Short Communication

Forkhead Box Protein-3 Gene Expression of Purified Human Peripheral Blood Mononuclear Cells Treated with Methicillin-Resistant *Staphylococcus aureus*-Somatic Antigens and Hepatitis B Virus Vaccine

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Abstract

Background: Optimal hepatitis B virus (HBV) vaccine efficacy linked to producing systemic and mucosal antibodies and cellular immunological memory requires a complete set of immunological responses. Numerous variables affect how strong the immunological response is important variables include the vaccine's kind and any additional adjuvants, which can dramatically enhance antigen presentation and cell activation. **Objectives:** This study aimed to illustrate the purified human peripheral blood mononuclear cells' immunological response against somatic antigens of methicillin-resistant *Staphylococcus aureus* (MRSA) and to study the immunodulatory effect of HBV antigens on this immune response represented by gene expression of forkhead box protein-3 (FOXP3) as a regulatory gene for immune cells. **Materials and Methods:** Case-control research was conducted on 75 peripheral blood mononuclear cells (PBMCs) samples. This work involved five experimental groups each group involving 15 samples. Group I involves separated PBMCs (1×10^6 cells/mL) alone as a control; Group II involves PBMCs stimulated with killed somatic MRSA antigen; Group III of PBMCs were stimulated with HBV vaccine only; Group IV involves PBMCs pretreated with HBV vaccine for 48 hr, and then with killed somatic MRSA antigen. Group V involves PBMCs stimulated with mixed of killed somatic MRSA antigen and HBV vaccine. The immune response against MRSA somatic antigens was assessed by the genetic parameter was done to detect the level of FOXP3 mRNA gene expression by real-time polymerase chain reaction (PCR). **Results:** The results of relative gene expression in the ($P < 0.05$) FOXP3 gene showed that the mean concentration of FOXP3 gene expression level was significantly increased in Group II, Group III, Group IV, and Group V, respectively, as compared to Group I. **Conclusion:** MRSA-somatic antigens and HBV vaccine are significantly increased the regulatory FOXP3 gene expression as an indicator for increasing the immune response.

Keywords: FOXP3 gene, HBV vaccine, MRSA, somatic antigens

Introduction

Forkhead Box Protein 3 (FOXP3) is a regulatory T cell transcription factor that plays an important function in the body's immune system balance. FOXP3+ regulatory T (Treg) cells have pleiotropic immune-regulatory functions that are important for immunological homeostasis, autoimmunity prevention and the control of inflammatory reactions caused by pathogens. T-cell receptor signaling plays crucial roles in Treg differentiation and FOXP3-mediated gene regulation. The transcription factor FOXP3 regulates Treg cell formation, differentiation, and function.[1]

The deficiency in, the FOXP3 gene, results in hyper-activation of CD4+ T cells, overproduction, of pro-inflammatory, cytokines, and massive, multi-organ

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pathology. FOXP3-expressing regulatory T cells (Tregs) have a role in both the beneficial reduction of immunological disease and the down-regulation of protective immune responses to infection.[1] As a reliable marker for cells with a suppressor function, FOXP3 has been proven to have a direct role in causing immune-suppression. As FOXP3 is also expressed in other cells (such as CD8+), with a suppressor role, it has been demonstrated in more recent investigations that these cells in people are not strictly CD4+ CD25, high naturally existing Tregs.[2]

This study aimed to investigate the purified human peripheral blood mononuclear cells' immunological response against somatic antigens of MRSA and to study the immunodulatory effect of HBV antigens on this immune response represented by gene expression of FOXP3 as a regulatory gene for immune cells.

Materials and Methods

Study design

Case-control study enrolled on 75 samples subdivided into five groups. Blood samples (5 mL) were collected aseptically by vein puncture from apparently healthy 15 males age range (20-35 years), and collected in anticoagulant tubes containing heparin. Blood samples left for 15 min to cool to room temperature before peripheral blood mononuclear cell (PBMC) separation using density gradient centrifugation.[3]

Data collection tools

Blood samples were taken from apparently healthy male subjects. An experimental analytical randomized controlled trial was conducted. All subjects involved in this work were not suffering from any health problems and did not receive any drugs. The excluded criteria included any person who had an infection or disease.

Testing groups

Purified human PBMCs separated from blood samples were cultured on RPMI1640 medium, and then each sample was split into five groups, as shown in Table 1.

Ethical approval

The research was done in accordance with the ethical guidelines found the Helsinki Declaration. Before taking a sample, the agreement was obtained verbally and in writing of every participant. According to document number 28, on June 28, 2023, a local ethics committee of Babylon medical college evaluated and approved the research protocol, subject information, and permission form.

Table 1: The testing groups in this study

Testing groups	No.	Treatment
Group I	15	PBMCs only.
Group II	15	PBMCs + killed MRSA antigen.
Group III	15	PBMCs + hepatitis B virus vaccine.
Group IV	15	PBMCs + pretreatment with HBV vaccine for 48hrs,then killed MRSA Ag added.
Group V	15	PBMCs + mix(MRSA Ag and HBV vaccine).

FOXP3 gene expression

The relative expression of the FOXP3 gene in volunteer PBMCs Samples were calculated based on Livak Method 2-(ΔΔCT), which normalizes quantitative reverse transcription polymerase chain reaction (RT-qPCR) (CT values) of tested genes using (GAPDH) as a reference gene in control and treatment groups.

Statistical analysis

IBM SPSS Statistics for Windows, Version 2010 (IBM Corp., Armonk, New York, USA) and Microsoft Excel (2010, Microsoft Corp., Armonk, New York, USA) were used for all statistical computations. According to reference,[4] a P value of 0.05 was deemed statistically significant.

Results

FOXP3 gene expression concentration expressed in Table 2 showed a significantly ($P < 0.05$) immunomodulation effect of MRSA-somatic antigens and HBV antigens on FOXP3 mRNA gene expression as indicated in the increasing the FOXP3 concentrations of the Ag-treated groups (II, III, IV, and V respectively) after 48 h of induction in comparison to Group I.

The highest concentration of FOXP3 mRNA gene expression was in Group V which includes PBMCs treated with both MRSA-somatic antigens and HBV antigens.

The results indicated a significant increasing in the concentration of FOXP3 in Group V as compared to Group II as well as between Group IV and Group II [Figures 1 and 2].

Group V exhibits a significant increasing in the FOXP3 gene expression as compared to Group IV [Figure 3].

Table 2: Concentrations of FOXP3 gene expression levels in study groups

Study group*	Concentration (pg/mL)	*P* value**
Group I	0.4871	<0.05
Group II	1.7980	<0.05
Group III	2.2468	<0.05
Group IV	4.3950	<0.05
Group V	6.3178	<0.05

*Group I (without treatment), Group II treated with MRSA somatic antigens only, Group III treated with HBV antigen only, Group IV pretreated with HBV then treated with MRSA antigens, and Group V treated with mixed of HBV antigen and MRSA somatic antigen

**Significant differences between treated group with untreated (Group I)

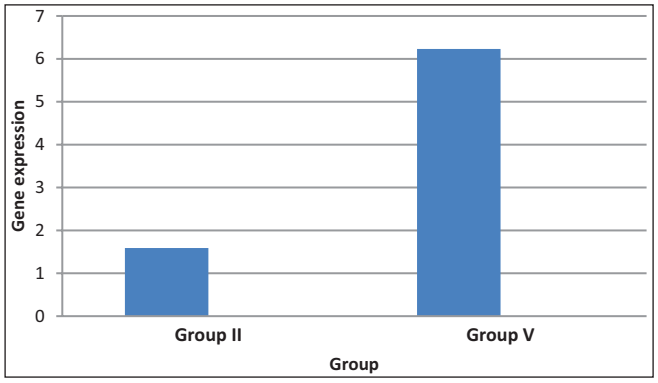

Figure 1: The correlation between Group II treated with MRSA somatic antigens only and Group V treated with mixed of HBV antigen and MRSA somatic antigen

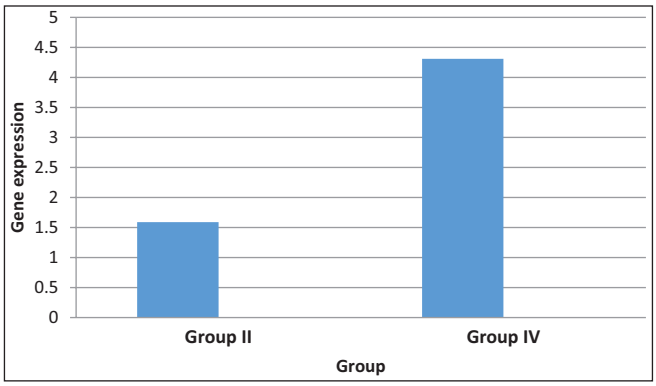

Figure 2: The correlation between Group II treated with MRSA somatic antigens only and Group IV pretreated with HBV, then treated with MRSA antigens

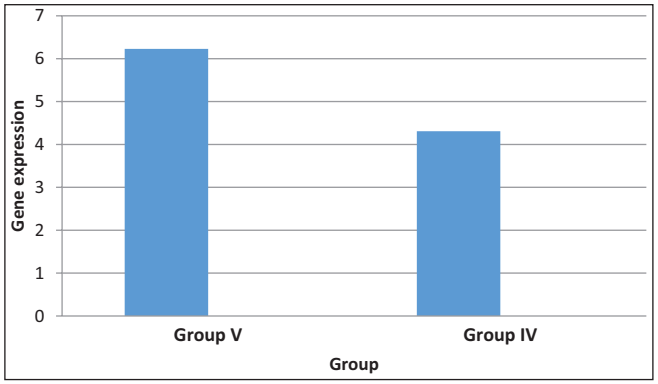

Figure 3: The correlation between Group V treated with mixed of (HBV antigen and MRSA somatic antigen). Group IV pretreated with HBV then treated with MRSA antigens

Discussion

The most precise molecular indicator of natural Tregs (nTregs), FOXP3, is associated with the immunosuppressive function of CD4+CD25+ Tregs. For CD4+CD25+ Tregs to mature and operate, FOXP3 expression is necessary. Peripheral Tregs, specifically CD4+CD25+ FOXP3+ Tregs, closely control B and T cell responses as well as auto-reactive responses. The most well-known immune cell type, FOXP3+ Tregs have the most potent inhibitory mechanism and a wide range of inhibitory targets. The prevalence of autoimmune and allergy illnesses is decreased, and FOXP3+ Tregs have anti-inflammatory capabilities.[5]

The most significant immuno-suppressive cells are without a doubt regulatory T cells (Treg), which express the transcription factor FOXP3. For the preservation and proper operation of Treg, the transcription factor FOXP3 is essential. It determines lineage during development.[6] Their proper growth and operation are essential for building peripheral (self-) tolerance and, consequently, for avoiding autoimmune disorders and immunological reactions to benign antigens or commensals. Treg can regulate immune responses to infections or sterile inflammatory illnesses to curb overreacting immune responses, (re-)establish immunological homeostasis, and guard against needless tissue damage. Treg can also aid in tissue healing. Because self-tolerance breaks broken, a variety of multiorgan autoinflammatory disorders are caused by Treg that are either under- or over-produced.[7]

Developments in immunology and molecular biology have revealed that asthma is not only associated with the imbalance of Th1/Th2 function[8] but also with Tregs, because imbalances in forkhead transcription factor P3 (FOXP3)+ Treg/Th17 and Th2/FOXP3+ Treg cells lead to asthma.[5] Tregs are a subset of CD4+ T cells that play an essential role in maintaining peripheral immune tolerance and controlling allergic diseases. Tregs, together with effector T cells (Teffs), cytokines, immune antibodies, and other cellular components, play an important role in maintaining immune balance.[9] As important immunosuppressive cells, CD4+CD25+ Tregs act in cell-cell contact-dependent inhibition patterns and ultimately inhibit immune diseases by inhibiting helper T cell activation and differentiation, and directly inhibiting B cell activation to produce antibodies.[10] FOXP3 expression is regulated by DNA methylation, histone modifications and posttranscriptional modifications.[11] The epigenetic regulation and methylation of FOXP3 play an important role in its stable expression.[12] Changes in the methylation level of the FOXP3 gene may affect Treg differentiation and regulate the occurrence of an immune response. FOXP3 is a key regulator of Treg formation and function. Demethylation at the CpG-rich island of FOXP3 upstream enhancers can alter FOXP3 expression, and may affect Treg function and response against triggering antigens.[13]

Conclusion

Thus FOXP3 gene expression is significantly increasing in Group V indicating positive effect for antigens of HBV on the immune response of PBMCs against MRSA antigen.

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Conflicts of interest
There are no conflicts of interest.

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