Chapter

Salmonella and the Immune System

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Abstract

The human body has many mechanisms to resist invaders like pathogenic bacteria to avoid harm according to the living creature's law "survival for the best". On the opposite; *Salmonella* as pathogenic bacteria have many weapons that they utilize to invade the human body. The resistance mechanisms expressed by the human body are called immunity which represented by the immune system that has many different types of resistance processes, either specific (adaptive immune response) or non-specific (Innate Immune Response) against certain pathogenic invaders. As far as these processes are strong they will be enough to avoid infections occurrence, otherwise, the human body will get infected with *Salmonella*, be ill, show the disease symptoms, transmit the disease to others, and may become a carrier for the pathogen according to many circumstances. Prevention is still stood the most effective way to avoid getting infected with *Salmonella* by personal hygiene or suitable vaccination if available.

Keywords: immune system, *Salmonella* virulence factors, *Salmonella* infection, *Salmonella* resistance and *Salmonella* vaccination

1. Introduction and overview

1.1 Introduction to immunology and immune system

Immunology is the study of our protection against foreign macromolecules or invading organisms and our responses to them. These invaders include viruses, bacteria, protozoa or even larger parasites. Any Human body is continuously exposed to pathogenic microorganisms. The immune system is composed of two major subdivisions of immune system, the innate or nonspecific immune system and the adaptive or specific immune system [1].

The innate immune system is our first line of defense against invading organisms while the adaptive immune system acts as a second line of defense and gives protection against re-exposure to the same pathogen. Each of the major subdivisions of the immune system has both cellular and humoral components by which they carry out their protective function and help each other to do these functions. Since pathogens may replicate intracellularly (viruses and some bacteria and parasites) or extracellularly (most bacteria, fungi and parasites), different components of the immune system have evolved to protect against these different types of pathogens [2].

1.2 Innate or non-specific defenses

Include first line of defense which acts before invasion of pathogenic microbes. And the second line of defense which acts after invasion. The anatomical barriers that works mainly against infections with microbial invaders. This first line of defense represented by the epithelial surfaces and skin form the physical barriers that are very impermeable to most infectious agents [1].

The shedding of skin epithelium also helps remove bacteria and other infectious agents that have adhered to the epithelial surfaces. Movement due to cilia or peristalsis helps to keep air passages and the gastrointestinal tract free from microorganisms. The trapping effect of mucus that lines the respiratory and gastrointestinal tract helps protect the lungs and digestive systems from infection [2].

Chemical barriers like Lysozyme and phospholipase found in saliva and other secretions can breakdown the cell wall of bacteria and destabilize bacterial membranes. The low pH of gastric secretions prevents the growth of bacteria [3].

The microbiota of the skin and in the gastrointestinal tract can prevent the colonization of pathogenic bacteria by secreting toxic substances or by competing with pathogenic bacteria for nutrients or attachment to cell surfaces. They represent the biological barriers of the innate immunity [2].

The anatomical barriers are very effective in preventing colonization of tissues by microorganisms. However, when there is damage to tissues the anatomical barriers are breeched and infection happens. Once infectious agents have penetrated tissues, another innate defense mechanisms comes into play, namely acute inflammation as the second line of innate immune defense. Many Humoral and cellular factors play an important role in inflammation against microbial invasion, which is characterized by edema and the activation of phagocytic cells [4].

These humoral factors are found in serum or they are formed at the site of infection. They contain Complement system, Interferons and Lysozymes. The most important humoral barrier is the Complement system, since it acts as with the phagocytic cells as a bridge between specific and non-specific immune response. Complement system represents a set of glycoproteins in blood. Once they are activated after rapid cascade events that can lead to increase vascular permeability, activation of phagocytic cells, opsonization of bacteria and lysis [5].

Complement glycoproteins are synthesized by liver cells (hepatocytes) and macrophages and many other cell (e.g. gut epithelial cells). All normal individuals have complement components in their blood. This system can be activated by [1, 2]:

a. Antigen-antibody complexes containing IgG or IgM activate complement by the classical pathway that starts with C1 (complement 1).

b. Membranes and cell walls of microbial organisms (e.g. Lipopolyccharides layer [LPS] of gram –ve bacteria) and many other substances can activate complement by the alternative pathway.

c. Proteolytic enzymes released either from microbes or from host cells during immune defence mechanisims, can also activate the complement system by breaking down critical components.

The complement system takes part in both specific and non-specific resistance and generates a number of products of biological and immunological importance. The functions of the complement system are summarized in **Table 1**, [3, 5]**:**

On the other side; the cellular factors are the main line of defense in the nonspecific immune system, they are listed in the **Table 2** [2, 5].

No.	Function
1.	Binding and neutralizing foreign substances that activate it.
2.	Induce the ingestion of complement-coated substances by phagocytic cells (help fn the opsonization process when C3b and C4b linked with the surface of microorganisms and attach to Complement receptor on phagocytic cells then induce phagocytosis).
3.	Activation of many cells including polymorphonuclear cells (PMNs) and macrophages.
4.	Have roles in regulation of antibody responses.
5.	Clearance of immune complexes and apoptotic cells.
6.	Have roles in inflammation and tissue damage.
7.	Some components (C3a, C4a and C5a), have role in Anaphylaxis (a dangerous case of type 1 hypersensitivity), hence they are called anaphylotoxins.
8.	Some complement components acts as chemotactic facters e.g. C5a and MAC.

Table 1.
The functions of the complement system.

No.	Cell	Function
l.	Neutrophils	Polymorphonuclear cells (PMNs) migrate to the site of infection where they phagocytose invading organisms and kill them intracellularly. In addition, PMNs contribute to tissue damage that occurs during inflammation.
2.	Macrophages	Tissue macrophages and activated monocytes, which differentiate into macrophages, also function in phagocytosis and intracellular killing of microorganisms. In addition, macrophages are capable of extracellular killing of infected or transformed cells (self-target). Furthermore, macrophages have role in tissue repair and act as antigen presenting cells APC, which are required for the induction of specific immune responses.
3.	Natural killer	NK cells can nonspecifically kill virus infected and tumor cells. These cells are not part of the inflammatory response but they are important in nonspecific immunity to viral infections and tumor surveillance.
4.	Eosinophils	Eosinophils have proteins in granules that are effective in killing certain parasites.

Table 2.
Cellular factors of the nonspecific immune system and their function.

1.2.1 Phagocytosis and intracellular killing

Phagocytosis is a very important process during non-specific immune response when specialized cells engulf foreign body like bacteria or molecule like toxin or virus. The phagocytosis has four steps, **Figure 1** [2]:

1. Chemotaxis. Phagocytic cells response and migrate to the site of infection or injury by the effect of complement products and cytokines released from tissue macrophages that have encountered bacteria or any foreign body in tissue.

2. Endocytosis. Starts with pseudopodia formation then phagocytic cells bind to the foreign body by: Fc receptors–Bacteria with IgG antibody on their surface have the Fc region exposed and this part of the Ig molecule can bind to the receptor on phagocytes. Complement receptors–Phagocytic cells have a receptor for the complement C3b. Scavenger receptors mainly for invading bacteria.

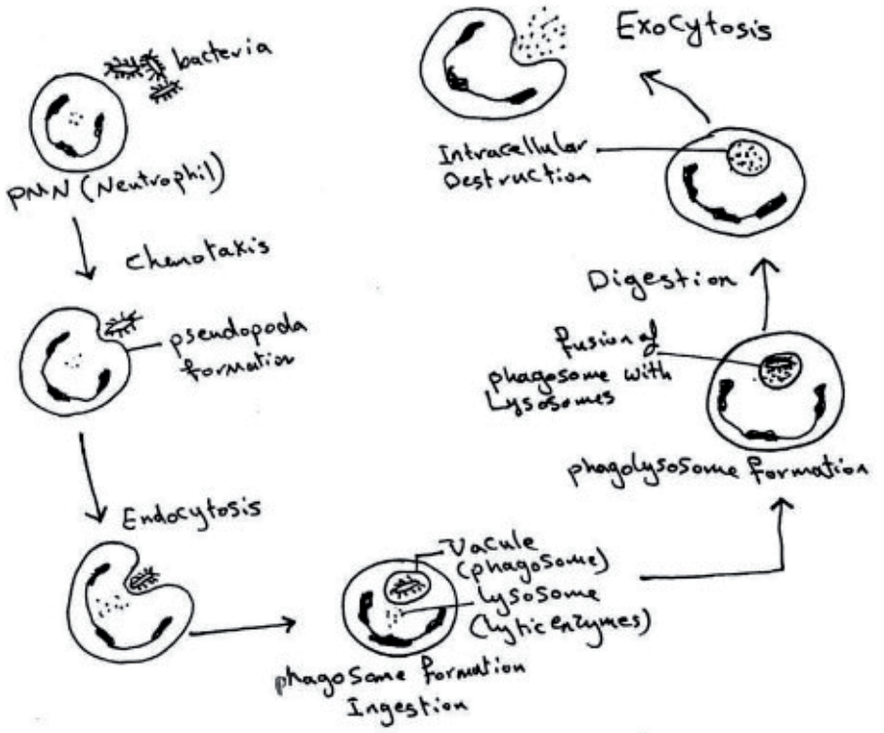

Figure 1.
Phagocytosis process steps.

3. Phagolysosome formation and degradation of foreign substances. After attachment of the bacteria the phagocyte begins to extend pseudopods around the bacteria and surround and engulf them forming the phagosome. During phagocytosis the granules or lysosomes of the phagocytes bind or fuse with the phagosome and empty their contents. The result is the foreign bodies or bacteria engulfed in the phagolysosome which have the contents of the granules or lysosomes.

Intracellular killing and Digestion (Lysis and excretion): There are three means of killing the microorganisms inside phagocytic cells; either Oxygen dependent killing by formation of NADPH using Oxygen, then production of the toxic oxygen compounds like H_2O_2 and hydroxyl radical (OH•). These compounds are toxic to microbes and kill them, Oxygen independent killing by production of toxic hypochlorite (OCl-) and singlet oxygen (1O2) from H_2O_2 using the enzyme Myeloperoxidase that released into the phagolysosome or Nitric oxide dependent killing by Toxic nitric oxide synthesis and production (NO) when microorganism binds to the macrophage because of cytokines release (TNF-α and IFN-γ) [3].

Oxygen -dependent killing and Oxygen -independent killing both are called the Respiratory burst. After killing, the enzymatic system of the cell will digest all the phagosome components then absorb the useful materials and excrete the residues to the environment (blood) by fusing the phagolysosome with the cell membrane.

The cells that able to do phagocytosis are (monocytes, macrophage, PMNs and dendric cells). The results of phagocytosis are either a complete destruction of foreign body and excretion (PMNs). Or a complete destruction of foreign body and some parts (polypeptides) of it will be processed and presented on the surface of the phagocytic cells (monocytes, macrophage and dendric cells) then the phagocytic cell will be antigen presenting cell (APC) [2, 3].

1.3 Cells involved in specific immune responses

1.3.1 Antigen presenting cell (APC)

These cells are the messengers between innate (non-specific) immunity and the adaptive (specific). Specialized APC are macrophage (MØ), B-cells and Dendric cells (DC) [3].

Roles of Antigen Presenting Cell (APC) can be summarized as [5]:

1. Engulfment of foreign Ag, processing it and presenting it (or a olypeptide from it) on the surface near the Major Histocompatibility Complex MHC class I or II.

2. Communication during the immune response between immune cells especially T- cells to induce the proper immune response cellular or humoral.

3. Secretion of cytokines which are substances (glycoproteins) that regulate the immune response.

1.3.2 Lymphocytes

B-cells or B-lymphocytes, T-cells or T-lymphocytes (T-helper cells including Th1 and Th2, T-Cytotoxic Tc and T-suppressor Ts) [3].

1.3.3 Natural killer cells (NK)

Natural killer cells (NK) have no CD markers on the surface so they are usually called null cells [3].

It is important to know that B-cells are able to be APC by internalization of Ag inside the cell and do the processing and presenting, which will be discussed later. Also Dendric cells (DC) are cells found only in the mammalian immune system; their function is to engulf and process Ag then present it on the surface to other immune cells. Found in tissues that in contact with external environment such as skin, lung, stomach and intestine [2].

1.4 Mucosal immune response

In the mucosal surfaces and sites, the mucosal immune response come to play role in resistance against infection establishment. Many lymphoid tissues are associated with mucosa which are usually called mucosa-associated tissues play major role in protection since they are rich with both T-cells and B-cells, produce many types of Lymphokines that acts as signals of the immune system actions, produce IgA (sIgA Secretory IgA); the main effective immunoglobulin type in the surfaces of the body and the most important part is that mucosal surfaces have the receptors of microbiota that play as a biological barrier and support innate immunity. Many secretions are also produced by the mucosa to protect surfaces like gastric acid and continuous mucous secretion and shedding helps in renewing normal flora population and shed colonized pathogens.

Mucosa-Associated Lymphoid Tissues (MALT) Include the lymphoid tissues of the intestinal tract, genitourinary tract, tracheobronchial tree, and mammary glands. All of the mucosa-associated lymphoid tissues are unencapsulated and contain both T and B lymphocytes [2, 5].

1.5 Gut-associated lymphoid tissue (GALT)

It is found along the digestive tract. Three major areas of GALT that can be identified are the tonsils, the Peyer's patches, located on the submucosa of the small intestine, and the appendix. In addition, scanty lymphoid tissue is present in the lamina propria of the gastrointestinal tract [3, 5].

1. Tonsils, located in the oropharynx, are predominantly populated by B-lymphocytes and are the site of antigenic stimulation.

2. Peyer's patches (PPs), they are lymphoid structures disseminated through the submucosal space of the small intestine

Physiological roles of secondary lymphoid organs:

a. The follicles of the intestinal Peyer's patches are extremely rich in B-cells, which differentiate into IgA-producing plasma cells.

b. T-lymphocytes are also present in the intestinal mucosa, the most abundant of them expressing membrane markers that are considered typical of memory helper T-cells. This population is involved in the induction of humoral immune responses (HMI) [1, 2].

1.6 Antibody mediated immune response (humeral mediated immunity, HMI)

B-cells have normal Ag receptors on the surface they are natural Igs, these Igs are able to form Ag-Ab complex on the surface of B-cell. This complex will be internalized inside B-cell, then the foreign Ag will be processed within B-cell and presented (or polypeptides from it) on the surface of B-cell near MHC class II and now B-cell is APC.

T-helper (Th) cells come near the APC B-cell and by the help of TCR and CD4; Th will interact and communicate with APC B-cell and Th cell will be activated and release cytokines or lymphokines (IL-2, IL-4, IL-5 and IFN-γ), these products will induce other B-cells for dividing, proliferation and differentiation. IgM will be the first Ig produced then B- cell will switch to make IgG. This response is called T-dependent Ag immune response. The other type of response is T-independent Ag immune response, this type of Ag stimulates B-cells without need for T-helper lymphocytes interfere [1, 2].

After B-cells activation, series of events happen (proliferation, clonal expansion, division and maturation), ending with Ab and memory B-cells production. These series of events called B-cell Maturation. During the second exposure to the same Ag that started the first immune response (perhaps after year from first exposure), the B-memory cells will remember the Ag and will be activated and divide into a clone of plasma cells to start the Secondary immune response (Memory response) [3].

Antibodies or Immunoglobulins (Ig) that are produced after specific humoral response are in five types; IgG; IgM, IgA, IgD and IgE based on differences in the amino acid sequences in the constant region of the heavy chains. In addition, the classes of immunoglobulins can be divided into subclasses based on small differences in the amino acid sequences in the constant region of the heavy chains [1].

IgG immunoglobulin: is a Monomer, have4 subclasses (IgG1, IgG2, IgG3 and IgG4), the subclasses differ in the number of disulfide bonds and length of the hinge region. IgG is the major Ig in serum and extra vascular spaces of total serum Igs. Able

to cross placenta transfer is mediated by receptor on placental cells for the Fc region of IgG, able to fix complement an binding to cells - Macrophages, monocytes, PMN's and some lymphocytes have Fc receptors for the Fc region of IgG. The term opsonin is used to describe substances that enhance phagocytosis. IgG is a good opsonin [2].

IgM immunoglobulin: is a pentamer, but it can also exist as a monomer. It is the third most common serum Ig and it is the first Ig to be made by the fetus and the first Ig to be made by B cells when it is stimulated by antigen. This type of Ig is a good complement fixing Ig. Thus, IgM antibodies are very efficient in leading to the lysis of microorganisms. Also a good agglutinating Ig. Thus, IgM antibodies are very good in clumping microorganisms for elimination from the body [5].

IgA immunoglobulin: is a monomer but IgA found in secretions is a dimer. When IgA is found in secretions is also has another protein associated with it called the secretory piece or T piece (sIgA), this secretory piece is made in epithelial cells and is added to the IgA as it passes into the secretions. The secretory piece helps IgA to be transported across mucosa and also protects it from degradation in the secretions. It is the second most common serum Ig and the major class of Ig in secretions - tears, saliva, colostrum, and mucus. Since it is found in secretions secretory IgA is important in local (mucosal) immunity but does not fix complement [5].

IgD immunoglobulin: is a monomer **and** found in low levels in serum; found on B cell surfaces where it functions as a receptor for antigen also does not bind complement [5].

IgE immunoglobulin: **is** a monomer and rare on serum. It has role in allergic reactions and does not fix complement [5].

1.7 Cell mediated immune response (cellular mediated immunity, CMI)

This response occurs against cells, which are called Target cells. During both HMI and CMI, T-helper cells recognize foreign Ag processed on the surface of APC. If this Ag was processed and presented near MHC class II, then Th cells will activate HMI by B- cells activation, but if the presented Ag on APC was near MHC class I, then Th cells will activate CMI by activation of Tc, NK and MØ. Th cells able to activate and regulate CMI and HMI by many cytokines production.

In addition, in both CMI and HMI, when Th cells recognize the foreign Ag, Th cells will start T-cells activation by series of events (expanding, clonal proliferation and differentiation), then become mature to give specific activated T-helper cells in HMI and give specific activated T-helper cells and memory T-cells in CMI [2, 5].

Role of CMI response: is the defense against Tumor cells or cancer cells, Grafts Rejection, against Intracellular parasite infected cells with foreign Ag presented near MHC class I. Target cell is the infected cell with parasite and Types 4 hypersensitivity (Delayed type of hypersensitivity) [5].

1.7.1 Activation of CMI cells

When T-helper cells recognize foreign Ag on the surface of target cell in association with (or near) MHC class I. The TCR and CD4+ play role in recognition. Then Th cell will be activated and produce cytokines (especially IL-2 and IFN- γ). These cytokines will activate Tc CD8+ cells, MØ and NK cells. This activation will increase these cells ability for killing and became more effecter.

1.7.2 Mode of action for killing target cells

After T-cytotoxic cells and NK cells activation by Th cells, T-cytotoxic cells come into close contact with target cell; they will bind to the Ag by their specific Ag

receptors. While NK cells will attach to Ag (on Target cell surface) by their non-specific receptors for Ag.

T-cytotoxic cells and NK cells will kill target cells by the following mechanisms [1–5]:

a. Direct contact killing: Production of perforin, which is a protein able to form pores in target cell membrane at the point of contact between Tc cell and target cell, lead to osmotic lysis of target cell.

b. Indirect killing: By secretion of a toxin protein in the space between the two cells, which causes fragmentation of target cell nuclear DNA, then the death of target cell by Apoptosis: the programmed cell death.

c. Antibody-dependent cellular cytotoxicity (ADCC) killing: it is specific mode of killing occurs when the parasites Ags have ability to induce both HMI and CMI, target cells will be coated with specific Abs formed after HMI against some parts of intracellular parasite like virus. These Abs will bring Tc and NK cells very close to the target cell by acting like a bridge because Tc and NK have receptors to the constant region of Ab. Then Tc and NK cells will be activated and kill the target cell by extracellular products (toxins and enzymes).

This type of CMI occurs when the foreign Ag persist for long time (e.g. *Mycobacterium tuberculosis* infection is long standing intracellular infection), also, against some kinds of cancer cells [2, 5].

1.8 Primary immune response

It is the first exposure to the Ag resulting of forming specific Abs and memory B-cells for HMI or T-cells and memory T-cells for CMI, the phases are, **Figure 2** [1–5]:

1. Latent Phase: start after first time exposure to an Immunogen or after induction, include the followings

   - No Ab level increase (Steady titer).
   - Recognizing Ag as foreign after processing the Ag inside APC.
   - Cellular proliferation and differentiation.
   - Duration of this phase (period) is variable depending on many factors (Ag immunogenicity, Ag dose, Ag solubility, Ag route of immunization or exposure).

2. Logarithmic phase: starts when Ab titer begin to increase (active biosynthesis of Ab), last for 10-14 days till reach peak.

3. Steady phase: starts when the rates of both formation (synthesis) and catabolism are equal, then serum concentration of Ab is constant.

4. Decline phase: starts when the Ab titer starts to fall down due to increase Ab catabolism rate than synthesis.

Note: during early primary response, IgM class antibodies is predominant and first rise than IgG appears later [2, 5].

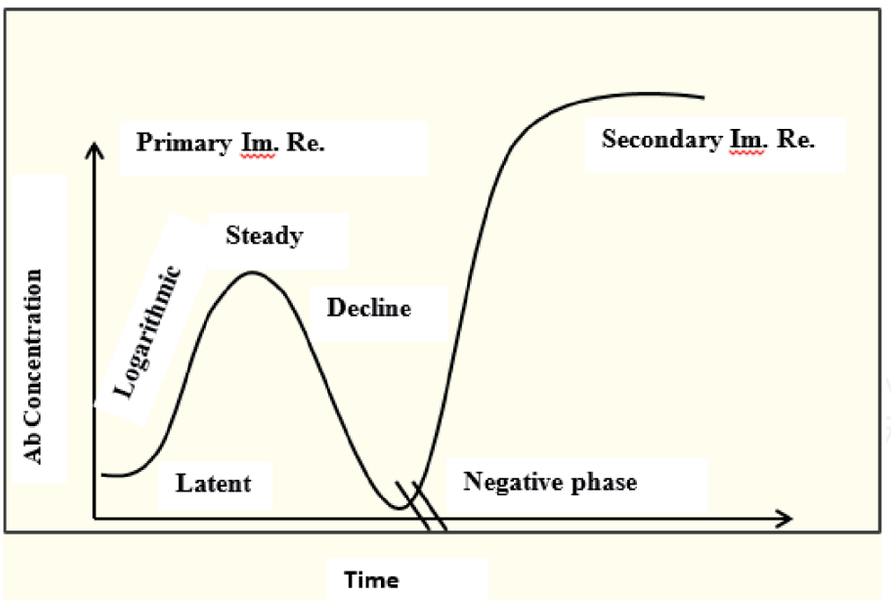

Figure 2.
Primary and secondary immune response.

1.9 Secondary immune response

It is the second exposure to the same immunogen that induced the first immune response (after booster dose of vaccination) may be after weeks, months, or even years later, includes [1–5]:

1. Accelerated or fast appearance of Abs.

2. Shorter latent period.

3. Rapid rate of Ab synthesis.

4. Higher peak titer of Ab.

5. More presence of memory cells.

6. Dose of immunogen needed is lower than primary.

7. Predominant Ab Class is IgG.

8. Long standing steady phase, whereas Ab titer will stay high longer time.

Negative phase: occur between primary and secondary Immune response when immunogen second dose is small and/or there is pre-existing antibodies from the first immune response (primary), then immunogen will be all consumed in Ag-Ab complex formation and phagocytosed then removed with no induction to secondary immune response [5].

2. *Salmonella* as a pathogenic Bacteria

Salmonella is a Gram-negative facultative rod-shaped bacterium in the same family as *Escherichia coli*, Enterobacteriaceae. *Salmonella* live in the intestinal tracts

of warm and cold blooded animals. In humans, *Salmonella* are the cause of two diseases called salmonellosis: enteric fever (typhoid), resulting from bacterial invasion of the bloodstream, and acute gastroenteritis, resulting from a foodborne infection/intoxication. Most common species related to human infections are *Salmonella Typhi* and *Salmonella Paratyphi* cause host-specific infections, being human the host. But other serotypes as *S.Typhimurium* and *S. Enteritidis*, mainly related to food products are also important serotypes that cause human diseases [5, 6].

Salmonella are found in the natural environment (water, soil, sometimes plants used as food). These bacteria are zoonotic, human or animal can excrete *Salmonella* either when they are infected with disease Salmonellosis and when they remain carriers. Also this disease is called food handling born disease due to the infected or carrier food handling workers [7].

Salmonella is intracellular parasite pathogen, do not multiply significantly in the natural environment (out of digestive tracts), but they can survive several weeks in water and several years in soil if conditions of temperature, humidity, and pH are favorable [5].

2.1 Antigenic structure and virulence factors

The bacterial antigens are the components or products of pathogens that are able to induce the immune defenses of the host to defend against, and to eliminate, the pathogen or disease. As with all *Enterobacteriaceae*, the genus *Salmonella* has three kinds of major antigens with diagnostic or identifying applications: somatic, surface, and flagellar [8–10].

2.1.1 Somatic (O) or cell wall antigens

Somatic antigens are the O side chain of LPS; they are heat stable and alcohol resistant. Cross-absorption studies individualize a large number of antigenic factors, 67 of which are used for serological identification. O factors labeled with the same number are closely related.

2.1.2 Surface (envelope) antigens

Surface antigens, commonly in enteric bacteria (e.g., *Escherichia coli and Klebsiella*), may be found in some *Salmonella*. Surface antigens in Salmonella may mask O antigens, and the bacteria will not be agglutinated with O antisera. One specific surface antigen is well known: the Vi antigen. The Vi antigen occurs in only three species *Salmonella Typhi*, *Salmonella Paratyphi* C, and *Salmonella dublin*.

2.1.3 Flagellar (H) antigens

Flagellar antigens are heat-labile proteins. Mixing *Salmonella* cells with flagella-specific antisera gives agglutination. Also, anti-flagellar antibodies can immobilize bacteria H antigens. Antigenic changes of the flagella known as the phase variation of H1 and H2 occurs in *Salmonella Typhimurium*.

2.1.4 Exotoxins

Salmonella strains may produce a thermos-labile enterotoxin that which has a limited relatedness to cholera toxin and *E. coli* (enterotoxin LT) in both structurally and antigenically characters. Additionally, a cytotoxin that inhibits protein synthesis. Both of these toxins play a role in the diarrheal symptoms of Salmonellosis.

2.2 *Salmonella* between pathogenicity and immunity

Innate immunity barriers play a good role in defense against *Salmonella* adhesion and colonization. But, upon infection specific immunity come to act; both humoral and cellular specific immune response will be activated to control this infection.

Primary infections with *S. Typhi* or *Salmonella ParaTyphi* usually induce a degree of immunity. Reinfection may occur but is often milder than the first infection. Circulating antibodies to O and Vi are related to resistance to infection and disease [11, 12].

Salmonella infections in humans vary with the bacterial species, the infectious dose upon ingested contaminated food, and the host health. The oral dose of at least 10^5 *Salmonella Typhi* cells are the most effective dose to cause typhoid in 50% of human volunteers as agreed by many references, whereas at least 10^9 *S. Typhimurium* cells (oral dose) are needed to cause symptoms of a toxic infection. Infants, immunosuppressed patients, and those affected with blood disease are more susceptible to *Salmonella* infection than healthy adults [12].

2.3 Salmonellosis (Typhoid fever)

In the **pathogenesis** of typhoid the bacteria enter the human digestive tract, penetrate the intestinal mucosa (causing no lesion), and stope in the mesenteric lymph nodes. Enteric Fever, Salmonellosis or Enterocolitis occurs after attachment to enterocytes of the ileum and colon. About 12-24 hours following ingestion of contaminated food (containing a sufficient number of *Salmonella*), the ingested *Salmonella* reach the small intestine, from which they enter the lymphatics and then the bloodstream. They are carried by the blood to many organs, including the intestine. Then *Salmonella* cells will attach to Microfold cells (M cells) and dendric cells of the jejunum. These specialized epithelial cells are found in the Peyer's patches and initiate mucosal immunity by endocytosis process for these bacteria antigens to the Macrophages and B-Lymphocytes to form APC specific for these antigens. Invasion can occur in this stage via the means of endocytosis, transfer, and exocytosis. Phagocytosis in the subserosa by macrophages and translocation into the mesenteric lymph nodes. Lymphogenous and hematogenous dissemination combined by immune cells proliferation and specific immune response is integrated. Of course the complement system upon these events is already activated, since LPS layer can activate the alternative pathway as soon as this endotoxin liberated from bacterial cells due to destruction leading to more inflammatory reactions at the site of invasion as described in the complement system roles. Moreover, the mannose residues that are found on the surface of *Salmonella* undergo lectin pathway activation of the complement system [5–12].

The organisms usually multiply in intestinal lymphoid tissue and are excreted in stools. However, in the case of *S. Typhi*, the bacteria survive ingestion by the phagocytes, and multiply within these cells. This period of time, during which the bacteria are multiplying within the phagocytes, is the 10–14 day is known as the incubation period. When huge numbers of bacteria fill an individual phagocyte, the bacteria are discharged out of the cell and into the bloodstream, where their presence begins to cause symptoms. Secondary foci in the spleen, liver, bone marrow, bile ducts, skin (roseola), and Peyer's patches then develop [5].

The presence of increasingly large numbers of bacteria in the bloodstream (called bacteremia) is responsible for an increasingly high fever, rising in stages throughout the first week to 39/40/41°C and may last throughout the four to eight weeks of the disease, in untreated individuals. Other symptoms include constipation (initially), extreme fatigue, headache and joint pain. Further symptoms:

leukopenia, bradycardia, splenic swelling, abdominal roseola, beginning in the third-week diarrhea, sometimes with intestinal bleeding due to ulceration of the Peyer's patches and inflammation of the gallbladder, severe irritation and inflammation of the lining of the abdominal cavity, called peritonitis, which is frequently a fatal outcome of typhoid fever [5, 12].

From the mesenteric lymph nodes, viable bacteria and LPS (endotoxin) will be released into the bloodstream resulting in septicemia. Moreover the effect of LPS as pyrogenic toxin, it causes activation of the complement alternative pathway which ends with membrane attack complex MAC, and that will increase LPS levels in bloodstream due to breakage of more bacterial cells leading to more harmful pyrogenic effects. The fever rises to a high plateau, and the spleen and liver become enlarged. Rose spots or rash usually on the skin of the abdomen or chest may be seen in some cases. Another scientific fact, LPS can induce both T-Dependent and T-Independent specific immune response. Specific antibodies against *Salmonella* antigens will be formed after primary infection occurrence, but, T-independent specific antibodies are with no memory B-cells formation. And that is the cause of short time specific immunity resultant after *Samonella* infections according to many scientists' opinions [7].

The complications of typhoid fever include liver and spleen enlargement (sometimes so extreme that the spleen ruptures), anemia (low red blood cell count due to blood loss from the intestinal bleeding), joint infections (especially frequent in patients with sickle cell anemia and immune system disorders), pneumonia (due to a superimposed infection, usually by *Streptococcus pneumoniae*), heart infections, meningitis, and infections of the brain (causing confusion and even coma). Untreated typhoid fever may take several months for full resolve. Spontaneous cure usually occurs [12].

2.4 Immune response features of *Samonella*

Due to that *Salmonella* behave as intracellular parasite inside host, these bacteria can survive inside phagocytic cells and escape the immune system meeting. Escape of destruction inside phagocytic cells like macrophage referred to the resistance of *Salmonella* to the oxidative burst used by these immune cells to kill and digest invading bacterial cells. Phagocytosis is the key process for induction of specific immune response but in the condition of contact with invading microbes and process the antigens to become APCs. Hence encountering of these bacteria sometimes can be late due to lack facing with immune cells and bacteria hide inside phagocytic cells. Also in certain circumstances as in immunocompromised patients like diabetic and old people, they usually suffer from late or weak immunological response against *Salmonella* and almost become carriers when the diagnosis and treatment are late [7, 8].

Salmonella induce both Th1 immunity (T-helper 1 or immunity or cellular mediated immunity) and Th2 immunity (T-helper 2 or humoral mediated immunity). When APC formed, then the immune response will be turned from innate or non-specific immunity to the specific humoral and cellular immune response, APC will present the processed Antigens of *Salmonella* to the cells of the specific immunity. Concerning Humoral mediated Immunity, specific IgG, IgM and IgA antibodies are formed against *Samonella* antigens, LPS- O antigen Vi antigen and H-antigen. Agglutinating antibodies can give positive reaction after one week post symptoms rise according to Gruber-Widal against H and O *Salmonella* antigens then the antibodies titer continue to elevate with infection time going. The white blood cell count can be found as normal or low at these stages [7, 8].

Antigenic variation can occur due to that *Salmonella* is able to generate genetic exchange and mutation abilities leading to the flagellar phase during infection course. This phenomena will cause the sero-variation and disease phases properties that is usually a characteristic of the infectious disease resultant from *Salmonella*.

Cellular mediated immunity is induced after APC formation, since *Salmonella* act as intracellular parasite and multiply inside macrophages. Then specific activated Cytotoxic T-cells will be produced and specific T-memory cells are released. Some scientists attribute joints inflammation that combined with Typhoid infections to the cellular immune response and due to accumulation of antigen-antibody complexes in patients' joints mainly during high bacterial load infections [7–12].

Cytokines of both Th1 and Th2 levels increase during Salmonellosis, Interlukins (IL-1, IL-2, IL-4, IL-6, IL-8, IL-9, IL-10, IL-13, IL-15, IL-17). Also Interferon-gamma (IFN-γ) play a great role during cellular immune response and its levels elevates in patients' blood even after cure. Another important cytokine is Tumor necrosis factor-alpha (TNF-α), its levels raise upon infection start and stay elevated along the disease time [5, 12].

2.5 Carriers

After manifest or subclinical infection, some individuals continue to harbor *Salmonella* in their tissues for different times (for example convalescent carriers or healthy permanent carriers). Three percent of survivors of typhoid become permanent carriers, harboring the organisms in the gallbladder; biliary tract; or, rarely, the intestine or urinary tract. Carriers of *S. Typhi* must be treated even when asymptomatic, as they are responsible for the majority of new cases of typhoid fever. Eliminating the carrier state is actually a difficult and require two different medications for four to six weeks at least.

In the case of carriers with gall stones, surgery may need to be performed to remove the gall bladder, because the *S. Typhi* bacteria are often housed in the gall bladder, where they may survive despite antibiotic treatment [7, 8, 12].

2.6 Re-infections and healthy carriers

Despite of that some patients with *Salmonella* will get spontaneous cure, *Salmonella* excretion by human patients may continue long after clinical cure. About 5% of patients clinically cured from typhoid remain carriers for months or even years. Antibiotics are sometimes ineffective on *Salmonella* but can reduce mortality which may reach was 10% [9].

However, relapses may occur in 2–3 weeks after recovery despite specific antibodies titer rise. Secretory IgA antibodies may prevent attachment of *Salmonella* to intestinal epithelium during next time exposure and avoid secondary infection establishment.

Some genetic factors can make person susceptible host for re-infection easier like persons with S/S hemoglobin (sickle cell disease) are susceptible to *Salmonella* infections. Persons with A/S hemoglobin (sickle cell trait) may be more susceptible than normal individuals (those with A/A hemoglobin) [9, 10].

The incidence of human disease decreases when the level of development of a country increases (like controlled water sewage systems, improve hygiene, pasteurization of milk and dairy products). Bad ways in having food like eating raw or undercooked egg can cause illness due to these bacteria called *Salmonella* Enteritidis Infection or Egg-associated salmonellosis which is an important public health problem.

Plasmid-borne antibiotic resistance is very frequent among and can be considered as a virulence factor upon ongoing infections. *Salmonella* strains can get resistance against ampicillin, streptomycin, kanamycin, chloramphenicol, tetracycline, and sulfonamides [11–13].

2.7 Vaccination against Typhoid fever

Vaccination is very good health measure in eradication of *Salmonella* infections. Tourists and visitors for countries endemic with *Salmonella* must be vaccinated with *Salmonella* vaccines as a prophylactic health measure.

Early research produced two vaccines made from the entire (whole-cell) bacterium. The first one became available in the 1890s, the second in 1952. Both protected about 65% of recipients. However, the frequency and severity of the adverse effects they caused dissuaded many countries from using them. These shortcomings, combined with drug treatment failures, as a result of increasingly widespread resistance to antibiotic therapy [12–14].

Before the end of the 20th century, two new-generation typhoid vaccines had entered the scene. One, named (Ty21) and first licensed in 1983, is given in three to four oral doses and consists of a live but genetically modified *S. Typhi* strain. The second, named "Vi" and licensed in 1994, is given by injection and consists of a sugar molecule (polysaccharide) located on the surface of the bacterium. In clinical trials and early field use, the duration of efficacy of both vaccines varied to some degree. Moreover, no evidence of efficacy has been reported in children under two years of age [15]. Both vaccines are licensed, internationally available, and safe, and both are effective enough not only to reduce the incidence of typhoid fever in endemic areas but also to control outbreaks [9–11].

Meanwhile, third-generation typhoid vaccines are under trial. One is a Vi conjugate vaccine that protects about 85% of recipients, according to late-stage clinical trials, and appears to be effective in children under two years of age. A second candidate vaccine, is, like Ty21a, a live attenuated vaccine but, unlike Ty21a, can be given in a single oral dose [15].

Three types of typhoid vaccines are currently available for use nowadays:

1. Oral live-attenuated vaccine.

2. Heat-phenol-inactivated vaccine; killed bacterial vaccine.

3. The Vi capsular polysaccharide vaccine for intramuscular use.

A fourth vaccine, an acetone-inactivated parenteral vaccine, is currently available only to the armed forces. While Typhoid fever vaccinations for tourists and travelers to the endemic areas is best be done with the oral attenuated vaccine Virotif Ty 21a.

Despite of that; No typhoid vaccine is 100% effective and provide only short-term protection (sometimes for a few months), it is not a substitute for being careful and elevate hygiene [15].

3. Conclusions

Salmonella, whatever species, is dangerous microbe that is able to invade human body due to many weapons owned. Good and healthy immune system can stand against these bacteria. But with bad nutrition, low hygiene and immunosuppression;

infection with *Salmonella* will occur upon exposure and may develop to a systemic disease. *Salmonella* induce human immunity with different types of resistance processes, either specific (adaptive immune response) or non-specific (Innate Immune Response) that act against these bacteria and lead to cure during treatment course. Despite of many effective vaccines that have been produced; elevation personal hygiene is still the best way to eradicate this infectious disease. Healthy carriers of *Salmonella* are a public health problem.

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