Effect of Stahylococcus Aureus on Phagocytic Ability in Healthy Huntans that Carried S.Aureus in Their Noses (in Vitro)

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Abstract

This study included eighteen healthy humans (h.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 h.hs carriage S.aureus (SPA+) and second subgroup included six h.hs carriage S aureus (SPA-) and ten h.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.aures). Significant increase (P< 0.005), in percentage of phagocytic cells and significant decrease (P< 0.005) in percentage of non phagocytic cells in h.hs that carriage S.aureus (SPA+),was found and there wasn't any significant differences in h.hs that carriage S.aureus (SPA-) when compared their results with control group. When the percentages of Neutrophils, Lymphcytes, 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

Sraphylococcus 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 -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). Superficial interactions between common pathogens S.aureus, Hemophilus influenzae and Pseudomonas aeroginosa and the respiratory epithelium are sufficient to cause inflammation as documented in many histopathological studies (7). These cells can adherent on 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 lumen even in the absence of viable bacteria (8).Presence of S.aureus in mucosal membrane may stimulate epithelial cells to production of IL – 8 by activated Ca^{++} dependent signalling cascade, but mutants S.aureus can not do that (9) Interleukin -8 is a prototype of the family of cytokines which are chemotactic for Neutrophils, Macrophages, Fibroblasts, endothelial cells and epithelial cells. Endotoxin (LPS) , tumer necrosis factor (TNF)α, IL-1, granulocyte macrophages - 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 subject s 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 recognise phagocytosis and destroy the pathogens. This is accomplished through opsonization followed by Fc mediated binding or antigen recognition using complement receptors. The pathogen is ingested and killed in the PMN 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 a potent stimuli of epithelial IL-8 expression by the airway cells (11).S.aureus

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virulence has been studied intensively but the mechanism remain obscure. The primary line of defence against Staphylococci is the PMNs which phagocytoses 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). Confounding 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(Coagglutination)(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 Igs can stimulate a large fraction of B-cells contributing to lymphocytes clone selection (14).

Materials and Methods

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

Isolation and identification of S.aureus: Colonies on the Staphylococcus 110 were sub cultured on the mannitol salt agar and Blood agar. Incubated at 37 °C for 24 h. identification of yellow colonies Bergy'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 10^9 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 seleconized test tubes. The tubes were incubated at 37 °C for half hour with gently shacking from time to time. Smears were made from every tube stained with Leishman stain, examined the percentages of phagocytic cells and non phagocytic cells of PMNs. were calculated.

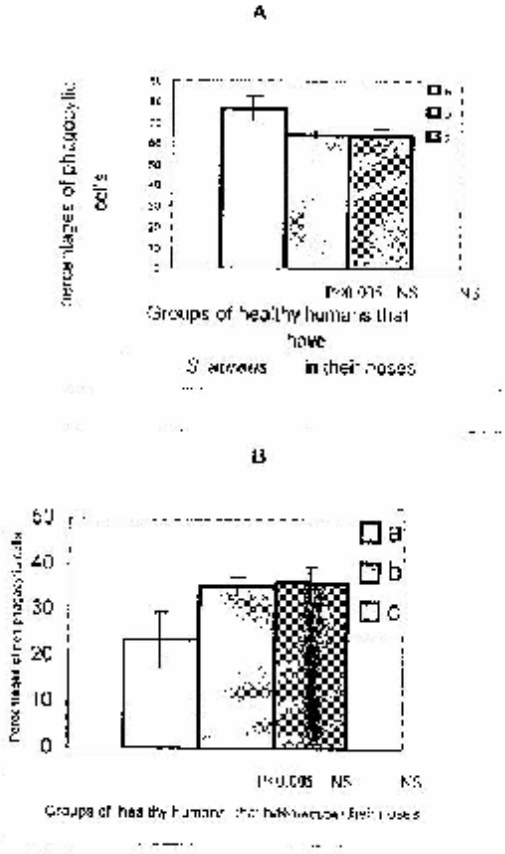
Differential count of peripheral blood of all cases and control group. : Smears were prepared for all cases and control and stained with Leishman stain. The percentages of Neutrophils, Lymphocyes, Monocytes, Eosinophils and Basophils were counted.

Determination of IL-8 concentrations: Concentrations of IL-8 were detected in sera of all cases and control group by the procedure of Backman coulter company method (Backman coulter, 13276 Marseille Cedex 9, France).

Statistical analysis: T- test was used to detect any significant differences in groups of cases when compared with control group.

Results and Discussion

This study showed that there was significant increase in percentage of phagocytic cells ($p< 0.005$) of the healthy humans carriage S.aureus and positive to Staphylococcus aureus protein –A (SPA+)group comparing with the results of the control. But there is not any significant differences in the percentage of phagocytic cells in h.hs. carriage S.aureus and negative to S.aureus protein –A (SPA-) when compare its result with control group figure -1 (A). Significant decrease in percentage of non-phagocytic cells in h.hs. that carriage (SPA+) was found, and there was not any significant differences in percentage of non-phagocytic cells in h.hs. that carriage (SPA-) when compare these results with control group. Figure -1(B).



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.hs. have 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.humans 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.

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+), H.hs. have S.aureus (SPA-)and control group.

Information	H.h carriage S.aureus with (SPA+),n: 12	H.h. carriage S.aureus with (SPA-),n:6.	Control n:10
Mean of percentages of Neutrophil	62.8 ± 6.87 N.S	69.3 ± 2.52 N.S	61.75 ± 10.8
Mean of percentages of Lymphocytes	33.2 ± 7.46 N.S	24.0 ± 1.63 N.S	32 9.5
Mean of percentages of monocytes	4.4 ± 1.14 N.S	5.33 ± 0.56 N.S	4.25 ± 1.26
Mean of percentages of Eosinophils	3.6 ± 1.34 N.S	1.33 ± 0.57 N.S	1.5 ± 1
Mean of percentages of Basophils	0	0	0

When we detected the concentration of IL-8 in sera of healthy humans carriage S.aureus (SPA+) group, h.hs. 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-.

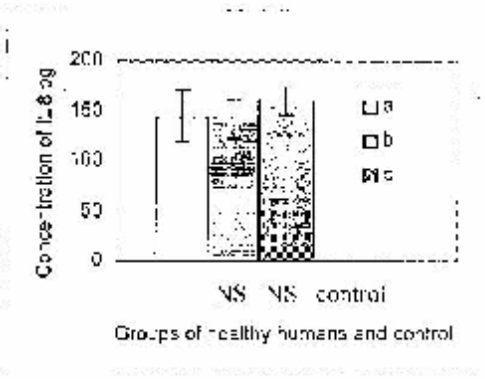


Figure-2- Concentration of IL-8 in peripheral blood of: a- Healthy humans have S.aureus (SPA+), b- Healthy humans have S.aureus (SPA-), C- Control group.

We used in phagocytosis experiment S.aureus isolate that has SPA. Because SPA has the ability to bind to the Fc region of Immunoglobulin G (IgG) in most mammalian species(17), this protein (SPA) has been shown to inhibit opsonization and phagocytosis of Staphylococcus in vitro (18).Thus we used S.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 non-immunoglobulins (Igs) independently of the light chain isotype and activate along proportion of the B cell repertoire and SPA can activate B cell by binding with V_H II of immunoglobulin that bind on B cell. (14, 20).SPA in the presence of IL-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 after activation can production 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 T_H 1 lymphocytes. It potentiates cell- mediated immunity and phagocytosis (22).SPA also exhibits diverse immunological properties, including an ability to activate complement components (23). Components of the complement pathway act as very potent opsonis because the phagocytes (Neutrophils) express surface complement receptors that will increase the phagocytic potent (24).Thus we can see increase in phagocytic cells percentage (engulfed S.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 S.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 variety in concentration of IL-8 in all groups that is meaning SPA can activate phagocytes independently IL 8. In this study we suggest, SPA can stimulate the phagocytic activity indirectly way. Purification of protein –A from S.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

1- Nouwen,J.L.,Van Belkum,A. and Verbrugh,A.H.(2001). Determinants of Staphylococcus aureus Nasal carriage. Neth.J.Med.59.pp: 126-133.

2- Dupeyron,C.,Carrpillo,B.,Bordes,M.,Faubert,E .,Richardet,J.P.and Mangeney, N. (2002).A clinical trial of mupirocin in the eradication of methicillin resistant Staphylococcus aureus nasal carriage in a digestive disease unit. J.Hosp.Infect., 52, 281-7.

3- Maraha, B., Van-Halteren,J. Verzijl,J.M., Wintermans,R.G.F. and Buiting, A.G.M. (2002_.Decolonization of methicillin- resistant Staphylococcus aureus using oral vancomycin and topical mupirocin.Cli. Microbiol. Infect. 8,pp: 671-5.

4- Tajima,Y.Nagasawa, Z., Tanabe, I., Yamada,H., Kusaba, K. and Tadano, J.(1992). An improved method for the serotyping of coagulase from Staphylococcus aureus. Microbiol. Immunol., 36.pp:1233-7.

5 Kluytmans,J.,Van Belkum,A. and Verbrugh,H.(1997). Nasal carriage of Staphylococcus aureus :epidemiology, underlying mechanism and associated risks. Clin. Microbiol. Rev. 10: 505 -20 [Abstract]

6- Vanderbergh, M.F., and Verbrugh, A. H.(1999). Carriage of Staphylococcus aureus : epidemiology and clinical relevance. J.Lab.Clin. Med. 133, pp: 525 – 34.[Medlin].

7- Burns,J., Gibson,R.L., McNamara,S., Yim,D., Emerson,J., Rosenfeld,M.,Hiatt,P., Castile, R.,Smith,A.L.and Ramsey,B.W. (2001).Longitudinal assessment of Pseudomonas aeroginosa in young children with cystic fibrosis. J.Infect. Dis. 183. pp: 444 52.

8- Di Mango,E., Zar,H.J., Bryan,R., Prince, A. (1995). Diverse Pseudomonas aeroginosa gene products stimulate respiratory epithelial cells to produce IL-8. J. Clin Invest. 96, pp: 2204 – 10.

9- Rastogi, D.,Ratner, A. J. and Prince, A. (2001). Host – bacterial interactions in the initiation of inflammation. Paediatric Respiratory Reviews, 2, pp: 245 – 252.

10- Oppenheim, J.J. and Horuk,R.(2001). Chemokines In : Medical Immunology eds : Parslow, T.G., Stites, D. P., Terr,A.I. and Imboden, J.B., Mc Graw- Hill. New York, USA. Pp: 167 – 75.

11- Tosi, M.F., Zakem,H. and Berger,M. (1990). Neutrophil elastase cleaves C3 bi on opsonized Pseudomonas as well as CR1 On Neutrophils to create a functionally important opsonin receptor mismatch. J. Cli. Invest, 86, pp: 300 – 8.

12- Rayan, J.L. and Projan, S.J. (2001). Bacterial disease. In Medical Immunology, eds. Parslow, T.G., Stites, D. P., Terr,A.I. and Imboden, J.B., Mc Graw- Hill. New York, USA, pp: 607 – 16.

13- Lowy,F.D. (1998). Staphylococcus aureus. N. Engl. J. Med. 339, pp: 520.

14- Graide, M., Sturs, E.A., Corper, A.L., Sutton,B.J., Taussig,M.J., Charbonnier, J. B. and Silverman, G.J.(200). Crystal structure of a Staphylococcus aureus Protien A domain complexed with the Fab fragment of ahuman IgM antibody : Structural basis for recognition of B- cell receptors and superantigen activity. PNAS. 97, pp: 5399 - 404.

15- Holt, J. G., Krieg, N. R., Sneadi, P.H.A., Staley, J. T. and Williams, S.T. (1994) " Bergey s manual of determinative bacteriology" •9th ed, William. Wilk., Maryl..

16- Furth,R.V., Theda, L. and Leijilt. P.C. (1985) In vitro determination phagocytsis and intracellelar killing by polmorphonuclear and mononuclear phagocytes Hand book of experimenral immunology, Black well, Scientific publication, 3th ed. 2, 1 - 4.

17- Brooks.G.F., Butel,J.S. and Morse,S.A. (2004) Medical Microbiology. 20th ed. McGrow Hill company, New york. USA. pp: 119-147.

18- Gemmell,C.G., Tree,R., Paul,A., O'Reilly,M. and Foster,T.J. (1991). Susceptibility to opsonophagocytosis of protein –A, α-hemolysin and β-toxin deficient mutants of S.aureus isolated by allel-replacement. Zentbl. Bakteriol. 21 (Suppl.): 273 – 277.

19 Patel,A.H., Nowlan,P., Weavers,E.D. and Foster,T. (1987). Virulance of protein-A deficient and alpha toxin deficient mutants of Staphylococcus aureus isolated by allele replacement.Infect. Immun. 55:3103 – 3110.

20- Sausse,K.,Laure,L.,Rachic,C., Marcou,D.L., Silverman,G.J. and Zouali,M.(1998).Structural basis of the gp 120 superantigen bind site on human immunoglobulins. J.Immunol 161, 6681 – 88.

21 Lipsky,P.E. (1980).Staphylococcal protein –A a T-cell regulated polyclonal activator of human B-cells. J.Immunol, 325 : 155.

22- Oppenheim,J.J.andRuscetti,F.W.(2001).Cytokines In : Medical Immunology eds: Parslow, T.G., Stites, D. P., Terr,A.I. and Imboden, J.B., Mc Graw- Hill. New York, USA, pp: 148-174.

23- Kozlowski,J.D. and Levinson,A.L. (1996).Complemen activeation by a B cell superantigen. J.Immunol. 157: 1300 – 6. [Abstract].

24- Parslow, T.G.and Bainton, D.F.(2001). Innate immunity. In: Medical immunology .eds:. Parslow, T.G., Stites, D. P., Terr,A.I. and Imboden, J.B., Mc Graw- Hill. New York, USA, pp: 19 – 39.

الخلاصة

شملت الدراسة ثمانية عشر شخاص اصحاء يحملون بكتريا العنقوديات الذهبية في انوفهم حيث قسموا هؤلاء إلى مجموعتين ثانويتين اعتمادا على وجود او عدم وجود بروتين أ في العنقوديات الذهبية المعزولة من انوفهم : المجموعة الأولى وتشمل ثنتا عشر شخصا يحملون العنقوديات الذهبية والتي تحمل بروتين أ والمجموعة الثانية وتشمل ستة(6) اشخاص اصحاء يحملون العنقوديات الذهبية ولكن لا تحوي على بروتين أ و عشرة اشخاص اصحاء لا يحملون العنقوديات الذهبية يمثلون مجموعة السيطرة. حيث تم حساب قابلية الخلايا البلعمية على الإبتلاع من خلال حساب النسبة المئوية للخلايا البلعمية (المصابة للعنقوديات الذهبية) حيث وجد زيادة معنوية في هذه النسبة ($P<0.005$) في الأشخاص الأصحاء الحاملين للعنقوديات الذهبية التي تحمل بروتين أ وكذلك وجد انخفاض معنوي في النسبة المئوية للخلايا غير المنشطة ($P<0.005$). ولم نحصل على اي فروقات معنوية في هذه النسب لدى الأشخاص الأصحاء الحاملين للعنقوديات الذهبية(التي لا تحمل بروتين أ) عند مقارنة نتائجهم مع مجموعة السيطرة. وعند حساب النسبة المئوية لخلايا المنشطة الخلايا المتفاوية و الخلايا الوحيدة و الخلايا المتعشية و الخلايا اللمفية لم نحصل على اي فروقات معنوية في هذه النسب لدى كل المجاميع عند مقارنة نتائجهم مع مجموعة السيطرة. وكذلك

لم نحصل على اي فروقات معنوية في تركيز الأنزيم بركين-S لدى كل المجاميع عند مقارنت نتائجهم مع مجموعة السيطرة. من هذه الدراسة استنتجنا ان البروتين أ يمكن أن يعمل على زيادة قابلية الخلايا المناعية على ابتلاع الأجسام الغريبة ولكن بطريقة غير مباشرة.