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Original Research Article

Systemic Analysis of Serum Interleukin [IL-9, IL-12 and IL-13], Chemokines [CXCL-1 and CCL-11] and Growth Factors by using Pathogenic Bacteria

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Abstract: Streptococcus pneumoniae or pneumococcus is a type of bacteria that may cause a great variety of diseases some of which are rather dangerous. Certain strains of streptococcal pneumoniae are more apt to cause a disease than others due to special characteristics they have. Due to several factors among which is the formation of capsules, surface proteins, and toxins, like pneumolysin, bacteria can cause tissue damage and enable them to evade the host immune system. The study was conducted with the use of the multiplex assay in order to examine the influence of this chronic inflammation around the implant on the expression level of the inflammatory cytokines on the organismal level. Cytokine systemic induction in the model of mouse was species specific. Compared to the tissues of individual infections or sterile implants, there was an entirely different manner in which infected implants elicited cytokines. Interactions between factors of tissue, implant materials and bacteria factors are major contributors in the pathophysiology of peri-implant disorders through high levels of inflammation. Most recent studies have associated conditions of long-term inflammation with the type of bacteria that lead to the peri-implant infections. Biomaterial-related infection usually led to the determination of pro-inflammatory cytokines including IL-9, IL-12, and IL-13. These facts of massive infiltration and long-term persistence of the host immune cells in cases of biomaterial-related infections raise a question that whether these infections contribute more to the development of inflammatory responses.

Keywords: Streptococcus pneumoniae, Interleukin, Chemokines, Growth factors.

INTRODUCTION

Pneumococcal Pneumonia, or a leading cause of pneumonia, sepsis, and meningitis, is caused by Gram-positive bacteria: Streptococcus Pneumoniae. Streptococcus pneumoniae kills many people across the world and in underdeveloped countries mostly those above the age of 65 years and children below the age of 5 years. Effective vaccines and medication are currently available in the treatment and prevention of invasive pneumococcal infection [1, 2]. The innate immune system uses pattern recognition receptors (PRRs) to detect harmful microbes by targeting particular, conserved markers (molecular) of the microbe (PAMPs) to do so. Until now, the studies have indicated that numerous pneumococcal proteins are capable of causing strong innate immune activations through TLRs. Consider, as an example, endopeptidase O (PepO) of Streptococcus pneumoniae. It brought about the activation of the innate immune response of the host through TLR2 and TLR4. Likewise, BMPs were also activated by pneumococcal DNA (DnaJ) but this activation was TLR4-dependent. Finally, there was an immune response of GHIP of Streptococcus pneumoniae mediated through TLR2. It is not a secret the significance of Toll-like receptors in the initial phase of host defence

in regard to infectious microbes. A small group of toll-like receptors (TLRs); principally, TLR2 and TLR4, are found to be involved in pneumococcal infection. The innate immune system induced by the cell walls of Streptococcus pneumoniae peptidoglycan and lipiteichoic acid has been found to be activated by Toll-like receptor 2. A study by Regine Landmann *et al*., demonstrated that TLR2 knockout mice had a retarded phagocytosis syndrome when infected and had more bacteria loads than the control WT mice [3], when initiated with a challenge of Streptococcus pneumoniae. The TLR4 also facilitates strepococcal infection by pneumolysin recognition and triggered increase in apoptosis development occasioned by pneumolysin. In the hosts, the control of the infections is also dependent on DC molecular pathways [4-6]. It is well acknowledged that release of cytokines is mediated by the activation and phosphorylation of NF-KB, AKT and MAPKs. It is also vehement that microRNA regulates the biological pathways of immune cells such as DCs and macrophages. For example, mir-155 is essential to pneumococcal clearance and forefront in DC functions; the mir-146 is a negative regulator of innate immune responses in an NF-kappaB-dependent manner; mir-27a is important to cytokine production by DCs. Even though there are more choices on vaccines and treatment, Streptococcus pneumoniae still poses a serious threat as an abortive cause of pneumonia and meningitis. The swiftness at which penicillin-resistant pneumococcal strains advance and metastasize to the rest of the world has led to the rekindling of the interest in pneumococcal infection treatments [7, 8]. Following penicillin, a few other drugs that include chloramphenicol, macrolides, sulfonamides and tetra-cyclines also go resistant. When MICs are less than 0.1 mg/ml, pneumococci are said to be 100 percent sensitive to penicillin; when the MICs are between 0.1 to 1 mg/ml, it is intermediate, and when the MICs roll over 1 mg/ml, it is very resistant to penicillin. It has been established that larger amounts of penicillin work in some infections but when there is resistance to its ease of use by the other beta-lactam antibiotics such as glycopeptides and broad-spectrum cephalosporins then other alternatives have been proposed. The length of time that the concentration of the antibiotic exceeds the MIC (T.MIC), is the most important pharmacokinetic parameter that demonstrates in vivo an effect of beta-lactam antibiotics against penicillin-sensitive pneumococci [9, 10], which as we have already shown in an experimental animal animal model. As to the application of the same pharmacodynamic recommendations in penicillin-resistant pneumococci, little experimental researches have been conducted and fewer still with more specific strains taken into consideration. In our present work we have aimed at analysing the pharmacokinetics of penicillin and the relationship between its in vitro activity and the in vivo effect against pneumococcal infection by bacteria, which were different in their penicillin susceptibility. The pneumococcal polysaccharide vaccine (PPV) should not be administered to children, those with impaired immune systems and older people due to the number of the capsular polysaccharides used [10]. The conjugate PCV vaccination reduces the burden of pneumococcal diseases, and it is pricey and needs serotype replacement, which is a challenge. Such ability to induce an immune reaction in both infants and the elderly gives the protein-based vaccinations a bright future [1,3,11]. Therefore, it is also imperative to discover other possible vaccine proteins and explore connection between these proteins and the host; which may result to invention of anti-bacterial methods and development of wider pneumococcal protein vaccines [12, 13]. This study was done by using pathogenic microorganisms and was focused on serum interleukin [IL-9, IL-12 and IL-13] and chemokines [CXCL-1 and CCL-11] and growth factors.

MATERIALS AND METHODS

Streptococcus pneumoniae serotype 19F is a type of bacterial infection that can result into a variety of diseases such as pneumonia, meningitis, and otitis media, and is usually prevalent amongst youngsters. One should be concerned about the possibility of multidrug-resistant clonal expansion of serotype 19F isolates that may exhibit antibiotics resistance. Control and management of pneumococcal illness is done by isolating and identifying this serotype and monitoring its antibiotic sensitivity typology. Inoculation of the specimens was done by placing them on Brain-heart infusion broth and blood agar. An inverted incubator set at a temperature of 37 o C with 5-10 % CO 2 was used to incubate the BA plates. The time of incubation was 18-24 hours. The incubated colonies with optochin discs were then again later treated with Grams stain and catalase after which the suspicious of alpha-hemolysis colonies that had normalized morphology (colour, edges, glistening, and mucous) were reincubated. The optochin-sensitive isolates were subcultured to the grounds of obtaining a pure culture. The process of diagnosis includes microscopical examination of Gram-stained samples, determining the size and shape of the pneumococcus cells, performing catalase test, morphological colony characterization (colour, shape, size, edges, consistency), optochin sensitivity test, bile solubility test and other confirmatory tests such as the agglutination latex test with Pastorex meningitis kit and the VITEK 2 system used to determine biochemical identification. They used ten pneumococcal clinical isolates, which belong to different parts of the world [14, 15]. The reason is to demonstrate the spectrum of bacteria against which penicillin has minimum inhibitory concentrations. Bacterial suspensions used in injection were prepared by allowing stock cultures to freeze then growing them fresh overnight on 5 percent blood agar plates. The optical density of 540 nm was turned to 0.5 which provided the inoculum with an approximate density of 108 CFU/ml. Before inoculation, the colonies were suspended in sterile beef broth medium. In an attempt to determine the inoculum size, we diluted the total growth 10fold in beef broth to begin every experiment. Next, using 5% blood agar we inoculated 0.1 ml on the plates and counted the

colonies. Beef broth served as the medium to make a 10% (wt/vol) saline stock solution of an enzyme extract of pig stomach as an adjuvant inoculation to the mice which was set up in 5% blood agar as well as on Mueller-Hinton agar with 5% lysed horse blood. The mucin was brought to a final concentration of 5% (wt/vol) through the 1:1 dilution of the mucin solutions using pneumococcal suspensions prior to inoculation. In the time-kill experiments, a beef broth that contained the diluted mucin to the concentration of 5% (wt/vol) was used. Peritonealonisis in male mice model. When conducting the research male and female ssc CF-1 mice that were outbred were used and the mice were aged between 8-12 weeks. All the cages were made to contain five mice, and the animals had the freedom of getting food and water. The inoculation was done through intraperitoneal injection of 0.5ml pneumococcal solution with the help of a 25-gauge syringe.

Infections and Subcutaneous Implants in a Mice Model

The 8-1.2 weeks old female mice, employed to conduct the research, were bred at Central Animal Facility. They were placed in separate air-ventilated cages and they were well fed and watered. Three different species were used. The animals were made to sleep by intraperitoneal injection of ketamine (Albrecht Company, Germany) and xylazine (4 and 10 mg/kg respectively). The fur was removed using a hair trimmer after which a 70/ethanol solution was used to clean the insertion sites. With the help of surgical scissors and tissue forceps, three 1 cm in diameter incisions were made. Subcutaneous pouches were made on these incisions. The sutures to seal the wounds consisted of simple interrupted sutures. The inoculation of 5 _L of bacteria suspension in the form of infection was carried out within 30 min after the closing of the surgical wound, irrespective of the presence or absence of implantation. Blood was obtained after three weeks of the implantation and then these animals were followed up regularly.

Blood Quantification of Cytokines

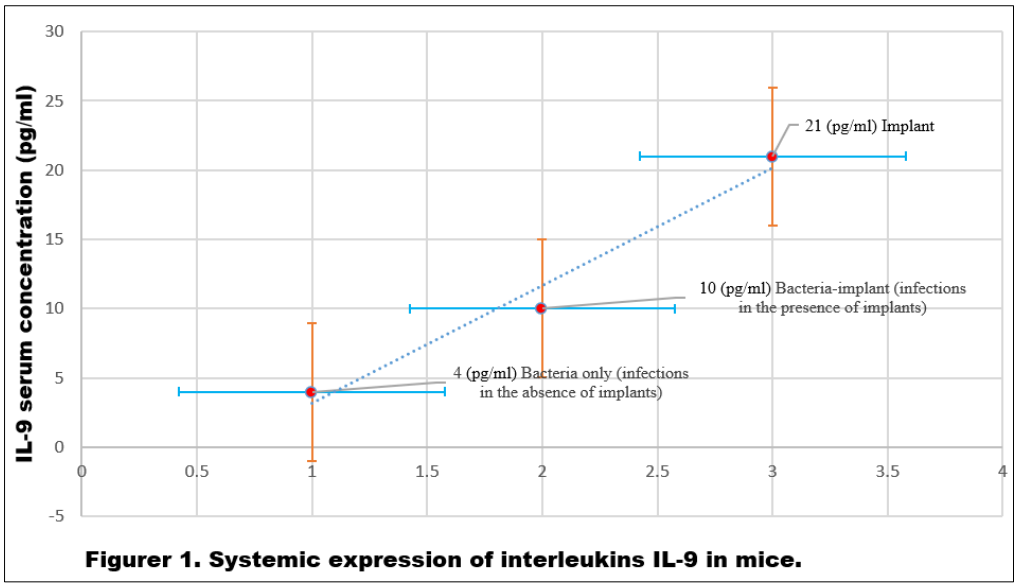
The implanted mice were subject to heavy anaesthetic and the blood sample collected without coagulant in total three weeks after implantation via extraction through their hearts. Blood samples were incubated in room temperature by placing them still and allowed to incubate for 30 minutes before centrifuging them at 1500 g for 10 minutes at 4 o C. We separated the serum by centrifugation and stored them at ~80 _ C prior to its use in other processing activities. The concentrations of the serum cytokine were determined using Bio-Plex Pro Mouse Cytokine Assay (Bio-Rad, Munich, Germany).

Statistical Analysis

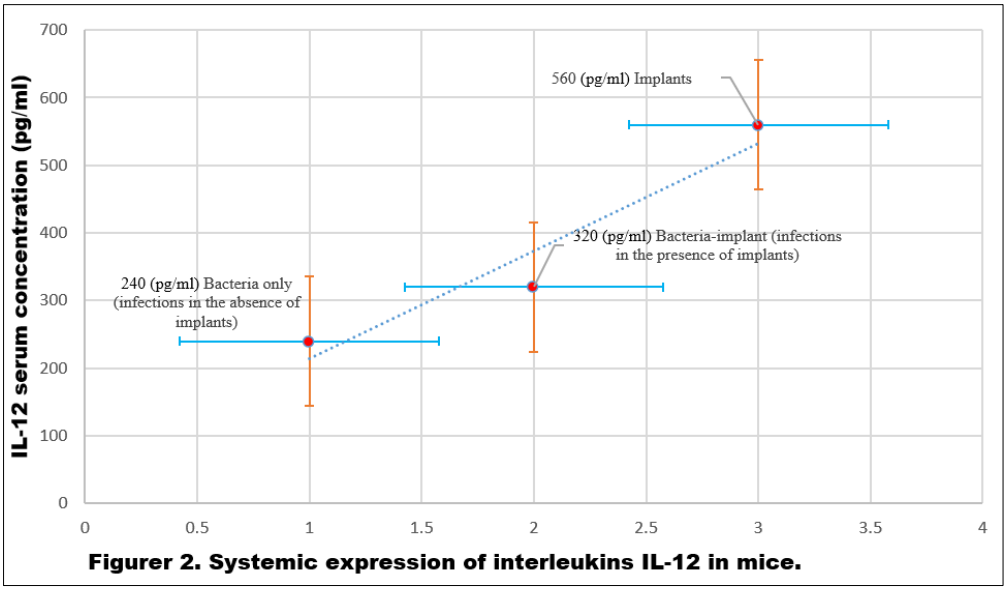
In order to conduct the statistical analysis, IBM SPSS Statistics (v. The use of 26) was used. To determine whether the subsequent categories of the population differed significantly among themselves, we applied rank-based nonparametric Kruskal-Wallis H test accompanied by Dunn post-hoc test.

RESULTS AND DISCUSSION

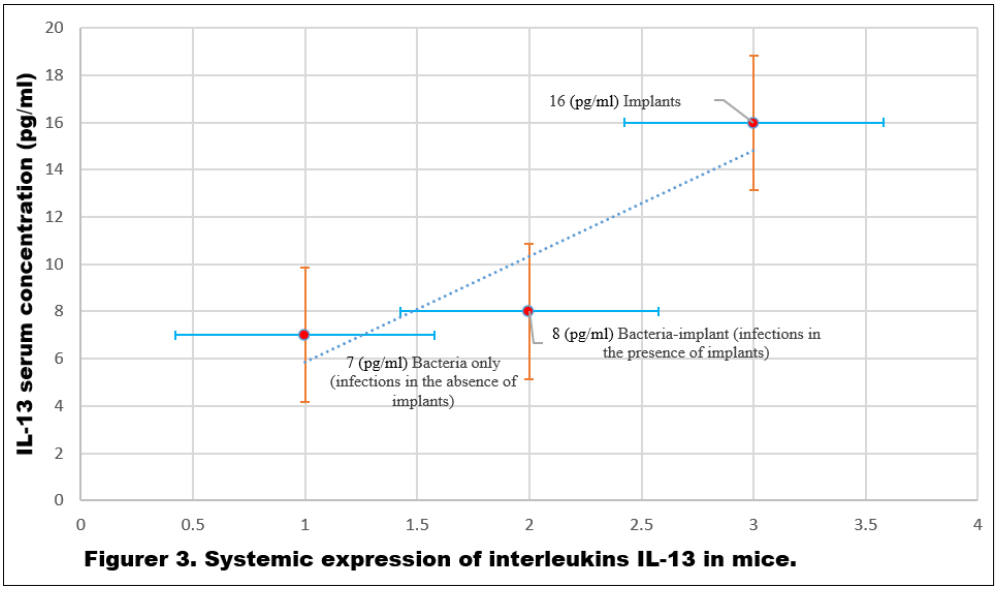
The streptococcus pneumoniae bacterium is often the cause of lower respiratory diseases in individuals with weakened immunity systems. According to a study, 10 percent of the people die due to invasive pneumococcal infections. Being the constituent of the upper respiratory tract commensal microbiota, S. pneumoniae achieves its introduction into the host by colonizing the nasopharynx without triggering any symptoms. In case the colonized bacteria is not eliminated by the immune system upon colonization, the bacteria will move horizontally into the lower airways and other parts of the body and tissues and later result into illness. The S. pneumoniae can be swept away before it manages to be pathogenic under the aid of a strong immune system together with an equilibrium between the resident flora and intruders. The defences of the host are low hence S. pneumoniae can colonize the host at regular and consistent rates leading to illness [16]. Through several of its traits, the bacteria avoids being removed by the local flora of the nasopharynx and it succeeds in escaping the detection process of the human immune system. It is hence of prime importance that the healthcare sector minimizes the impact of this bacterium and prevents further infections. And being an opportunistic agent S. pneumoniae feeds on those people, whose immune system is rather weak, immature, or failing. This is the reason that the deaths linked with S. pneumoniae infection are more among the elderly, in persons with a compromised immune system [17], and among children below the age of two. It is essential to understand how the immune system ages to come up with efficient medicines to be put against the colonization of vulnerable hosts. Multiplex assay was able to identify a number of inflammatory cytokines present in mouse serum such as interleukin, chemokines, and growth-stimulating factors. Binding of interleukins IL-9, IL-12 and IL-13 concentration (pg/ml) of serum Implant, Bacteria-implant (infections with the presence of implant) and Bacteria only (infections without the presence of an implant) records as [21, 10 and 4 (pg/ml)] concentrations of IL-9 respectively. Whereas measured [560, 320 and 240 (pg/ml)] serum level of IL-12 correspondingly. In the same period, it recorded [16, 8 and 7 (pg/ml)] concentration of IL-13 on serum respectively. Important p-value before Bonferroni correction respectively between sterile and infected implant. Important infections between infections with and without implants.



Figurer 1. Systemic expression of interleukins IL-9 in mice.

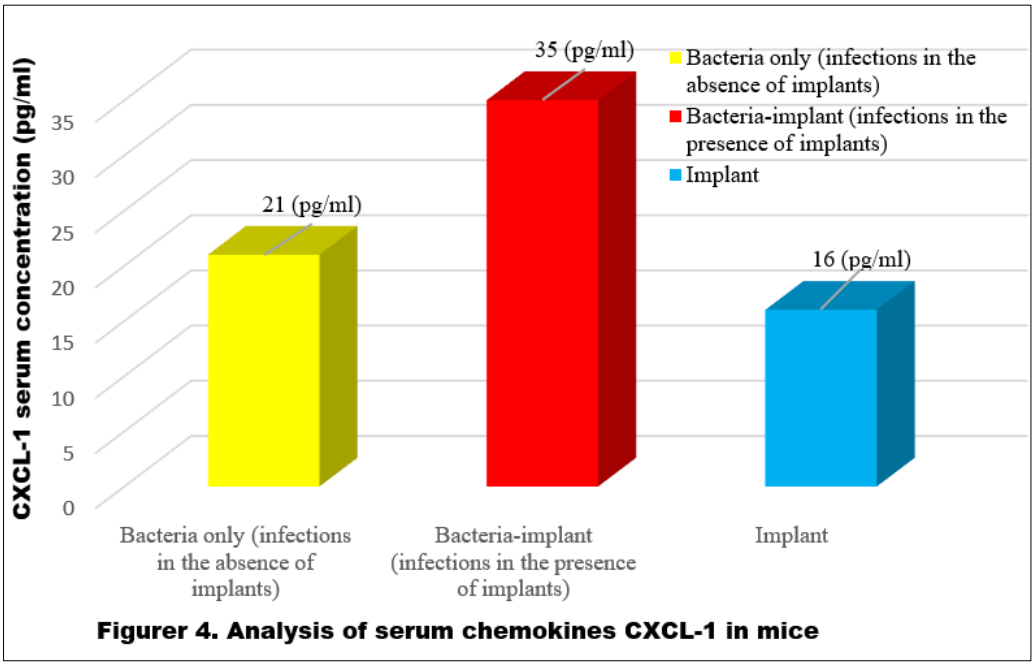


Figurer 2. Systemic expression of interleukins IL-12 in mice.

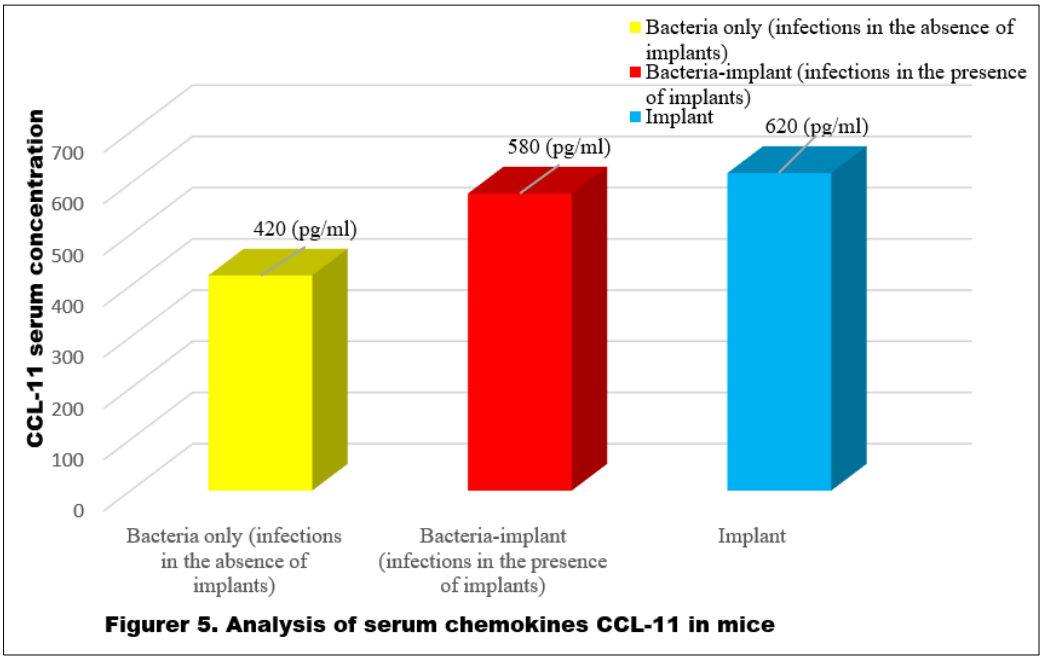


Figurer 3. Systemic expression of interleukins IL-13 in mice.

One important family of the cytokines is chemokines that play an important role in host immunity. The multiplex assay monitored the systemic concentrations of these chemokines that were shown to be bacteria specific and different in profile upon presence of implants. The addition of an implant changed the expression of chemokines. Chemokine induction was observed to be species-specific in majority cases. CXCL-1 and CCL-11 (pg/ml) levels were found on the serum-CXCL-1 [16,35 and 21 (pg/ml) in Implant, Bacteria-implant (infections in the presence of implants) and Bacteria only (infections in the absence of implants) respectively and CCL-11 [184, 127 and 255 (pg/ml) in Implant, Bacteria-implant (infections in the presence of implants) and Bacter Whereas they were measured [620, 580 and 420 (pg/ml)], respectively, with respect to CCL-11. Respectively, between sterile and infected implants there are significant p-value before Bonferroni correction. Major disparities between the infections where there are and where there are no implants.



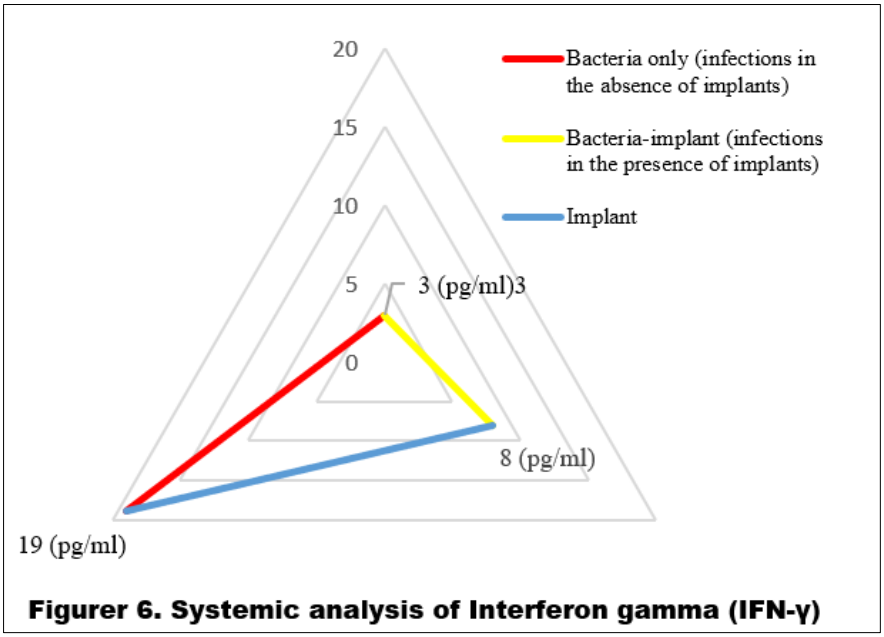
Figurer 4. Analysis of serum chemokines CXCL-1 in mice



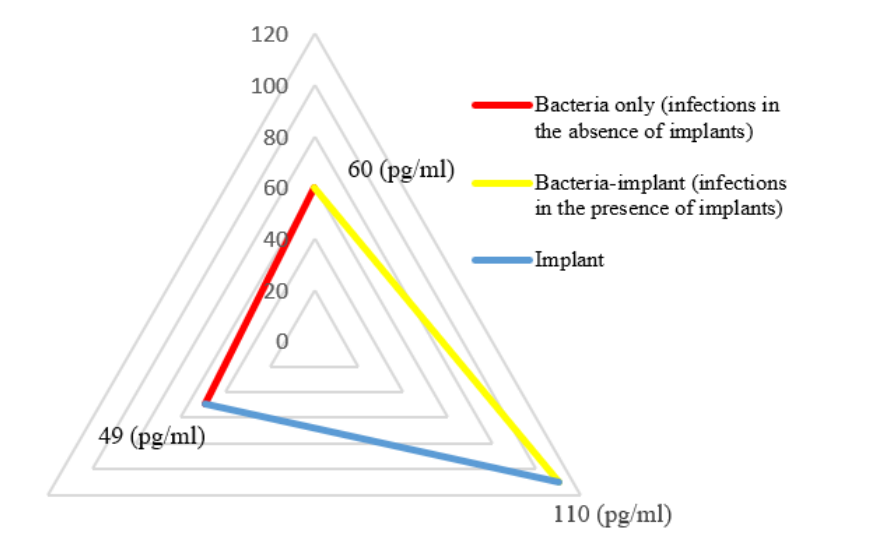
Figurer 5. Analysis of serum chemokines CCL-11 in mice

Multiplex was also applied in the systemic scrutiny of host inflammatory response, namely IFN-gamma and granulocyte-colony-stimulating factor (G-CSF). Systemic evaluation of IFN- gamma, and granulocyte colony-stimulating factor (G-CSF) (pg/ml) 19, 8 and 3 (pg/ml) measured in the Implant, Bacteria-implant (infections with implants) and Bacteria only (infection without implants) respectively, in the case of

IFN- gamma. On the other hand, it was recorded [49, 110 and 60 (pg/ml)], respectively, in G-CSF. Interferon gamma (IFN-gamma) is a cytokine or a protein, which is vital in the immune system. It also helps in balancing the immunological reactions including responses against infections and cancers, and is secreted by stimulated lymphocytes and natural killer cells. Among the popular functions of IFN-gamma is that one enhances the cellular immunity and initiates immune response to combat the tumors. A key protein in fighting against infections, granulocyte colony-stimulating factor (G-CSF) promotes bone marrow to produce a higher number of white cells, mostly neutrophils. Use it pre and post transplanting stem cells, and it cures neutropenia-A disease [18], where the count of white blood cells is low which could be as a result of chemotherapy or as a result of other diseases.



Figurer 6. Systemic analysis of Interferon gamma (IFN-γ)



Figurer 7. Systemic analysis of granulocyte colony-stimulating factor (G-CSF)

Kinetics of interferon synthesis depended upon the species of bacteria but were less in two-species infections and prolonged when implants were used than when they remained absent. Streptococcus pneumoniae is frequently used to infect mice with subcutaneous inoculation to study pneumococcal infection and to screen promise candidates of vaccines. This is the process through which the bacteria or the vaccine constituents can be injected beneath the skin of the mouse. The result of the infection due to the dosage and strain may be local or systemic. When Streptococcus pneumoniae gets inoculated on mice, there exist immune

responses in the form of interleukins. These interleukins are involved in several roles of the host defence system and such that involve [IL-9, IL-12 and IL-13]. It appears that IL-6 contributes to the host by dampening the inflammatory response, IL-10 may be harmful by diminishing the immune response and impeding the ability to rid the body of bacteria. The role played by IL-17 is not straightforward; it offers protection against some strains of pneumococcal, with less thick capsules, but it endangers the thicker ones [19, 20]. When inoculated with Streptococcus pneumoniae, mice and hamsters can experience all kinds of immunological reactions such as an increase or decrease in interleukin levels. Infection of mice with S. pneumoniae causes an upsurge of the cytokines which include IL-6, IL-2, and IFN-gamma which drives inflammation, and interleukin-10 cytokine which reduces inflammation. Some of the factors that may influence these reactions are the kind of bacteria that causes it, the mode of infection and the immune system of the host. The hamster presents with a strong inflammatory response after exposure to S. pneumoniae based on the observed better accumulation of neutrophils in the lungs and better upsurge of MPO activity. On the other hand, many host defences have been seen to recognize S. pneumoniae, react promptly and kill the bacterium even before it can cause diseases of pneumococcus. The protection against S. pneumoniae depends on the immune system hosted by the individual. Age factors in the efficacy of the immune system in eradicating the S. pneumoniae infection. Pneumococcal infections are more common among the elderly and young children who are below the age of five years. The answer to this is the fact that the immune system of newborns differs as compared to the immune system of the elderly who are in the process of immune-senescence [21-25]. The innate (primary defence) immune response and the adaptive immune response both deal with variety of immune cells. In the subsequent paragraphs, a brief description of the most important elements of the humoral and immune cells response to the attack of pneumococcal pathogens in a host is presented with some indication of how each of these elements could be affected by the process of ageing. B cells respond to antigen by releasing antigen specific antibodies that form part of the humoral immunity [26, 27]. T cells are cells that are involved with cell mediated immunity and help in recruiting other immune cells to directly kill harmful cells a process referred to as T cell mediated recruitment. These immune cells are produced by the bone marrow; in particular, the B cells mature in there into the antibodies which are specific to antigens producing cells (plasma cells). Pneumococcal bacteria specific antibodies can be used to inhibit infections in mucosal sites. It inhibits opsonization. Upon the cleavage of Fab, the unclipped fragment binds to the cell membrane. This enhances cell adhesion, decreases the negative charge on capsule, and reveals CBPs. Studies show that the Fab has the ability to neutralize negative charge of the capsule that facilitates easier attachment with cells. Also, the complement (C3) activates B lymphocytes. When the naive B cells are infected with an antigen, they are differentiated into IgM+ memory B cells. Class switching produces different immunoglobulins necessary in clearing out infection. Rather, T cells are fully developed in the thymus where they arise as cytotoxic (CD8+) or helper (CD4+) T cell. The T cells are activated to induce an immune reaction when the antigen-presenting cells (APCs) and the major histocompatibility complex (MHC) proteins combine to transfer the antigens, better yet peptides. Pneumococcal infection implies the activating influence of APCs and the co-stimulatory molecules upon CD4+ T lymphocytes [28-31]. Activated helper T cells result in production of Th1 and Th2. Cell-mediated immune response is activated through the production of cytokines such as interferon-gamma (IFN-g) by Th1 helper cells which recruit and activate other immune cells such as macrophages. Th2 helper cells also deal with B cells in the formation of their antibodies and also help in development of IL-4 cytokines that are set to promote a humoral immune response. Cytotoxic T cells have the responsibility of killing infected cells directly. Better still, memory B and memory T cells could form through activation of B and T cells hence faster elimination of second-order infections. The natural killer T-cells also aid pneumococcal clearance. More specifically, the immune response against S. pneumoniae is antibody-mediated held by the CD4 + T cells. Also, regulatory T cells (Tregs) and T-helper 17 (Th17) are also involved in pneumococcal infections. The Th17 cells express the pro-inflammatory cytokine IL-17. The IL-17 is required to recruit and engage monocytes, neutrophils and macrophages to the infections sites to complete the clearance of S. pneumoniae [32, 33]. The augmented generation of IL-17 has been associated with the reduction of the density of S. pneumoniae in mice and children nasopharynxes. Tregs are needed to regulate the level of IL-17 produced through the Th17. Autoimmune disease may occur with the disproportion of regulatory T cells (Tregs) and effector T helper 17 (Th17) cells that lead to overly inflamed conditions. T cells of infants respond weakly to non self-antigens because they did not generate many non maternal specificities as a result of prenatal exposure. Children also demonstrate unbalanced Th2 response when they are exposed to foreign antigens. To compensate to this, newborns possess a number of γδ T cells producing IFN- 9, thus providing them with the phenotype of Th1 type of immune response. The B lymphocytes of babies react poorly to antigens since their co-receptor expression levels are low. Moreover the switching of immunoglobulin classes is less efficient in infants and the somatic hypermutations are less than in adults. Immunoglobulin defence against the capsular polysaccharides of S. pneumoniae also gets regulated during development. The ability of the new born to protect itself by producing a steady antibodies is delayed until the age of two following the exhaustion of the maternal antibodies. Differently, infants that were infected with S. pneumoniae carried that antibody, IgM.

Similarly to how booster effects of vaccination stimulate production of antibodies, the re-exposure to the pathogen stimulates production of antibodies as well. With development, there is the maturation and memory development of adaptive immune cells accompanied by a reduction in S. pneumoniae infections. Adaptive immune cells used by people become ineffective when they age. There are aging-related changes that lend itself to colonization by S. pneumoniae and these include decreased antibody production, immunoglobulin class flipping, and maturation of cells. Aged related immune deterioration leads to a loss of the antibodies to capsular polysaccharide [34-36]. Moreover, a transient decrease in the number of naive T cells due to thymus involution is typical of an aging person. Besides, it was observed that the ratio of regulatory T cells (Tregs) to CD4+ cells increased. The enhanced impact of the diseases caused by pathogenic pneumococcal bacteria in the age groups subjected to risks can be explained by the weakened activity of the adaptive immune cells. As signaling molecules, these molecules are released by immune cells (both innate and adaptive) which direct other immune cells to points of infection. Chemokine is one of the types of cytokines that attracts cells to an infected site. They do not only promote cell recruitment, but also inflammation. TNF- a, a well studied pro-inflammatory cytokine, reduces the growth and dispersion of pneumococci. The TNF-alpha and IFN-gamma have a potential of working synergistically to enhance clearance of pathogens by stimulating phagocytes. TNF-a is made by macrophages, monocytes and T cells. Autolysin associated with pneumococcal activity can inhibit cytokines activating phagocytes. Persistent low-level inflammation associated with ageing is a typical symptom of old age [37]. It involves availability of low concentration with the persistence of pro-inflammatory cytokines such as TNF-alpha and IL-6. Senescence escalates the inflammatory status of the elderly through enhanced activation of NF-kB followed by secretion of other pro-inflammatory cytokines such as TNF-k. The higher the rates of TNF-a, the higher the rates of disease occurrences. When there is inflammation, the likelihood of being affected by S. pneumoniae is high because the inflammation causes an expression of host proteins that enhance the adherence of S. pneumoniae. Additional morbidities are also an accompaniment of inflammation.

CONCLUSION

There is an association in the serum interleukin (IL) level during systemic examination in inflammatory disorder as well as in the infectious disorder with the pathogenic microorganisms. Possible biomarkers of sepsis and other diseases are ILs, in particular IL-9, IL-12 and IL-13, which are commonly elevated among bacterial infections. When the immune system reacts to the presence or abundance of some microorganisms, ILs are produced to release the immune response. Other researches have demonstrated that interleukin (IL)-9, interleukin-12 (IL-12) and interleukin-13 (IL-13) is a superior indicator of sepsis on bacterial infections, other studies have demonstrated that there is no significant difference between the bacteremic and non-bacteremic patients. Having inoculated mice with four different periodontal pathogens, this study described the condition of inflammatory cytokines [CXCL-1 and CCL-11] and growth factors and subsequently examined them systematically in chronically inflamed mouse model of the implant site. A complex interaction between bacteria and the tissues could not be sufficiently investigated in a test tube experiment. Regulation of inflammatory cytokine expression was systemic and infection-specific and implant-dependent. Intervention in infections due to biomaterials is already hard enough to include systemic management of inflammatory cytokines. Moreover, our findings endorse the notion that future animal models that depict disorders linked to biomaterials must include multi-species studies. Notable signs of inflammatory processes associated with implants are high levels of release of inflammatory cytokines including [IL-9, IL-12 and IL-13]. Various kinds of infection, cross talk between bacteria, the implants used are just some of the possible sources of these dis-regulation of inflammatory cytokine expressions. Findings of this subcutaneous model indicate that the use of inflammatory cytokines needs further research in the future where mouse models would be used where infected implants would be placed in the mouth bones.

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