Effect of *Staphylococcus aureus* Thermostable DNase on Some Immunological Parameters

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Abstract

Thermostable DNase was purified from *Staphylococcus aureus* isolated from local clinical source. The current study includes different immunological assays to evaluate the role of purified DNase. The phagocytic activity showed that the group of mice infected with *P.aeruginosa* recorded the highest values with significant differences (P <0.05) in comparison with the group that received mixed injection of 1 µg /ml of DNase and *P. aeruginosa* showed decrease in phagocytic activity followed by group that injected by 1 µg /ml DNase only with phagocytic activity 2%. DNase has a positive immunomodulatory effect on IL-10 and IL-4 level. The data showed that the highest value of IL-10 is in group that injected with 1 µg /ml DNase only (IV group) was 86.79±1.68. While the highest value of IL-4 was in group that injected with both DNase (1 µg /mouse) and *P.aeruginosa* gave the highest values (III group) was 84.02 ±1.19. In Arthus reactions(Immediate type hypersensitivity), the group that have been injected with both DNase and *P. aeruginosa* (group III) gave the highest values (4.50±0.70) while in delayed type hypersensitivity the same group III showed higher values after 24 and 48 hrs (5.00±0.00) , (4.00±0.00) respectively.

Key words: *Thermostable DNase, Interleukin-4, Interleukin-10, Arthus, DTH, Phagocytic activity.*

Introduction

Staphylococci are important microorganisms that influencing ecosystems, industry, animal, and human health. Their diverse lifestyles, whether as commensals or opportunistic pathogens, promote their prevalence in different environments, both nonpathogenic and pathogenic can survive for short or extended periods, thus it contribute in spread and difficult eradication [1, 2].

Staphylococcus aureus is a notorious pathogen known to cause numerous acute and chronic diseases [3]. The diversity of the tissues affected and infection potency is due in part to the many secreted virulence factors produced by *S. aureus.* These secreted factors include toxins, super antigens, and a suite of exoenzymes proteases, lipases, hyaluronidase, phospholipase, and nuclease [4].

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Staphylococcal nuclease also referred by many different names, such as micrococcal nuclease, thermonuclease, deoxyribonuclease and DNase which is regarded as an important virulence factor that hydrolyzes DNA and RNA in host cells [5]. It has been postulated to play a role during invasion or establishment of an infection [6]. Bacterial extracellular nucleases have multiple functions in processes as diverse as nutrient acquisition, natural transformation, biofilm formation, or defense against neutrophil extracellular traps NETs [7]. In response to infection, neutrophils play a key role. In addition to phagocytosis and intracellular killing of pathogens, they have been shown to actively release extracellular netlike structures (NET) that consist of a nuclear DNA backbone, histones, and granular proteins, that can entrap, immobilize and even kill gram-positive and gram-negative bacteria [8].

DNase mediate bacterial escape from NETs which is an innate immune defense mechanism by which DNA released from dying neutrophils immobilizes and facilitates killing of invading pathogens [9].

Material and Methods

Extraction and purification DNase from *S. aureus*

The selected isolate *S. aureus* was grown on nutrient broth medium and inoculated at 32 °C, using 100 rpm shacking rate for 24 hrs. Extraction was carried aout according to Ohsaka [10]. DNase was extracted by ammonium sulfate precipitation and Dialysis of crude enzyme and Purified by Ion exchange chromatography and gel filtration chromatography according to Ibraheem and Al-Mathkhury (2015), [11].

Laboratory animals

Twenty male of albino mice, aged between 8-12week and 26–30 g weight were used in this study. The mice were obtained from Iraqi National Center for Drug Control and Research and kept in plastic cages, housed under a standard condition in the animal house of the Department of Biology/College of Science/ Mustansiriyah University. The mice left for 2 weeks for adaptation before the experiments began.

Animals groups and injection schedule

The current study was included four groups, all mice injected intraperitoneally for seven days as follows:

Ø Group I: Mice injected with 100 µl of phosphate buffer saline only as negative control

Ø Group II: Mice injected with 100 µl of 1.5 x 10^8 CFU / ml of *P. aeruginosa* only as positive control.

Ø Group III: Mice injected with 100 µl of purified DNase (1µg/mouse) only.

Ø Group IV: Mice injected with 100 µl purified DNase (1µg/mouse) after infection.

Blood collection

After 7 days of treatment, mice were anesthetized with sodium pentobarbital and killed by cervical dislocation, blood samples were obtained by cardiac puncture and divided; serum was obtained and stored at – 20 °C for next assays.

Measurement of serum cytokines levels

The levels of IL-4, IL-10 in the serum of mice were measured through ELISA. All the procedures were performed according to the manufacturer's instructions of Elabscience.

Phagocytic activity

The procedure was done depending on a method presented by Zakaria, *et al* 2011, [12] peripheral Blood Leucocytes 1×10^6 cell/ well (100 *ul* Blood, with 175 *ul* MEM media) added to each well of microtiter plate , 25 *ul* of NBT was added and incubated two hours at 28 °C. Then supernatant was removed carefully. Cells were fixed with methanol 100% (v/ v) for 5 min in each well .and washed twice with 125 ml with Methanol 70%, then drying at air overnight. Finally adding 125 ml Potassium hydroxide (2N) and 150 ml DMSO add to each well to dissolve NBT. Then Read by Elisa at 650-wave length.

Arthus and Delayed hypersensitivity test

Each mouse in the groups was injected with 50 ul of *P.aeruginosa* in day 5 in the right foot pad and normal saline in the left foot pad (except control group), arthus reaction was observed and determined as an increase in footpad swelling 4 hrs while delayed type hypersensitivity peak after 24 and 48 hours by a digital vernia and given in a unit of millimeter, as suggested by (Tansho *et al*., 2002) [13].

Statistical Analysis

A one way analysis of variance ANOVA was performed to test whether group variance was significant or not. Data were expressed as mean ±standard deviation (SD) and statistical significances were carried out using SPSS program version 20 [14].

Result and Discussion

Production, extraction, and purification of thermostable DNase

The crude enzyme of *Staphylococcus aureus* was purified by precipitation with ammonium sulphate at (65-85 %) saturation then dialysis, after that using ion exchange chromatography in CM cellulose and gel filtration by using Sephadex G150. The DNase activity for each step was 38, 31, 32, 49 and 42 U/mg respectively.

Phagocytic activity of DNase

Results in table (1) shows that the group of mice

infected with *P.aeruginosa* recorded the highest values with significant differences (P<0.05) but the group that received combination injection of 1 µg of DNase and *P. aeruginosa* showed decrease in phagocytic activity followed by group that injected only with 1 µg of DNase with phagocytic activity 2%.

Table 1: The phagocytic activity index of DNase in 1 µg /mouse

Treated groups	Mean± SD	Phagocytic activity%
Group (I): Negative Control	0.44±0.01 d	0
Group (II): Positive Control	2.722±0.14 a	5.1
Group (III): DNase and P. aeruginosa	1.82±0.09 b	3
Group (IV): DNase only	1.33±0.18 c	2

The different letter = high significant difference at p ≤ 0.05, The similar letter = non-significant at p≤ 0.05

The antimicrobial strategies employed by neutrophils include the phagocytosis of bacterial invaders, the release of toxic effectors, and the production of neutrophil extracellular traps (NETs) [15]. A significant feature of extracellular traps is that their expression is degraded by the addition of DNase 1. DNase 1 which breaks down extracellular DNA, and found that DNase 1 was highly effective in degrading extracellular trap formation both in vitro and in vivo. In addition, DNase 1 reduced the numbers of macrophages in the lung tissue induced by cigarette exposure [16]. *Staphylococcus aureus* produces a nuclease that degrades DNA and RNA to 5-phosphomononucleotides and dinucleotide, which are subsequently converted to deoxyadenosine (dAdo) through the AdsA enzyme. The dAdo product is cytotoxic to macrophages and therefore protects *S. aureus* by restricting these innate immune cells from entering staphylococcal abscesses [17].

Determination the level of cytokines IL-10 and IL-4

The results reported a positive effect of DNase on IL-10 and IL-4 level, as noticed group that injected in both DNase (1 µg/ml)and *P.aeruginosa* gave the highest value according to IL-4 while IL-10, the highest value was in group that injected with DNase only table (2).

Table 2: The levels of Interleukins 4 and 10 in studied groups injected with DNase enzyme (1µg/mouse).

Treated Groups	Cytokines concentration Pg/ml (mean ± SD)	
	IL-4	IL-10
Group (I): Negative Control	37.90±5.51 d	36.76±0.15 d
Group (II): Positive Control	51.50±0.14 c	53.22±2.20 c
Group (III): DNase and P. aeruginosa	84.02 ±1.19 a	66.66±1.66 b
Group (IV): DNase only	65.45 ±0.63 b	86.79±1.68 a

The different letter = high significant difference at p ≤ 0.05, The similar letter = non-significant at p≤ 0.05

Extracellular DNA, also called cell-free DNA, released from dying cells or activated immune cells can be recognized by the immune system as a danger signal causing or enhancing inflammation. The cleavage of extracellular DNA is crucial for limiting the inflammatory response and maintaining homeostasis. Deoxyribonucleases as enzymes that degrade DNA are hypothesized to play a key role in process as a determinant of the variable concentration of extracellular DNA [18]. Interleukin-4 and IL-10 are pleiotropic anti-inflammatory cytokines that function mainly by suppressing the proinflammatory milieu [19], when DNase degrades DNA in the abscess or NETs, the degradation products, monophosphate nucleotides, become substrate for another enzyme, adenosine synthase A (AdsA) that converts the degraded DNA to deoxyadenosine, which induces caspase-3 activation, leading to apoptosis of macrophages surrounding the abscess or the NET [19]. Gao et al. (2019), [20] showed that deoxyadenosine is one of the major bioactive metabolites that possesses multiple beneficial effects of anti-inflammatory, anti-cancer, anti-viral, and anti-fungal activities in addition and Deoxyadenosine enhanced the expression levels of IL-4 and IL-10, and decreased the expression levels of IL-1β and TNF-α.

Arthus and Skin Delayed Hypersensitivity Reactions

Table (3) exhibit the index of Arthus reactions where the group 5 that has been injected with both DNase and *P. aeruginosa* gave the highest values in comparison with other groups as in group 5 (4.50±0.00).

Table (3): Results of Arthus reaction/mm in studied groups

Treated Groups	Mean± S.D
	After 4 hrs.
Group (I): Negative Control	3.12±0.17 [b]
Group (II): Positive Control	4.00±0.00 [ab]
Group (III): DNase and *P. aeruginosa*	4.50±0.70 [a]
Group (IV): DNase only	4.25±1.060 [ab]

The different letter = high significant difference at $p \leq 0.05$, The similar letter = non-significant at $p \leq 0.05$

Arthus involves the in situ formation of antigen-antibody complexes after the injection of an antigen with high levels of circulating antibodies. It manifests as local vasculitis due to the deposition of IgG-based immune complexes in dermal blood vessels [21].

In DTH the highest values after 24 hrs were positive control group and in group that have been injected with both DNase and *P. aeruginosa* (5.00±0.00) and (4.50±0.70) respectively while after 48 hrs, the values of these groups were decreased table (4).

Table 4: Delayed hypersensitivity reaction after 24 and 48 hrs. in the studied groups

Treated Groups	Mean± S.D	
	After 24 hrs.	After 48 hrs.
Group (I): Negative Control	3.12±0.17 d	3.12±0.17 cd
Group (II): Positive Control	4.00±0.00 c	3.2±0.355 abc
Group (III): DNase and P. aeruginosa	5.00±0.00 ab	4.00±0.00 ab
Group (IV): DNase only	4.50±0.70 bc	3.75±0.35 b

The different letter = high significant difference at $p \leq 0.05$, The similar letter = non-significant at $p \leq 0.05$

During the sensitization phase, the host's antigen presenting cells (APC) take up, process, and present antigenic peptides to T-cells at the site of infection. Sensitized T-cells formed as a result of the initial exposure, and, upon secondary challenge with the same antigen, a vigorous response by the sensitized Tcells accompanied with the release of several cytokines and tissue swelling. Because DTH relies on antigen processing by APC and subsequent presentation to T-cells at least 24–72 h are needed for the response to occur, which may take only minutes to manifest [22]. DNase has been shown to increase the production of IL-4 that enhances Th2 immunity by inhibiting Th1 responses through the repression of IL-12 signaling [19]. Classically, IL-4 drives CD4+ T cell polarization in the Th2 phenotype together with suppression of interferon (IFN)-γ– producing Th1 cells [23].

Conclusion

The purified DNase had immunostimulating activities on the murine immune system. This effect is reflected by phagocytic activity, Arthus and DTH reactions. Also had immunomodulator effect with significant differences (P <0.05) in elevated level (IL-10 and IL-4).

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