RESEARCH ARTICLE

The Study Around Effects Cell Wall of *Streptococcus pyogenes* on the Immune System Response

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Received: 23rd December, 2022; Revised: 16th January, 2023; Accepted: 03rd February, 2023; Available Online: 25th March, 2023

ABSTRACT

Many of the most common diseases affecting humans are caused by *Streptococcus*. The study of the cell wall of these bacteria is directly related to the immune response. In our study, about 20 throat swabs were collected for children aged 5 to 15 years, according to the procedures and protocol of the World Health Organization (WHO), to search for *Streptococcus pyogenes.* We cultured bacteria on blood agar media and then used HA and HAI to obtain the main component of the bacteria wall, peptidoglycan, to determine its immune response. Whereas the study we carried out proved that the injection of Ags S/C after one month and collecting serum from these labs gives titers in group 1 1:1280 and in group 2 1:640, indicating that antigens are highly virulence (complex antigens), composed of peptides and carbohydrates.

All samples we tested gave positive results and generated an immune response to the bacteria's cell wall peptidoglycan material.

Keywords: *Streptococcus, Streptococcus pyogenes*, Immunity, Cell wall, Ags S/C.

International Journal of Drug Delivery Technology (2023); DOI: 10.25258/ijddt.13.1.48

How to cite this article: Hussain MJA, Mahmoud FA, Mshachal MA. The Study Around Effects Cell Wall of *Streptococcus pyogenes* on the Immune System Response. International Journal of Drug Delivery Technology. 2023;13(1):297-299.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Streptococcus bacteria are mainly found in the skin and in the digestive and respiratory tracts of humans.[1] *Streptococcus* is gram-positive and grows in facultative anaerobic conditions. *Streptococcus* causes many diseases to humans, especially in children aged between 5–15 years.[1,2] *S. pyogenes* known as group A Streptococci, is considered as the causative agent of different diseases.[3] Mild pharyngitis, impetigo and post-streptococcal sequelae are examples of GAS infections, which is characterized by a variety of clinical symptoms.[4,5]

Immunological studies represent a favorable method for the detection of GAS.[6] These methods are very important and depend on the activity of the cellular components of the immune system, which include macrophages, dendritic cells, and neutrophils.[7,8]

Some modern methods have been conducted in the USA to extract and prepare peptidoglycan from the *S. pyogenes* cell wall .strain Sv (Group A, M type 3) was used for this purpose.[9,10]

As the main purpose of this study, as we mentioned previously, is to know the relationship between the components of the cell wall of *S. pyogenes* and the emergence of the immune response against it and measure the titer of antibodies in laboratory animals.

MATERIALS AND METHODS

Collection of Samples

Where throat swabs were taken from pediatric patients aged 5 to 15 years at Sheikh Zayed Hospital in Baghdad.

Culture Media

The swabs were cultured on sheep blood agar, considered an enriched medium as it contains 5% of sheep blood and tri metho prime-sulfametho xazole as a nutritional supplement for bacteria. Then we put the plates in the incubator at 37°C for 24 hours. Then the bacterial colonies, which appeared in small grayish-white color, as well as complete lysis of blood and from beta type, were tested for the bacteria's possession of streptolysin enzymes, as well as the bacteria were gram-positive, catalase-negative, and also sensitive to bacteria.[11,12]

Preparation of Antigen Dilutions

One pure colony + 1-mL broth (nutrient broth) cultured at 37°C for 24 hours. Re-subculture in blood agar to be sure no contamination.[17] Repeat this procedure 1-mL broth growth +9 mL nutrient-broth to give 10mL growth and recheck similarly. 10 mL growth + 90 mL nutrient broth to give 100 mL growth of bacteria, 100 mL broth growth + 900 mL nutrient broth to gives one liter of bacteria growth.[2,13]

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Then lyophilize after the breakdown of cells by ultrasonic and get cell walls and separate them by biochemical levels to get peptidoglycan.

Take 4 mg peptidoglycan and dissolve it in 1-L of distilled water.

Animals Lab. Grouping

group 1: S/C injection 15 mL of antigen.
group 2: S/C injection 8 mL of antigen.
group 3: control group (non-injected).
After 1-month collect serum from these lab animals to be examined by HA and HAI.

Principle HA and HAI (Hemagglutination and Hemagglutination Inhibition)

An antigen-encoded surface protein of RBC (sheep) agglutinates them and settles irregularly at bottom of the tube or microliter plate formation (lattice particles).[14] Here in this study, the antigen used is called peptidoglycan, which is hung to surface of RBC and formation lattice (hemagglutination).[14-16]The identification of immune response of the lab-animals by hemagglutination and hemagglutination inhibition (HA and HAI).[15,16]

The basis of the HAI are antibodies formed in response to Ag that will prevent attach of Ags to RBC (HAI).[15,16]

- HAI liter

The highest dilution of antibodies that prevent agglutination:

- The serum did not contain antibodies that react with peptidoglycan, as seen in the wells (Hemagglutination).
- The antibodies will react with Ags (Hemagglutination inhibition) as demonstrated.[14,16]

Procedure

- RBC used from sheep mixed with heparin to prevent lysis RBC.
- Bovine albumin is used as a diluent.
- Preparation of Ags as mentioned.
- Preparation of 2-fold dilution of lab serum with diluent from (200 mL diluent for each well + 200 mL serum and make 2-fold dilution) 1:10, 1:20, 1:40, up to 1:1280.
- Add peptidoglycan (Ags) fixed to all wells except controls. (10 mL of Ags).
- Incubate at room temperature for 60 minutes at 37°C.[14,16]

Add guinea pig RBC and incubate in refrigerator for 24 hours (25 mL of sheep RBC), as shown in (Figure 1).

RESULTS AND DISCUSSION

The results of all swaps were positive for *S. pyogenes* bacteria, which includes complete lysis of blood and from beta type, through streptolysin enzymes. The bacteria were gram-positive, catalase-negative, and sensitive to bacteria.

Where the injection of Ags S/C after one month and collecting serum from these lab gave titers in group 1 and group 2 .

- It was found group 1 S/C injection 15 mL for each one gave titer of 1:1280.
- S/C injection 8 mL for each group 2 gave titer 1: 640

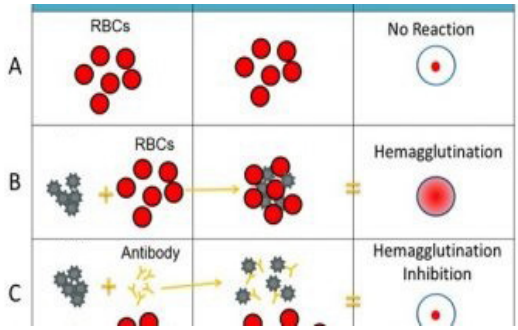

Figure 1: Passive Agglutination –Haemagglutination inhibition[17]

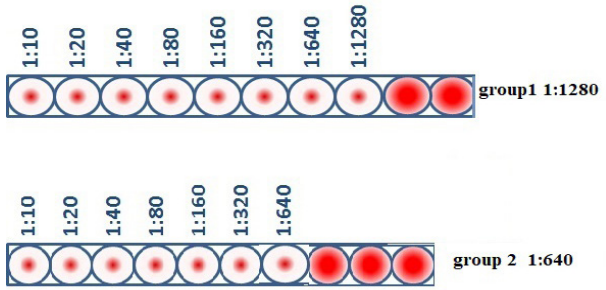

Figure 2: Serial dilution of serum for Rabbit and Guinea pig with Specific Ags.[18]

- It is found that S/C injection of group 1 gave immune response liter higher than group1, as shown in (Figure 2).
- Titer of group 1 more than group 2 may be due to highly immunogenic.

Complement proteins and antibodies act to attack to the bacteria when the bacteria are inside the host.[14,19]

The scientific study showed that the injection of Ags S/C after one month, the collecting serum from the rabbit and guinea pig gave titers as follows: in Rabbit 1:1280 and in guinea pig 1:640. The injection leads to stimulating a humoral immune response (macrophages). The macrophage major histocompatibility complex (MHCII) presents the antigen to the helper T cell and converts it to an activated cell. The activated helper T cell produces interleukin 1, leading to B cell activation.[20] The activated B cell is converted to a plasma cell and produces (interleukin 4 for proliferation and interleukin 5 for differentiation), and leads to the production of specific antibodies.[21] The difference between titer antibodies of rabbits and guinea pigs depends on viability of the immune system and memory cells.[22]

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