Microbiology Research Journal
4(1): 50-62, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Effects of Gamma Interferon on Intestinal Epithelial Cells Stimulated with *Salmonella Enterica serovar* Typhimurium and *Candida albicans*

Florence A. Okafor[1*], Ebony I. Weems[1] and Leonard L.Williams[2]

[1]Biological and Environmental Sciences Department, Alabama A&M University, Normal AL 35762, United States.
[2]North Carolina State University Center for Excellence in Post-Harvest Technologies, United States.

Authors' contributions

Authors FAO and EIW designed the study, managed the literature searches, wrote the protocol, performed the experimental analysis and wrote the first and final drafts of the manuscript respectively. Part of the study served as authors EI Weems MS thesis. Author LLW supervised and analyzed the flow cytometry and ELISA assays. All authors read and approved the final manuscript.

Research Article

Received 23th May 2013
Accepted 7th September 2013
Published 5th October 2013

ABSTRACT

Aims: The objective of this study was to determine the level of secretion of gamma-interferon by interferon-primed and unprimed Caco-2 intestinal epithelial cells and their survival or growth following infection with *Salmonella enterica subsp. enterica serovar* Typhimurium (ATCC 14028), *Salmonella enterica subsp. enterica serovar* Typhimurium DT104 (ATCC 700408), *and Candida albicans* (Robin) Berkhout, anamorph (ATCC 10231) as well as the survival of the test microorganisms following infection.
Study Design: Controlled laboratory experiments were performed using two different species of *Salmonella* and adenocarcinoma Caco-2 cells. Untreated/ unprimed Caco-2 cells served as control; Caco-2 cells' growth and interferon production were then determined using, Enzyme Linked Immunosorbent Assay (ELISA) and flow cytometry.
Place and Duration of Study: Department of Biological & Environmental Sciences Alabama A& M University and Center for Excellence in Post-Harvest Technologies, North Carolina A&T University USA April 2008 and February 2010.

**Corresponding author: Email: florence.okafor@aamu.edu;*

Methodology: Cell culture supernatants of Caco-2 intestinal epithelial cells, primed and unprimed with IFN-γ were infected with either wild-type *Salmonella* Typhimurium 14028, *Candida albicans*10231 or multi-drug resistant *Salmonella* Typhimurium DT104 were collected and analyzed. ELISA and flow cytometry were used to determine apoptosis, cell growth and interferon production. The Bioscreen-C Automated Growth Curve Analysis System was used under controlled environment to determine the growth of the microorganisms in the presence of different concentrations of IFN-γ.
Results: Secretion of IFN-γ from Caco-2 cells that were previously treated with 50µg/ml, 20µg/ml, 10µg/ml, 5µg/ml, and 2.5 µg/ml of IFN-γ were not concentration dependent. However, the amount of IFN-γ released from Caco-2 cells was dependent on microbial stimulus type. Cells that were pretreated with 5 µg/ml and 2.5 µg/ml of IFN-γ and then infected with *Salmonella* Typhimurium DT104 showed an increase in the amount of IFN-γ in the culture medium after 5 minutes. IFN-γ induced CaCo-2 cell death was dose-dependent for S.Typhimurium DT104 and *Candida albicans*. Results are reported as mean ± SEM fortriplicate values from three independent experiments at each time point and IFN-γ dose.
Conclusion: These findings indicate that IFN-γ may serve as alternative antimicrobial compounds to reduce the persistence of multi-drug resistant microorganisms such as *S.* Typhimurium DT104. Induction of interferon-gamma production may be related to microbial virulence/pathogenicity. The potential of IFN-γ as a natural therapeutic for persistent infections in the immune-compromised populations still needs to be further investigated.

Keywords: Interferon-gamma; Salmonella Typhimurium DT104; Candida albicans; Caco-2 Adeno-carcinoma cells.

1. INTRODUCTION

Salmonella enterica serovar Typhimurium is a pathogenic Gram-negative bacteria found mainly in the gastro-intestinal tract [1]. Its toxicity is due to an outer membrane consisting largely of lipopolysaccharides (LPS) which protect the bacteria from the environment. *Salmonella* is responsible for frequent and reoccurring diseases in Africa, Southeast Asia, and South America [2]. Some of the pathogenic strategies of *S.* Typhimurium include penetration of the mucosal barrier and invasion of non-phagocytic cells of the intestinal mucosa. The ability of *S.* Typhimurium to survive ~~to~~ host defense mechanisms and to cause disease has been directly linked to genes encoded in pathogenicity islands, which are large horizontally acquired regions of the chromosome. The emergence of drug-resistant *Salmonella* continues to be a major public health problem. Multi-drug resistant *Salmonella enterica serovar* Typhimurium DT104 also termed *Salmonella* Typhimurium DT104 has been shown to express multiple drug resistant genes [3,4] and is resistant to a spectrum of drugs. These drugs include ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline [5]. It has been reported that infections with drug-resistant *Salmonella* accounted for over 30,000 infections in a pool of over one million cases of gastroenteritis [6]. *Salmonella* infection has also been found to be recurrent in HIV- infected patients and immune-compromised patients [7,8]. *Salmonella enterica serovar* Typhimurium 14028 (now termed *S. enterica subsp. enterica serovar* Typhimurium ATCC 14028), is a descendant of CDC 60-6516, a strain isolated in 1960 from pools of hearts and livers of 4-week-old chickens [9].

Candida albicans is a dimorphic fungus that is part of the human normal flora. *C. albicans* is a frequent causative agent of the opportunistic infections (candidiasis) that could prove fatal. Ruhnke et al. [10] described *Candida albicans* as a common causative agent of nosocomial

infections. This fungus causes opportunistic infections in the human oral and genital tracts. *Candida albicans* may also cause systemic infections in immunocompromised individuals such as individuals with HIV, diabetes, cancer, or individuals in the extreme age groups, and organ transplant patients. The incidence of candidiasis continues to increase as a result of an expanding immuno-compromised population [11].

The Caco-2 cell line is a well characterized intestinal in vitro model. The Caco-2 cell line is a continuous line of heterogeneous human epithelial colorectal adenocarcinoma cells, developed by the Sloan-Kettering Institute for Cancer Research. Morphologically and functionally, Caco-2 cells resemble the enterocytes lining the small intestine and they produce enzymes similar to those produced by the enterocytes. The use of the Caco-2 cells for permeability assays and screening of drug-discovery strategies is on the increase [12]. It is ideal for this study since intestinal epithelium has been shown to secrete pro-inflammatory molecules, including IFN-γ [13,14]. Interferons are cytokines that function in the inhibition of pathogens that invade the immune system and in the activation of macrophages. These cytokines are multifunctional and are classified into two types, type 1 and type II [15]. Interferon-α, interferon-β, IFN-ε, IFN-κ, IFN-ω, IFN-σ, and IFN-τ are classified as type I interferon while interferon-gamma (IFN-γ) is classified as type II interferon. IFN-γ is produced and secreted by natural killer cells and activated T lymphocytes. Some of its biologic effects include induction of the production of cytokines, up-regulation of the expression of class I and II MHC antigens, Fc receptor and leukocyte adhesion molecules. IFN-γ also augments Helper T cell expansion and may be required for Helper T cell differentiation [16]. Researchers suggest that the production of IFN-γ during ~~the~~ initial infection is important for immunity [17]. Gamma-interferon is said to inhibit the growth and replication of intracellular bacteria by reducing the amount of intracellular iron and tryptophan in the host cell [18].Although there is a large amount of information in literature of the role of IFN-γ during infection with *S.* Typhimurium and *C. albicans*, there is no documented study to date on the role of interferon in CaCo-2 cells during infection with multi-drug resistant (MDR) -*S.* Typhimurium DT104. Therefore, the objective of this study was to determine the level of the secretion of interferon-gamma by Caco-2 intestinal epithelial cells as well as the survival of cells following their treatment with gamma-interferon during stimulation with *Salmonella* Typhimurium 14028, *Salmonella* Typhimurium DT104, and *Candida albicans*. This study will possibly shed light on the effectiveness of INF-y as a natural antimicrobial therapeutic in place of antibiotics in the clinical setting and also in animal husbandry.

2. MATERIALS AND METHODS

2.1 Microorganisms

Salmonella enterica subsp. *enterica serovar* Typhimurium ATCC 14028, *Salmonella enterica* subsp. *enterica serovar* Typhimurium DT104 ATCC 700408 and *Candida albicans* ATCC 10231 were used for this study [19]. The two bacterial organisms will henceforth be referred to as *Salmonella* Typhimurium wild-type and *Salmonella* Typhimurium DT104. The bacterial samples were cultured according to the American Type Culture Collection (ATCC) product sheets. The *Salmonella* samples were grown individually in Brain Heart Infusion broth and incubated at 37^0C for 24 hours. Each bacterial suspension was centrifuged for 10 minutes at 600 x g and resuspended in Phosphate Buffered Saline (PBS). Each suspension was adjusted to10^5CFU/ml by a serial dilution and plating method. *Candida albicans* ATCC 10231 was grown in Sabouraud Dextrose Broth (SDB) at 37°C for 24 hours. The fungal

suspension was also centrifuged for 10 minutes at 600 x g and resuspended in PBS. The fungal solution was adjusted to 10^5 CFU/ml by a serial dilution and plating method.

2.2 Tissue Culture Cells

Colorectal Adenocarcinoma cells (Caco-2) isolated from a 72 year old Caucasian Male (American Type Culture Collection, Rockville, MD, USA) used in this investigation were maintained at 37°C, 5% CO_2 in growth medium RPMI 1640 (Gibco) supplemented with 10% Fetal Bovine Serum (FBS). Cells were passed in complete growth medium every 48 hours until infection formed ~ 70 % confluence. Prior to infection Caco-2 intestinal epithelial cells were harvested and adjusted to 1.5 x 10^6cells/ml and 100 µl of cell suspensions were then seeded into a 24 or 96-well plate as appropriate.

2.3 Infection Methods

Infection was carried out by seeding 50µl of either the bacterial or fungal suspensions on the Caco-2 cells for 24hours at 37°C, 5% CO_2. The production of IFN-γ in Caco-2 cells infected with *S.* Typhimurium wild-type, *S.* Typhimurium DT104, and *C. albicans* was then determined using the ELISA assay (R&D). To determine the effect of IFN-γ in the induced cell death of intestinal epithelial cells during stimulation with *S.* Typhimurium wild-type, *S.* Typhimurium DT104, and *C. albicans* 10231, the Caco-2 cells were initially exposed/primed to varying concentrations of IFN-γ, infected with the particular microorganism and then analyzed by ELISA. Unprimed but infected Caco-2 cells served as control. Aliquots of IFN-γ (5µl/ml, 10µl/ml or 20µl/ml) were added to each well containing 100µl of Caco-2 cell suspension for a contact time of 48hours at 37°C, 5% CO2. After the 48 hour treatment, media containing IFN-γ was removed and the monolayers were washed with 0.5 ml of PBS and challenged with *S.* Typhimuriumwild-type*, S.* TyphimuriumDT104, *and C. albicans* 10231.

2.4 Bioscreen Analysis

To observe the function of IFN-γ as an antimicrobial agent, *S.* Typhimurium wild-type, *S.* Typhimurium DT104, and *C. albicans* were incubated for 24 hours in varying amounts of IFN-γ using the Bioscreen-C Automated Growth Curve Analysis System. Aliquots of 100 µl of the bacterial or fungal cells in appropriate microbiological growth medium (10^5 CFU/ml) were added to a 96-honey comb well plate specific for the Bioscreen C Analyzer. Then 50µl of interferon solution containing 50 µg/ml gamma-interferon, 20µg/ml IFN-γ, 10 µg/ml IFN-γ, 5µg/ml IFN-γ, 2.5µg/ml IFN-γ, and 0.312µg/ml IFN-γ was added to each corresponding well plate and the plate was incubated for 24 hours at 37°C. The optical density at 600 nm (OD_{600}) was measured every 3hours. Microbiological growth curve data were collected and analyzed. All determinations were performed with five replicates, and results are presented as mean values. Each assay was performed three times.

2.4 Flow Cytometry

The modified method of Nicoletti et al. [20] was used for the flow cytometry analysis of cell death. To measure the IFN-γ induced cell death of Caco-2 cells infected with either *S.* Typhimurium wild-type, *S.*TyphimuriumDT104, or *C. albicans the* cells were stained with propidium iodine and analyzed by flow cytometry. Briefly, the Caco-2 cells ($1x10^6$) were washed in PBS and centrifuged at 200xg for 5 minutes. The cell pellet was resuspended in

100ml PBS and fixed in 900ml of 70% (vol/vol) cold ethanol and incubated for 30minutes. The cells were then centrifuged, the ethanol decanted and cells resuspended in 1.5 ml PBS at room temperature for 10minutes and centrifuged again at 200xg for 5min. The cell pellet was resuspended in 1ml of DNA staining solution [10 ml of PBS + 200 mg Propidium Iodide (Sigma Aldrich) + 2 mg RNase] and incubated for 30minutes at room temperature in the dark. The samples were then analyzed by flow cytometry (BD Biosciences).

2.5 Statistical Analysis

The results were analyzed using the two-way analysis of variance (ANOVA). The Bonferroni test was used as Post Hoc analysis. P=0.05 was considered to be significant. Results are reported as mean ± SEM for triplicate values at each time point and IFN-γ dose for three independent experiments.

3. RESULTS AND DISCUSSION

It was observed in this study that *S.* Typhimurium wild-type, *S.* Typhimurium DT104, and *C. albicans* all induced interferon production by Caco-2 intestinal epithelial cells. This shows that IFN-γ may be important in immune activities against intracellular bacterial infections. Similar reports have shown that non-immune cell types such as the epithelial cells may secrete interferon-gamma [21]. This is in agreement with observations of Schroder et al 2004 [16]. The amount of IFN-γ released in the supernatant of Caco-2 cells infected with wild-type *S.* Typhimurium (Fig. 1) was significantly higher (P=0.05) than the amount observed in cells infected with multidrug-resistant *S.* Typhimurium DT104 and *C. albicans*.S. Typhimurium wild-type although a less invasive form of *Salmonella* [22]appears to stimulate Caco-2 cells to produce higher levels of interferon-gamma than the DT104 *and C. albicans* both of which are resistant to several antibiotics and antifungal drugs respectively. This suggests a possible relationship between microbial drug resistance and IFN-y production by infected cells.

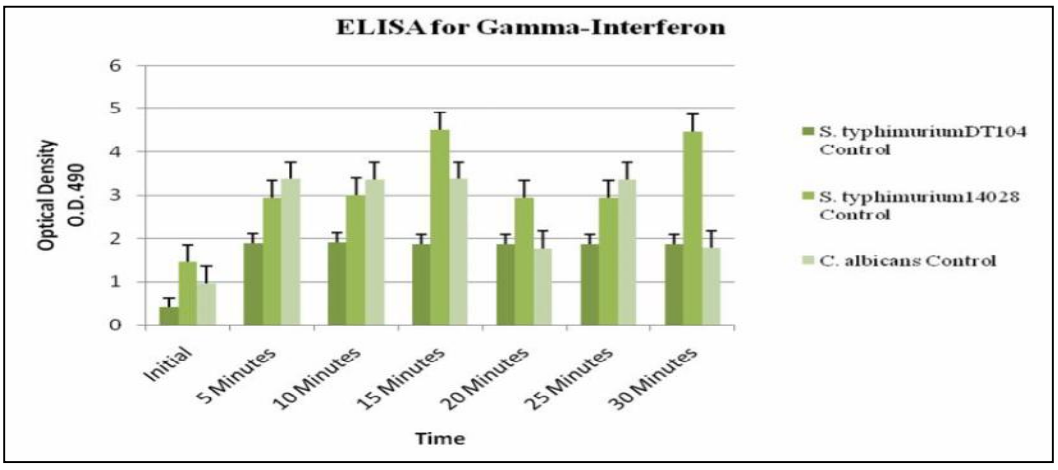


Fig. 1. Production of IFN-γ in Caco-2 cells during infection with *S.* Typhimurium14028, *S.* Typhimurium DT104, and *C. albicans*. IFN-γ release in cell culture supernatants during infection was quantified using the Quantikine Colorimetric Sandwich ELISA assay (R&D Systems). Data represents the mean of triplicate experiments

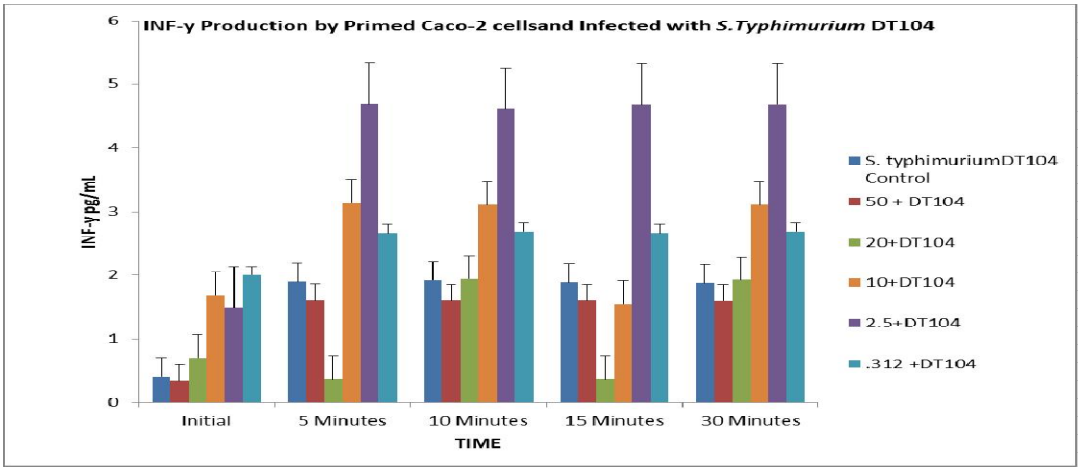


Fig. 2. Production of IFN-γ from Caco-2 intestinal cells during exposure to multidrug resistant *S.* Typhimurium DT104. Caco-2 cells were treated with 50 µg/ml (50+), 20 µg/ml (20+), 10 µg/ml (10+), 2.5 µg/ml (2.5+), and .3125 µg/ml (0.312+) of IFN-γ for 48 hours and stimulated with *S.* Typhimurium DT104. Data represents the mean of triplicate experiments. Untreated Caco-2 cells +*S.* Typhimurium DT104 served as control.

Pretreatment of Caco-2 cells with high levels of IFN-y (10, 20, 50ug/ml) did not result in significant higher levels of interferon production following infection with DT104. However, pretreatment with 2.5 and 5ug/ml of INF-y resulted in high levels of post-infection INF-y production (~4.8ug/ml) after 5minutes of exposure. Detection level remained constant for the 10 and 15 minute time points. By the 30minute time point the level of post infection INF production had become quite low (Fig. 2). This indicates that 2.5ug/mL pretreatment was enough to stimulate the intestinal epithelial cells to produce high levels of INF-y and higher levels might actually reduce the host cells ability to produce interferon over time.

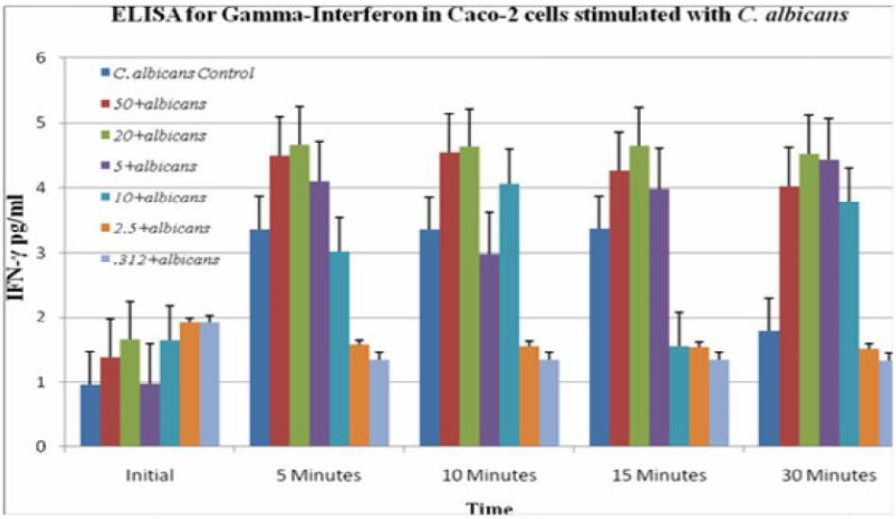


Fig. 3. Interferon gamma Production by Caco-2 cells previously primed with varying levels of IFN-y and Infected with *C. albicans*.

The graph (Fig. 3) shows the levels of IFN-γ released from cells primed/pretreated with varying amounts of IFN-y (0.312 to 50ug/mL).Cells that were not primed with IFN-y prior to infection with *C. albicans* served as control. Data represents the mean of triplicate values.

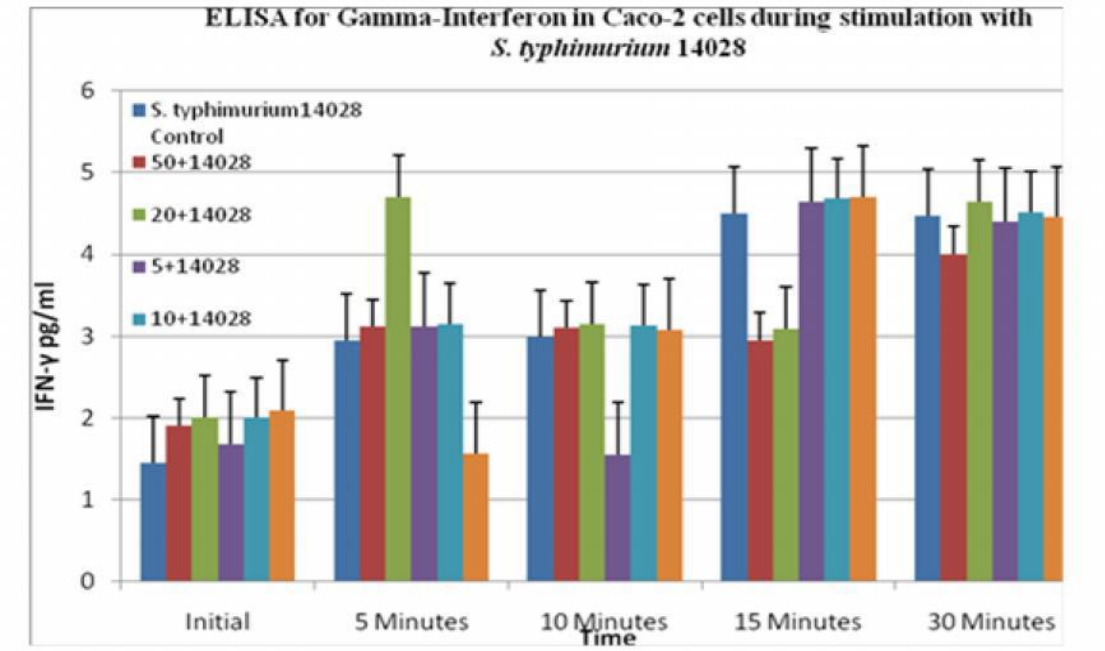


Fig. 4. Interferon Production by Caco-2 cell primed with IFN-y prior to infection with *S.* Typhimurium wild-type (ATCC 14028).

Cells that were not pretreated with IFN-y prior to infection with S. Typhimurium wild-type served as control. Values are mean of triplicate experiments.

Increase in IFN-γ production during challenge with wild-type S. Typhimurium14028 *was observed.* The production of interferon-gamma appeared to be dose dependent and increased after 5 minutes of exposure, but leveled off over time indicating that pretreatment was unnecessary for infections with *S.* Typhimurium wild-type, since the control cells that were not pretreated also showed high levels of IFN-y (Fig. 4).Secretion of IFN-γ from Caco-2 cells that were previously treated with 50µg/ml, 20µg/ml, 10µg/ml, 5µg/ml, and 2.5 µg/ml of IFN-γ were not concentration dependent. However, the amount of IFN-γ released from Caco-2 cells was dependent on microbial stimulus type. Cells that were pretreated with 5µg/ml and 2.5 µg/ml of IFN-γ and then infected with *Salmonella* Typhimurium DT104 and *C. albicans* showed an increase in the amount of IFN-γ in the culture medium after 5 minutes, leveled off and the amount of IFN-y became depleted after 30minutes of exposure. On the other hand, *Salmonella* Typhimurium wild-type apparently stimulated the Caco-2 cells into production of interferon-gamma regardless of absence or presence of prior pretreatment with the cytokine. *S.* Typhimurium as described by Godinez et al. (2008)[23] stimulated the up-regulation in mRNA transcription of T-cell derived cytokines and chemo-attractants in infected host cells. These cytokines include type I interferon (IFN –α and IFN-β) and type II interferon (IFN-γ).

IFN-γ role as Antimicrobial Agent: microbial growth in the presence of varying amounts of IFN-y was analyzed using the Bioscreen C microbial growth analyzer as well as flow cytometry. This was to determine if IFN-y had antimicrobial effects on extracellular test

microorganisms. Based on readings from the Bioscreen C microbial growth analyzer, there was no significant difference in the absorbance readings measured for wild-type *S.* Typhimurium, *S.* TyphimuriumDT104 and *C. albicans* incubated with varying concentrations of IFN-γduring the first 6 hours of incubation (Figs. 5-7). However there was an increase in the absorbance (growth) measured after 6 hours in both the wild-type and *S.* Typhimurium in all concentrations of IFN-γ.

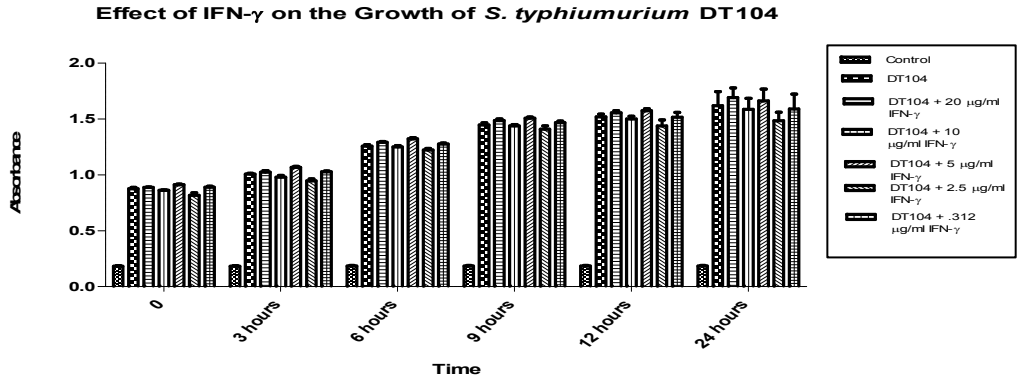


Fig. 5. Growth of *S.* TyphimuriumDT104 exposed to IFN-γ.

Values represent the mean and SEM of triplicate experiments. Absorbance was at OD_{600}

The growth of the bacterial cells appeared to be inhibited in the first six hours of exposure to IFN-γ. After six hours of incubation slight growth was observed. The same was observed for *C. albicans* and *S.* Typhimurium wild-type (Figs. 6 &7).This shows that IFN-y slightly inhibited the growth of the test microorganisms extracellularly. It has been reported that IFN-γ may have a microbicidal effect on many microorganisms [24], including species of *Salmonella* [25]. Based on the BioScreen readings, IFN-y in this study inhibited the growth of the test microorganisms. It has also been reported that IFN-y inhibited bacterial growth when added after infection of macrophages, although the magnitude of the anti-brucellae effects was less than that when it was added before infection [26].

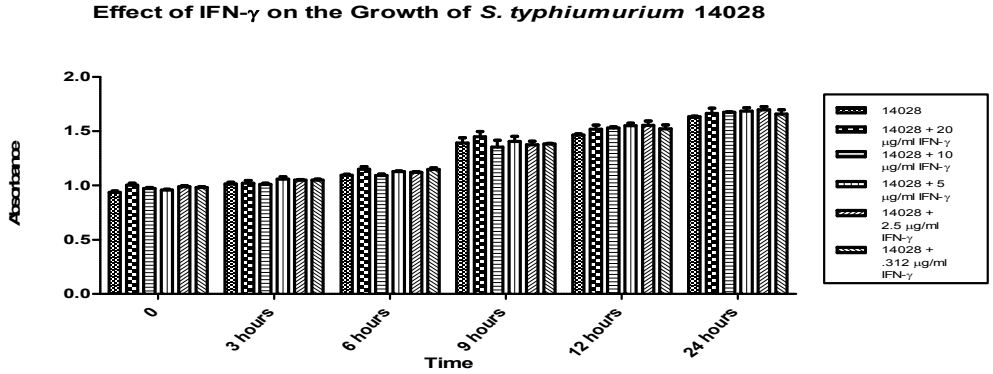


Fig. 6. Growth of *S.* Typhimurium14028 exposed to varying concentrations of IFN-γ.

There was no significant increase in the microbial growth of the bacteria in the first six hours of exposure to IFN-γ. However after six hours there was an increase in the absorbance measured during exposure. Values represent the mean and SEM of triplicate experiments

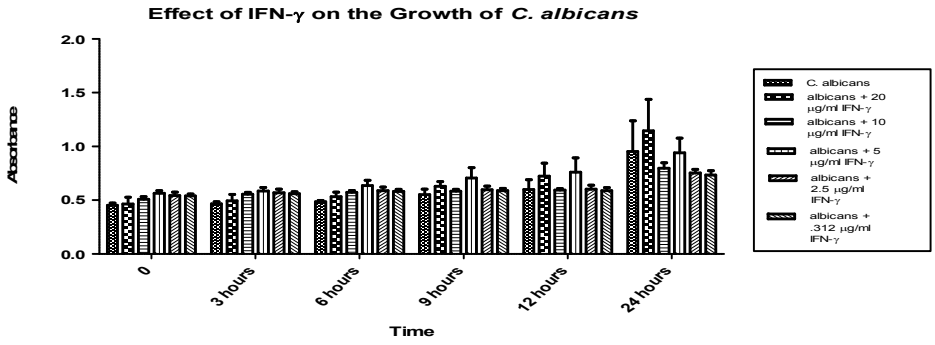
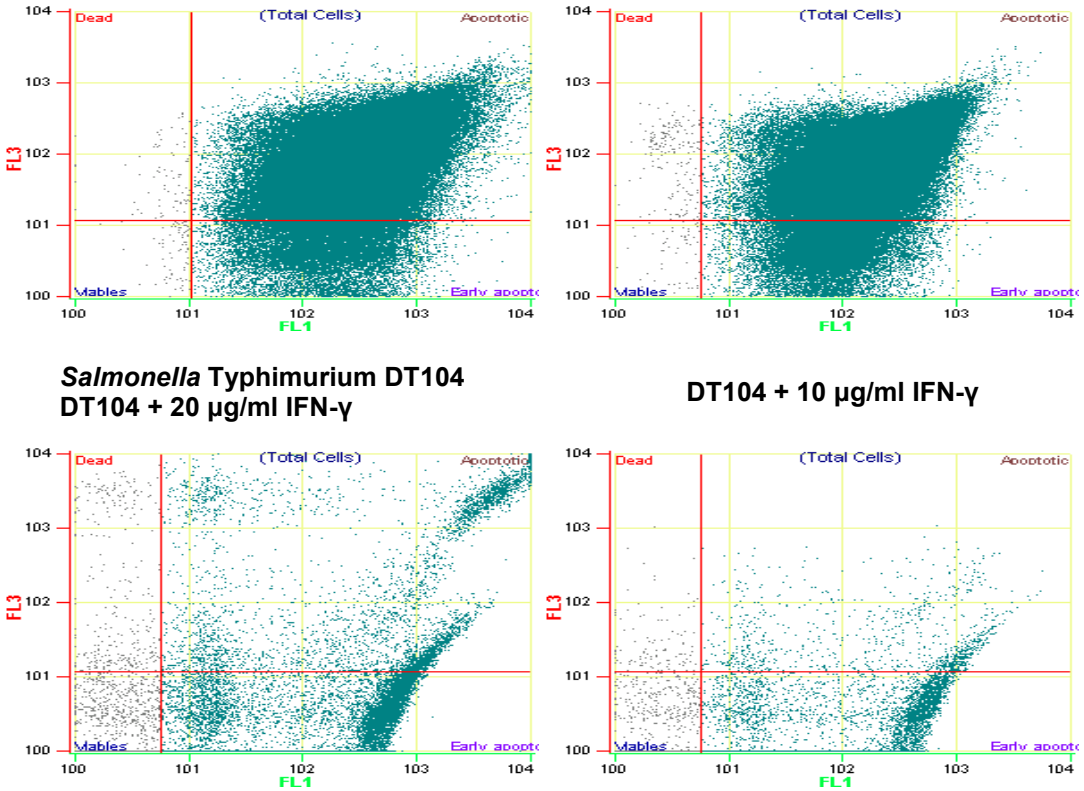


Fig. 7. Inhibition in the microbial growth of *C. albicans* by IFN-γ. Absorbance was OD_{600}. Values represent the mean and SEM of triplicate experiments

The various levels of IFN-y appeared to suppress the growth of *C. albicans* in the first 12 hours of exposure to IFN-γ**.** This indicates that *C. albicans* growth was temporarily inhibited for 12hours.Since the antimicrobial effects of IFN-y could not be determined directly by absorbance measurements; the microbial cells were incubated with varying concentrations of IFN-y and stained with propidium iodide (PI) and analyzed by Flow Cytometry (Beckman,).



***Salmonella* Typhimurium DT104**
DT104 + 20 µg/ml IFN-γ

DT104 + 10 µg/ml IFN-γ

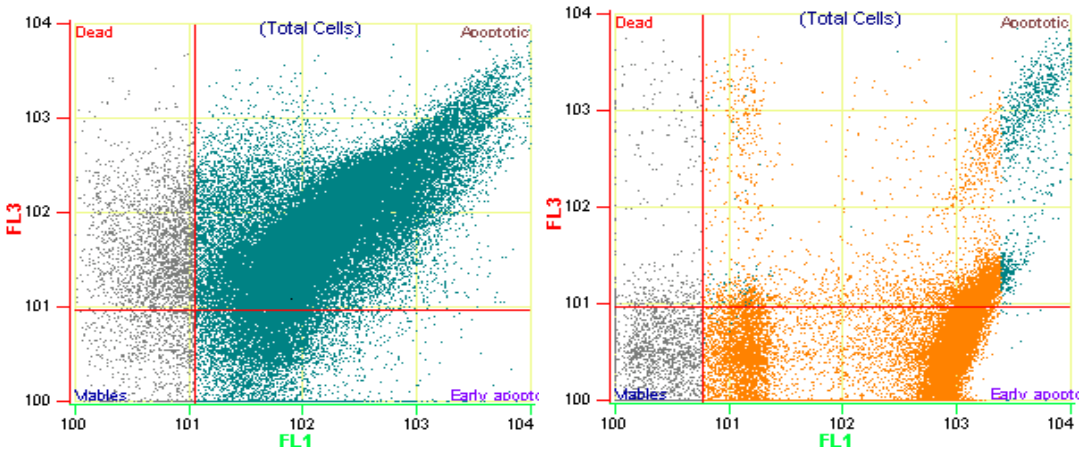
DT104 + 2.5 µg/ml IFN-γ

DT104 + .312 µg/ml IFN-γ

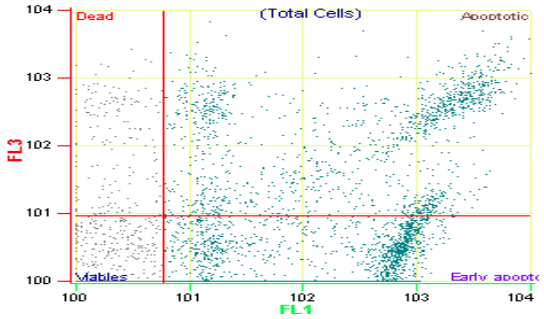
Fig. 8. Antimicrobial effects of IFN-γ on *S.* Typhimurium DT104

S. Typhimurium DT104 cells were exposed to 20 µg/ml IFN-γ, 10 µg/ml IFN-γ, 2.5 µg/ml IFN-γ, .312 µg/ml IFN-γovernight. The cells were then analyzed with flow cytometry. There appears to be an increase in the amount of cells undergoing cell death or damage following exposure to higher concentrations (20 µg/ml IFN-γ)

In the Flow cytometry analysis it was observed that the induction of cell death in *S.* Typhimurium14028, *S.* TyphimuriumDT104, and *C. albicans* was concentration dependent. For the DT104 bacterial cells (Fig. 8.), the amount of cells that were undergoing cell damage decreased from 469,796 damaged cells/ 50 µg/ml IFN-γ to 937 damaged cells/.312 µg/ml IFN-γ. This correlation was also observed in *C. albicans* (Fig. 9*)*. Thus more of the DT104 cells were damaged when exposed to high concentrations of IFN-γ, so the antimicrobial effects of IFN-γ was dose dependent. Jiang & Baldwin 1992 [24] found similar antimicrobial effects for IFN-γ working with *Brucella abortus* and macrophages



***C. albicans* + 20 µg/ml IFN-γ** **C. albicans + 10 µg/ml IFN-γ**



***C. albicans*+ .312 µg/ml IFN-γ**

Fig. 9. Interferon-gamma induced cell damage/death observed in *C. albicans*

C. albicans was exposed to 20 µg/ml IFN-γ, 10 µg/ml IFN-γ, and .312 µg/ml IFN-γ and incubated overnight. The culture was then analyzed with Flow Cytometry. Cell death or damage was concentration dependent.

4. CONCLUSION

Caco-2 cells infected with *S.* Typhimurium14028, *S.* Typhimurium DT104, and *C. albicans* released IFN-γ; however the amount of IFN-γ released was higher with *S.* Typhimurium wild-type infection than infections with multidrug-resistant *S.* Typhimurium DT104 and for *C. albicans*. *S.* Typhimurium14028 induced more IFN-γ production by Caco-2 cells than either *S.* Typhimurium DT104 and for *C. albicans*. Induction of interferon-gamma production may be related to microbial virulence/pathogenicity. Interferon-gamma had antimicrobial effects on the test microorganisms.

5. ACKNOWLEDGEMENTS

A part of this project served as the master's degree thesis of Ebony Weems. The project was part supported by funds from the former Dean, School of Arts & Science Alabama A&M University. The authors would like to thank ~~and~~ Dr. JU Johnson' who supplied most of the cell culture media. They also wish to thank Drs. Jeanette Jones, Jacob Savage and Conwin Vanterpool for their very valuable suggestions towards the completion of this project.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lawley TD, Bouley DM, Hog YE, Gerke C, Relman DA, Monack DM. Host transmission of Salmonella *enterica Serovar* typhimurium is controlled by virulence factors and indigenous intestinal microbiota. Infection and Immunity. 2008;76(1):403-416.
2. Boyle EC, Bishon JL, Grassl GA, Finlay BB. Salmonella: from pathogenesis to therapeutics. Journal of Bacteriology. 2007;189(5):1489-1495
3. Threlfall EJ. Epidemic Salmonella typhimurium DT 104-a truly international multiresistant clone. J Antimicrob Chemother. 2000;46:7–10. Available: http://dx.doi.org/10.1093/jac/46.1.7
4. Izumiya H. Terajima J. Yamamoto S. Ohnishi M. Watanabe H. Kai A, etal.Genomic analysis of Salmonella *enterica serovar* Typhimurium definitive phage type104 [letter]. Emerg Infect Dis [Internet]. 2013. Available: http://dx.doi.org/10.3201/eid1905.121395
5. Glynn MK, Bopp C, Dewitt W, Dabney P, Mokhtar M, Angulo FJ. Emergence of multidrug- resistant Salmonella Enterica serotype typhimurium DT104 infections in the United States. The New England Journal of Medicine. 1998;338(19):312 pgs.1333-1338.
6. WHO Global Salm-Surv Progress Report; 2000-2005.
7. Gordon MA, Jack DL, Dockrell DH, Lee ME, Read RC. Gamma interferon enhances internalization and early nonoxidative killing of Salmonella *enterica serovar* typhimurium by human macrophages and modifies cytokine responses. Infection and Immunity. 2005;73(6):3445-3452.

8. Bodey GP, Fainstein V. Infections of the gastrointestinal tract in the immunocompromised patient. Annual Review of Medicine. 1986;37:271-281.
9. Jarvik T, Smillie C, Groisman EA, Ochman H. Short-term signatures of evolutionary change in the Salmonella *enterica serovar* typhimurium 14028 genome. J Bacteriol. 2010;192:560–567. [PMC free article] [PubMed]
10. Ruhnke, Markus. Epidemiology of Candida albicans infections and role of non-Candida albicans yeasts. Current Drug Targets. 2006;7(4):495-504.
11. Gozalbo D, Gil ML. IFN-gamma in *Candida albicans* infections. Front Biosci. 2009;14:1970–1978
12. Angelis ID, Turco L. Caco-2 cells as a model for intestinal absorption. Curr Protoc Toxicol. 2011;20:6. doi: 10.1002/0471140856.tx2006s47
13. Rouabhia M, Ross G, Page N, Chakir J. Interleukin-18 and gamma interferon production to *Candida albicans* or lipopolysaccharide stimulation. Infection and Immunity. 2002;70:7073-7080
14. Saarinen M, Ekman P, Ikeda M, Virtala M, Gronberg A, Yu DTY, Arvilommi H, Granfors K. Invasion of Salmonella into human intestinal epithelial cells is modulated by HLA-B27. Rheumatology. 2002;41:651-657.
15. Ganes CS, Lengyel P. The interferon system. The Journal of Biological Chemistry. 1992;267:5017-5020
16. Schroder K, Hertzog PJ, Ravasi T, Hume DA. Interferon-gamma: an overview of signals, mechanisms and functions. J. Leukoc. Biol. 2004;75(2):163–89. doi:10.1189/jlb.0603252. PMID 14525967]
17. Arase, H, N Arase, T Saito. Interferon-γ production by natural killer cells and NK1.1+T cells upon NKR-P1 cross-linking. J Exp Med. 1996;183:2391–2396, pmid:8642351
18. Fratti RA, Ghannoum MA, Edwards JE, Filler SG. Gamma interferon protects endothelial cells from damage by Candida albicans by inhibiting endothelial cell phagocytosis. Infection and Immunity. 1996;64(11):4714-4718
19. Clark L, Perrett CA, Malt L, Harward C, Humphrey S, Jepson KA, Jepson MA. Differences in Salmonella *enterica serovar* Typhimurium strain invasiveness are associated with heterogeneity in SPI-1 gene expression. Microbiology. 2011;157(7):2072-2083.
20. Nicoletti I, Migliorati2 G, Pagliacci1 MC, Grignani1 F, Riccardi2 C. A rapid and simple method for measuring thymocyte apoptosis by propidium iodide staining and flow cytometry. Journal of Immunological Methods. 1991;139,Issue 2(3):271–279
21. Gordon MA, Jack DL, Dockrell DH, Lee ME, Read RC. Interferon-y enhances phagocytosis and early non-oxidative killing of *Salmonella enterica serovar* Typhimurium by human macrophages and modifies cytokine responses. Infection and Immunity. 2005;73:445-52.
22. Clark L, Perrett CA, Malt L, Harward C, Humphrey S, et al. Differences in *Salmonella enterica serovar* Typhimurium strain invasiveness are associated with heterogeneity in SPI-1 gene expression. Microbiology. 2011;157:2072–2083.
23. Godinez I, Haneda T, Raffatellu M, George MD, Paixão TA, Rolán HG, et al. T cells help to amplify inflammatory responses induced by *Salmonella enterica serotype* Typhimurium in the intestinal mucosa. Infect. Immun. 2008;76:2008–2017.

24. Yoshihara R, Shiozawa S, Fujita T, Chihara K. Gamma interferon is produced by human natural killer cells but not T cells during Staphylococcus aureus stimulation. Infect Immun. 1993;61(8):3117-22.
25. Lalmanach AC, Lantier F. Host cytokine response and resistance to Salmonella infection. Microbes and Infection. 1999;9(1):719–726
26. Jiang X, Baldwin CL. Effects of cytokines on intracellular growth of *Brucella abortus.* Infect. Immun. 1993;61(1):124-134.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=275&id=8&aid=2183