Effect of *Staphylococcus Aureus* on Phagocytic Ability in Healthy Humans that Carried *S. Aureus* in Their Noses (in Vitro)

Ayad, K. Zgair
Department of Biology, College of Science, University of Baghdad

Abstract

This study included eighteen healthy humans (15 hs) carriage *S. aureus* in their noses. This group had been divided into two subgroups depending on presence or absence of *S. aureus* protein-A (SPA). The first subgroup included twelve hs. carriage *S. aureus* (SPA+), and second subgroup included six hs. carriage *S. aureus* (SPA-), and ten hs. as a control group (They do not have *S. aureus* in their noses). The phagocytic ability was calculated by the percentage of phagocytic cells (Phagocytes engulfed *S. aureus*). Significant increase (P < 0.005) in percentage of phagocytic cells and significant decrease (P < 0.005) in percentage of non-phagocytic cells in hs. that carriage *S. aureus* (SPA+), was found and there wasn't any significant differences in hs. that carriage *S. aureus* (SPA-) when compared their results with control group. When the percentages of Neutrophils, Lymphocytes, Monocytes, Eosinophils and Basophils in all groups were calculated there weren't any significant differences in these percentages when compare their results with control group. There weren't any significant differences in concentration of IL-8 in all groups when compare with control group. From all that we can suggest, SPA may activate the phagocytosis in vivo indirectly and independently of IL-8.

Introduction

*Staphylococcus aureus* nasal carriage has been extensively studied in patients and healthy individuals (1). It is one of the regular flora of human body surface, and it is transmitted from person to person by touch, which is an important infection route of nosocomial infection (2, 3). In the studies on *S. aureus* carriers, samples from the nasal vestibulum have usually been examined although *S. aureus* is widely distributed in the human body, including the nose, throat, intestine, skin etc (4). Between 20 and 70% of adult individuals carry *S. aureus* in the nose, some of these individuals are permanently colonized and others are only transiently colonized (5, 6). Suppurative interactions between common pathogens *S. aureus*, Hemophilus influenzae and *Pseudomonas aeruginosa* and the respiratory epithelium are sufficient to cause inflammation as documented in many histopathological studies (7). These cells can adhere to the epithelial cells and capable to stimulate pro-inflammatory response, epithelial production of IL-8 is particularly important in recruiting and stimulating polymorphonuclear cells (PMN) in the lung and is widely used as a clinical marker of inflammation. Several laboratories have examined which bacteria components activate epithelial IL-8 expression to recruit PMNs into the airways. These include both adherent intact organisms as well as isolated bacterial gene products which may be present in the airways lining even in the absence of viable bacteria (8). Presence of *Staphylococcus aureus* in mucosal membrane may stimulate epithelial cells to production of IL-8 by activated CD4+ dependent signalling cascade, but mutants *S. aureus* can not do that (9). Inter-leukin-8 is a prototype of the family of cytokines which are chemotactic for Neutrophils, Macrophages, Fibroblasts, endothelial cells and epithelial cells. Endothelin (ET-1), tumor necrosis factor (TNF)-α, IL-1, granulocyte-macrophage colony stimulating factor (GM-CSF), lectins, immune complexes and phagocytes all stimulate IL-8 production (9, 10). Macrophages play a central role in host bacterial interactions. They are most prevent non-parenchymal cells in the airways of normal subjects and are important regulatory of airways inflammation (9). Neutrophils are the most important cells recruited to the airways after exposure to a pathogen. Their primary function is to recognize phagocytosis and destroy the pathogen. This is accomplished through opsonization followed by Fc- mediated binding or antigen recognition using complement receptors. The pathogen is ingested and killed in the PMNs phagosome through the expression of peptides and reactive oxygen intermediates. Neutrophils release lipid mediators, leukotrienes and reactive oxygen species which are important in bacteria killing and in the inflammatory response, PMN elastase is up-regulated by epithelial IL-8 expression by the airway cells (11). *S. aureus*
Vindaré has been studied extensively but the mechanism remain obscure. The primary line of defence against Staphylococci is the PMNs which phagocytose and kills the bacteria. S. aureus produces a vast number of virulence factors, including secreted toxins which have been shown to contribute to its pathogenicity (12). Contouring the immune response to S. aureus protein - A (SPA) it is a cell wall component of many S. aureus strains that binds to the Fc portion of IgG molecule except IgG3. The Fab portion of IgG bound to protein - A is free to combine with a specific antigen. Protein - A has become an important reagent in immunology and diagnostic laboratory technology for example protein - A with attached IgG molecules directed against a specific bacterial antigen will agglutinate bacteria that have that antigen (Agglutination) (13). S. aureus produces a virulence factor, protein - A (SPA) that contains five homologous Ig-binding domains. The interactions of SPA with the Fab region of membrane - anchored IgG can stimulate a large fraction of B-cells contributing to lymphocytes clone selection (14).

Materials and Methods
Healthy humans: This study included eighteen healthy humans (hhs) carriage S. aureus in their noses divided into two subgroups dependent on type of S. aureus isolates: Twelve hhs. carriage S. aureus have Staphylococcus aureus protein - A (SPA+) and six hhs. carriage S. aureus that do not have SPA (SPA-). Control group include ten hhs. negative to S. aureus (Nasal swabs Negative to S. aureus).

Isolation and identification of S. aureus: Colonies on the Staphylococcosis 110 were sub cultured on the mannitol salt agar and Blood agar. Incubated at 37 °C for 24 h. Identification of yellow colonies Blood's manual of determinative Bacteriology (15).

Determination of Protein-A activity: fresh cultures of Staphylococci grown on Mueller Hinton agar for 18-24 h. were suspended with 25μl of Latex reagent coated with IgG (Kit for detection protein - A Bio-Kit) on slides and the formation of agglutination within 2 minutes was considered as positive. Control positive and negative were performed.

Preparation of bacterial suspension: Staphylococcus aureus (SPA-) was cultured on nutrient broth at 37 °C for 18 hrs. The growth was collected and washed three times with normal saline. Number of bacteria was adjusted to 10⁹ cells/ml. Bacteria suspension stored in -20 °C until time of experiment (16).

Phagocytosis: 1ml of whole heparinized peripheral bloods (collected less than 2 h.) was mixed with 1 ml of bacteria suspension in sealed flasks test tubes. The tubes were incubated at 37 °C for half hour with gently shaking from time to time. Smeared were made from every tube stained with Leishman stain, examined the percentages of phagocytic cells and non phagocytic cells of PMNs.
Table 1: The mean of percentages of many types of leukocytes in peripheral blood of Healthy humans have S. aureus positive to protein A (SPA+) and H. influenzae carriage S. aureus (SPA-) and control group.

<table>
<thead>
<tr>
<th>Information</th>
<th>H. influenzae carriage S. aureus with (SPA+)</th>
<th>H. influenzae carriage S. aureus with (SPA-)</th>
<th>Control cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean of percentages of Neutrophils</td>
<td>62.8 ± 6.87</td>
<td>60.3 N.S</td>
<td>61.75 N.S</td>
</tr>
<tr>
<td>Mean of percentages of Lymphocytes</td>
<td>31.2 ± 7.46</td>
<td>24.0 N.S</td>
<td>32 N.S</td>
</tr>
<tr>
<td>Mean of percentages of Monocytes</td>
<td>4.4 ± 1.44</td>
<td>5.33 N.S</td>
<td>4.75 N.S</td>
</tr>
<tr>
<td>Mean of percentages of Eosinophils</td>
<td>7.6 ± 1.34</td>
<td>7.37 N.S</td>
<td>1.5 N.S</td>
</tr>
</tbody>
</table>

When we detected the concentration of IL-8 in sera of healthy humans carriage S. aureus (SPA+) group, H. influenzae carriage S. aureus (SPA-) and control group. We didn't find any significant differences in concentration of IL-8 in sera of all two first group when compared their results with control group Figure 2.

Figure 2: Concentration of IL-8 in peripheral blood of a) Healthy humans have S. aureus (SPA-). b) Healthy humans have S. aureus (SPA+). c) Control group.

NS: Non significant differences.
S: Significant differences (P<0.005).

Figure 1: The percentage of the phagocytic cells (A) and nonphagocytic cells (B) in all studied groups and control: a) Healthy humans have S. aureus (SPA+), b) H. influenzae carriage S. aureus (SPA-), c) Control group (Do not have S. aureus).

Lymphocytes, Monocytes, Eosinophils and Basophils in peripheral blood of healthy humans carriage S. aureus (SPA+) and H. influenzae carriage S. aureus (SPA-) and control group. We didn't find any significant variety in all these groups when compare their results with control group. Table 1.
We used in phagocytosis experiment. Staphylococcus aureus include that has SPA. Because SPA has the ability to bind to the Fc region of immunoglobulin G (IgG) in most mammalian species; this protein (SPA) has been shown to inhibit complement and phagocytosis of Staphylococcus aureus in vitro (18); thus we used Staphylococcus aureus (SPA+) to prevent that during phagocytic experiment. But this factor (SPA) may contribute to the virulence of human in vivo (19). SPA also exhibit diverse immunological properties, including an ability to activate B cells (B-cell super antigen) and interact with human nonimmunoglobulins (Igs) independently of the light chain isotope, and activate allograft progression of the B cell repertoire and SPA can activate B cells by binding with Vh11 of immunoglobulin that bind on B cells (14, 20). SPA in the absence of H-2 directly stimulates B-cell activation, proliferation, and differentiation (21). From all that we can suggest that SPA stimulate high number of B-cell and that B-cell activation can produce many types of interleukins one of these is IL-12 this is a critical regulator of both innate and acquired immunity. By selectively promoting differentiation of Th1, lymphocytes, Th1-polarized cell-mediated immunity and phagocytosis (22). SPA also exhibit diverse immunological properties, including an ability to activate complement components (23). Components of the complement pathway act as very potent enhancers because the phagocytes (Neutrophils) express surface complement receptors that will increase the phagocytic potential (24). Thus we can see increase in phagocytic cells percentage (engulfed Staphylococcus aureus). The activation of B cells that will increase in Immunoglobulins and it will increase in the immune complex (ICs) and similarly, ICs have specific receptors on phagocytes (Fc receptors) and this binding will stimulate and increase in activity of phagocytes to engulf antigens (S.aureus) (24). There is high affinity between SPA and Fab region in spite of the affinity between SPA and Fc region on Immunoglobulin, that mean may be percent competition between Fab and Fc to bind with SPA and the binding between SPA and Fab will stimulate the immune system indirectly (14). We didn't find any increase in number of Neutrophil and others Leukocytes table 2 but we found increase in activity of phagocytes percentage of phagocytosis that is meaning the people who carriage Staphylococcus aureus (SPA+) have high activity of phagocytosis but without increase in number of these cells. Interleukin 8 play an important role in phagocytosis because it is chemotaxis to phagocytes (22). But in this study we found there isn't any variation in concentration of IL-8 in all groups that is meaning SPA can activate phagocytes independently H-2. In this study we suggest, SPA can stimulate the phagocytic activity indirectly way. Purification of protein-A from Staphylococcus aureus and study the effect of it on phagocytic ability in vivo and in vitro that will support the results that were presented in this research, so this steps are very important to do in future studies.

References


elexalam


لا يمكنني قراءة النص العربي من الصورة المقدمة.