ORIGINAL ARTICLE

Meta-Analysis of Human Molecular Responses to Staphylococcus Aureus Components

Sidra Younis[1,3,5*], Farah Deeba[2], Syeda Mehpara Farhat[1], Mahwish Ali[1], Qamar Javed[3,4], Miroslav Blumenberg[5]

ABSTRACT

Objective: This study is aimed to identify genes and pathways that are upregulated or downregulated by *Staphylococcus aureus* components using meta-analysis.

Study Design: Meta-analysis of microarray Data.

Place and Duration of Study: The study was conducted at R.O. Perelman Department of Dermatology, NYU Langone Medical Center, New York, USA, from January 2015 to March 2015.

Materials and Methods: Public repository "GEO Datasets" was searched using key term "*Staphylococcus aureus*" for data sets covering effects of *S. aureus* infection in *Homo sapiens* cells. Meta-analysis was performed using microarray data for immune cell responses to S. aureus components and analyzed using RankProd, RMAExpress, and DAVID software.

Results: The secreted factors from biofilm and planktonic cultures predominantly induce adaptive immune process and suppress mitotic cell cycle. The biofilms conditioned media treated keratinocytes upregulate anti-apoptosis genes and immunity while planktonic cultures conditioned media treated keratinocytes upregulate cell cycle as major cytoprotective process. Similar to the secreted factors from *S. aureus* cultures, superantigens induce adaptive immunity and suppress innate immunity in challenged cells. *S. aureus* components Panton Valentine Leukocidin (PVL) and iPVL induce adaptive immune system as a defensive mechanism. Importantly, these *S. aureus* components increased microbicidal activity in host cells.

Conclusion: PVL could be a potential priming agent for myeloid cells against virulent *S. aureus* infections. Further investigations into bactericidal ability of PVL will provide efficient therapy against community-associated Meticillin-resistant *Staphylococcus aureus* (CA-MRSA) infections.

Keywords: *Biofilms, Meta-Analysis, Panton Valentine Leukocidin, Staphylococcus Aureus.*

How to cite this: *Younis S, Deeba F, Farhat SM, Ali M, Javed Q, Blumenberg M. Meta-Analysis of Human Molecular Responses to Staphylococcus Aureus Components. Life and Science. 2023; 4(3): 197-215. doi: http://doi.org/10.37185/LnS.1.1.232*

[1]*Department of Biological Sciences*
National University of Medical Sciences, Rawalpindi, Pakistan
[2]*Department of Biochemistry and Biotechnology*
The Women University, Multan, Pakistan
[3]*Department of Biochemistry*
Quaid-i-Azam University, Islamabad, Pakistan
[4]*School of Life Sciences*
University of Bedfordshire, United Kingdom
[5]*The R.O.Perelman Department of Dermatology*
Department of Biochemistry and Molecular Pharmacology,
NYU Langone Medical Center, New York, USA

Correspondence:
Dr. Sidra Younis
Associate Professor, Biological Sciences
National University of Medical Sciences, Rawalpindi, Pakistan
E-mail: sidra.younis@numspak.edu.pk

Funding Source: NIL; Conflict of Interest: NIL
Received: Sep 29, 2021; Revised: Apr 05, 2023
Accepted: Apr 07, 2023

Introduction

Staphylococcus aureus (*S. aureus*) is an important infectious agent in humans responsible for nosocomial and hospital acquired infections leading to high morbidity and mortality. *S. aureus* secretes disease-causing toxins *e.g.* superantigens, exfoliative and membrane-active toxins including Panton Valentine leukocidin (PVL). There are 19 known *S. aureus* super-antigens including toxic shock syndrome toxin (TSST-1), staphylococcal enterotoxins (SEG-SEJ, SEASEE) and the staphylococcal enterotoxin-like toxins (SElU, SElK-SElR). Superantigens cause toxic shock syndrome and also contribute to septic shock.[1] Peripheral Blood Mononuclear Cells (PBMC's) form major cellular

fraction of the innate immunity and play primary role in combating *S. aureus* infection. PVL forms pores within the plasma membrane of peripheral blood mononuclear cells (PBMC's) and may contribute to lysis of PBMC's.[2]

S. aureus biofilms have been found in chronic skin ulcers. These biofilms are unique in their phenotype, formation of complex structures and release of the toxins in extracellular matrix than their planktonic counterparts. The biofilms are more resistant to antibiotics and initiate a different immune response. The regulatory system that controls genes for biofilm formation also controls expression of virulence factors, that directly effects human cells.[3] In patients with comorbidities, wounded epithelium is highly susceptible to colonization and biofilm formation. The host immune system is not very effective in clearing the infections from infected chronic wounds and are also antibiotic resistant.[3]

S. aureus secretes enterotoxin gene cluster (egc) and non-egc super-antigens during different phases of its interaction with the human host. The cells exposed to egc super-antigens produce different cellular response as compared to the cellular population exposed to non-egc super-antigens. The non-egc super-antigens may induce adaptive immune response in the cells. Most of the studies showed that egc and non-egc super-antigens are mostly similar in inducing immunity processes for example gene expression, cytokine production and secretion, T cells activation but their release from bacteria is regulated differently.[1]

S. aureus and associated toxins are recognized by a number of surface and sub-cellular receptors in host cells, such as Toll-like receptors (TLR), RIG and NOD like receptors. The activation of these receptors and costimulatory molecules results in cascades of reactions which lead to activation of NFkB or IFNγ through MyD88-dependent or independent pathways. Subsequent production of cytokines, chemokines and complement molecules activate either innate or adaptive immune system, based on triggering factor. Additionally, pathogen-containing phagosomes are processed by endoplasmic reticulum and presented as peptides on the surface of antigen presenting cells as a complex with major histocompatibility molecules (MHCI or MHCII) or heat shock proteins. Some heat shock proteins can process pathogen specific peptides independent of endoplasmic reticulum and Golgi apparatus, thus giving extra advantages in antigen presentation and activation of innate immune specific proteins and pathways.[4-6]

Although considerable information is available about *S. aureus* components mediate immune responses and signaling. However, the regulatory molecules that may be targeted are still obscured. To explore this problem, we performed meta-analysis on the microarray data available on public repositories to compare the differential gene expression to *S. aureus* components.

Materials and Methods

We have compared transcription differences between untreated (control) cells *vs.* those challenged with *S. aureus* biofilm *vs. S. aureus* planktonic products, and the specific effects of the *S. aureus* super-antigens and PVL-challenged cells. We have also combined the data from previously published study[7] and current study for global analysis of the *S. aureus* and its components effects on the human epithelial and immune cells.

Searching for the Meta-analysis-appropriate studies in public repositories

Public repository "GEO Datasets" was searched using key term *"Staphylococcus aureus"* for data sets covering effects of *S. aureus* infection in *Homo sapiens* cells. We selected studies comprising effects of secreted components from *S. aureus* cultures in epithelial or immune cells. The cells were treated for different time periods.

Expression analysis in cells challenged with *S. aureus*-secreted components

This analysis included 4 studies, 9 data sets, 87 microarrays and 22276 genes. Affymetrix platforms GPL570 and GPL571 were used in this analysis. Differential expression was observed in leukocytes, PBMC's and keratinocytes challenged with *S. aureus* PVL, inactive PVL (iPVL), superantigens, and cultures from biofilms and planktonic *S. aureus*. Cluster analysis using Multiple expression Viewer software (MeV) for average difference between controls and challenged microarrays for individual data sets indicated that biofilm and planktonic cultures-treated samples cluster together, but separately

from the *S. aureus* PVL- and super-antigens treated samples. Hence, these analyses were separated into sub-groups. We separately compared 2 data sets challenged with super-antigens, containing 12 microarrays and 54675 genes. For analysis of PVL and iPVL comparison, 2 data sets 42 microarrays and 54675 were combined and processed (Figure 1).

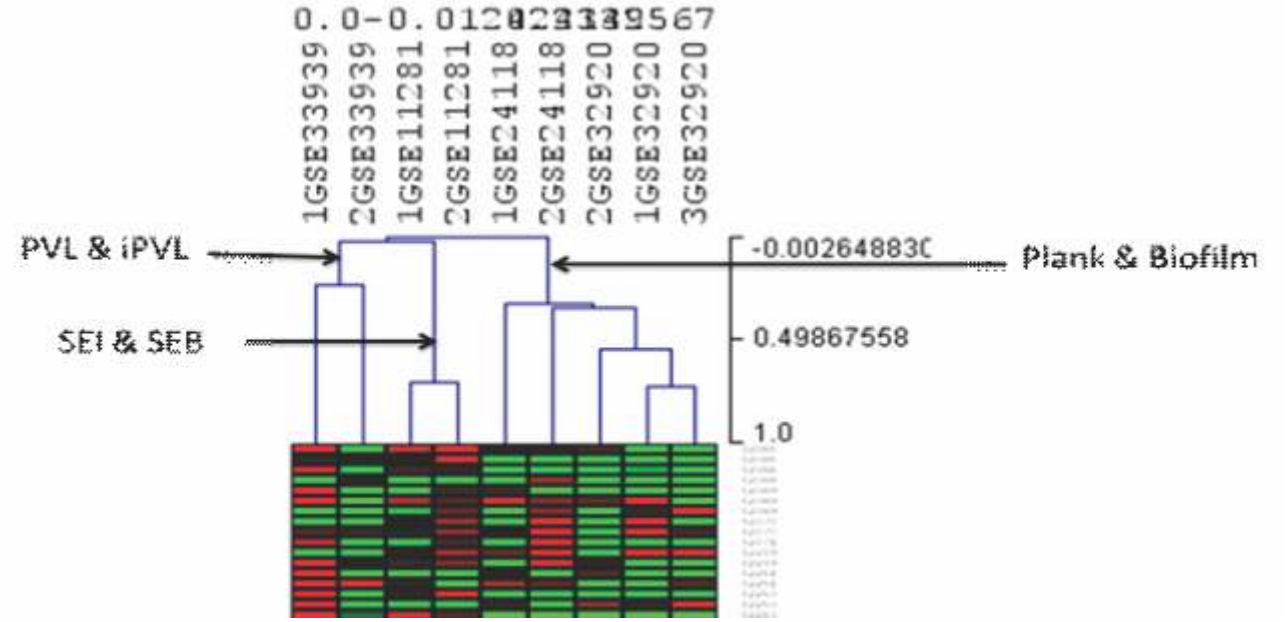


Fig A.1: Data sets selection for RankProd analysis of *S. aureus* components challenged *vs.* control human cells (PVL & iPVL, S. aureus cytotoxin; SEI & SEB, S. aureus superantigen; Plank & Biofilm, Secreted factors from S. aureus planktonic and biofilm cultures)

In biofilm-planktonic combined analysis we analyzed 5 data sets, 33 microarrays containing 20697 genes. Biofilm and planktonic cultures-treated microarrays were also checked in separate analyses. In biofilm-challenged microarrays analysis, we analyzed 2 data sets and 12 microarrays containing 22277 genes. For planktonic culture-challenged analysis, we checked 3 data sets, 21 microarrays and 22277 genes. Biofilm-challenged microarrays *vs.* planktonic cultures-treated ones were also compared in a separate analysis. This analysis did not include non-challenged, control microarrays and was performed on 2 data sets, 18 microarrays and 22277 genes altogether (Table A.1).

Table A.1 Data sets used in comparison of S. aureus components challenged vs. control cells

Sr.	Acc. No.	MA C+T	Cell type	*S. aureus* component	Platform
i) Biofilm + planktonic					
1a	GSE32920	3+3	keratinocytes	Biofilm secreted factors	GPL571 Affymetrix
1b	GSE24118	3+3	keratinocytes	Biofilm secreted factors	GPL571 Affymetrix
1c	GSE24118	3+3	keratinocytes	Planktonic secreted factors	GPL571 Affymetrix
1d	GSE32920	6+3	keratinocytes	Planktonic secreted factors	GPL571 Affymetrix
1e	GSE32920	3+3	keratinocytes	Planktonic secreted factors	GPL571 Affymetrix
Total		33 MA	22,277 Genes		
ii) Biofilm *vs.* planktonic					
2a	GSE32920+24118	6+12	keratinocytes	Biofilm *vs.* Planktonic	GPL571 Affymetrix
Total		18 MA	22,277 Genes		
iii) Super-antigens					
3a	GSE11281	3+3	PBMCs	*S. aureus* SEB	GPL570 Affymetrix

3b	GSE11281	3+3	PBMCs	*S. aureus* SEI	GPL570 Affymetrix
Total		12 MA	54675 Genes		
iv) Cytotoxin					
4a	GSE33939	12+9	PMNL	*S. aureus* PVL	GPL570 Affymetrix
4b	GSE33939	12+9	PMNL	*S. aureus* iPVL	GPL570 Affymetrix
Total		42 MA	54675 Genes		

GSE, Data series; MA, Microarrays; C, Control; T, Treated; PMNL, Polymorphonuclear cells; PBMCs, Peripheral blood mononuclear cell, S. aureus, Staphylococcus aureus; PVL, Panton Valentine Leukocidin; iPVL, Inactivated Panton Valentine Leukocidin; SEB, S. aureus enterotoxin B; SEI, S. aureus enterotoxin I; GPL, Gene platform

Global analysis of differential expression in *S. aureus*-challenged cells

We have published meta-analysis of human cells responses to live and inactive *S. aureus* and from those with infection.[7] In present study, we included data from current and previous studies. Microarray data was synchronized from 15 studies comprising 24 data sets, 532 microarrays and 15600 genes. These studies used Affymetrix (GPL96, 97, 570, 571, 6106 and 6244) and Illumina (GPL10558) platforms. Overall distribution of studies based on challenging treatments were as following: 7 studies for live *S. aureus*, 2 for heat or UV-inactivated *S. aureus* and 4 for *S. aureus* secreted f actors or components. In 2 studies RNA expression was observed in blood of *S. aureus*-infected patients *vs.* controls. These separate studies were conducted on keratinocytes, epithelial, endothelial, immunocyte and hepatocellular carcinoma cell lines. The two data sets utilizing the Illumina arrays were not used for the global comparison but only in analyses of specific subsets due to incomplete overlap of raw data in microarrays (Table A.2).

Table A.2 Datasets used in global comparison of *S. aureus* and *S. aureus* components challenged *vs.* control cells

Studies	Acc. No.	MA C+T	Cell type	Challenge	Platform
1	GSE39889	4+4	Neutrophils	*S. aureus*	GPL570 Affymetrix
2	GSE13670	15+15	Macrophages	*S. aureus*	GPL570 Affymetrix
3a	GSE13736	1+5	Endothelial cells	*S. aureus*	GPL570 Affymetrix
3b	GSE13736	1+5	Endothelial cells	*S. aureus*	GPL570 Affymetrix
3c	GSE13736	1+1	Endothelial cells	*S. aureus*	GPL570 Affymetrix
4	GSE16837	20+78	PMNL	*S. aureus*	GPL570 Affymetrix
5	GSE25504	44+17	Blood	*S. aureus*	GPL570 Affymetrix
6	GSE33341	43+32	Blood	*S. aureus*	GPL571 Affymetrix
7	GSE2405	27+3	PMNL	*S. aureus*	GPL96 Affymetrix
8	GSE16129	10+42	PBMC	*S. aureus*	GPL96, 97 Affymetrix
9a	GSE44943	3+3	HCCC	*S. aureus*	GPL6244 Affymetrix
9b	GSE44943	3+3	HCCC	*S. aureus*	GPL 6244 Affymetrix
10	GSE6802	3+2	Epithelial cells	Inactive *S. aureus*	GPL571 Affymetrix
11a	GSE44720	15+15	Dendritic cells	Inactive *S. aureus*	GPL10558 Illumina
11b	GSE44720	15+15	Dendritic cells	Inactive *S. aureus*	GPL10558 Illumina
12a	GSE33939	12+9	PMNL	*S. aureus* PVL	GPL570 Affymetrix
12b	GSE33939	12+9	PMNL	*S. aureus* inactive PVL	GPL570 Affymetrix
13a	GSE11281	3+3	PBMCs	*S. aureus* super-antigen SEB	GPL570 Affymetrix

13b	GSE11281	3+3	PBMCs	*S. aureus* super-antigen SEI	GPL570 Affymetrix
14a	GSE24118	3+3	keratinocytes	Secreted Factors *S .aureus* Biofilm	GPL571 Affymetrix
14b	GSE24118	3+3	keratinocytes	Secreted factors from Planktonic Cultures	GPL571 Affymetrix
15a	GSE32920	3+3	keratinocytes	Secreted Factors *S .aureus* Biofilm	GPL571 Affymetrix
15b	GSE32920	6+3	keratinocytes	Secreted factors from Planktonic Cultures	GPL571 Affymetrix
15c	GSE32920	3+3	keratinocytes	Secreted factors from Planktonic Cultures	GPL571 Affymetrix
Total	532 MA		15600 Genes		

GSE, Data series; MA, Microarray; C, Control; T, Treated; PMNL, Polymorphonuclear cells; HCCC, Hepatocellular carcinoma cell line; PBMCs, Peripheral blood mononuclear cell, *S. aureus*, *Staphylococcus aureus*; PVL, Panton Valentine Leukocidin; SEB, *S. aureus* enterotoxin B; SEI, *S. aureus* enterotoxin I; GPL, Gene platform

Meta-analysis procedure

The data for transcriptional profiling were found deposited in respective gene expression series as CEL or TXT files. RankProd software was used to identify the genes differentially expressed in *S. aureus*-challenged cells with p-values better than 10^{-2}. RankProd analysis for each study group produced a table representing induced or suppressed genes in challenged cells. The DAVID software was used to get tables, charts and clusters for the induced or suppressed genes with p-Value better than 10^{-4} obtained from RankProd output tables.

Results

The number of differentially expressed genes in analyzed groups is given in Figure A.2.

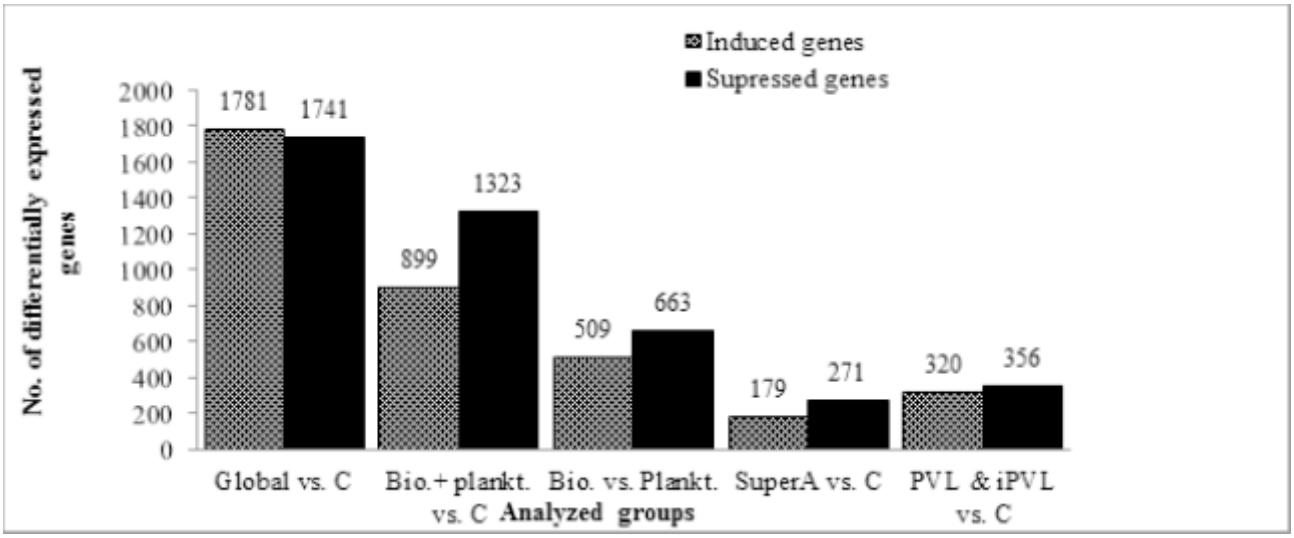


Fig A.2: Number of induced and suppressed genes in analysed groups

Comparison of differential expression in *S. aureus* biofilms and planktonic cultures secreted factors challenged keratinocytes

S. aureus forms surface associated complex communities characterized as biofilms and secretes ECM proteins within these communities. *S. aureus* biofilms are phenotypically different from *S. aureus* planktonic cultures and are more resistant to antibiotics. The *S. aureus* biofilms associated with chronic skin ulcers are important in delaying wound healing process. The regulatory elements for biofilm formation also control the gene expression of virulence factors. The influence of biofilm formation on *S. aureus* virulence is disputable.[8] In following

analysis we have compared the differential expression in the keratinocytes challenged with secreted factors from *S. aureus* biofilms and planktonic cultures through Meta-analysis procedure.

The Table B.1 presented keratinocytes responses to secreted factors from *S. aureus* biofilm and planktonic cultures challenge versus control cells. Principally processes including gene transcription, response to organic substance and program cell death were induced.

Table B.1: Gene ontologies induced and suppressed in bioplanktonic cultures treated keratinocytes

	a) Bioflim planktonic Challenged : induced			b) Bioflim planktonic Challenged : Suppressed	
Sr	**Gene Ontologies**	***p* Value**	**Sr**	**Gene Ontologies**	***p* Value**
1	7.22		1	41.69	
	RNA polyII transcription R.	1.73E-09		nuclear lumen	3.72E-51
	pos. R. of gene expression	2.75E-08		membrane-enclosed lumen	3.70E-49
2	5.85		2	26.78	
	response to organic substance	6.94E-09		nucleolus	7.12E-33
	transcription regular activity	1.33E-07		non-membrane-bounded organelle	8.05E-25
3	5.83		3	10.77	
	RNA polyII R. of transcription	1.73E-09		mitotic cell cycle	4.90E-14
	transcription regular activity	6.49E-08		cell cycle phase	1.01E-12
4	5.57		4	8.49	
	R. of PCD	1.56E-08		RNA processing	9.04E-22
	Neg. R. of apoptosis	1.78E-07		RNA splicing	3.61E-13
5	5.46		5	6.47	
	death	2.06E-06		response to DNA damage	1.21E-08
	PCD	3.93E-06		DNA metabolic process	1.59E-08
6	4.59		6	6.2	
	neg. R. of macromolecule metabolism	1.57E-06		chromosome	1.40E-08
	Neg. R. of biosynthetic	2.80E-06		chromosomal part	6.13E-08
7	3.98		7	5.91	
	response to extracellular stimulus	3.87E-06		RNA complex biogenesis	3.17E-10
	response to nutrient levels	1.05E-05		ncRNA processing	4.47E-08
8	3.49		8	4.55	
	response to inorganic substance	9.78E-07		Cell cycle checkpoint	5.77E-07
	response to ROS	6.55E-04		DNA damage response	1.46E-06
9	3.26		9	4.52	
	tube development	3.32E-05		R. of apoptosis	2.72E-06
	respiratory tube development	8.69E-04		R. of PCD	4.01E-06
10	3.16		10	4.46	
	response to steroid hormone	2.96E-05		transcription	1.97E-06
	response to estradiol	3.28E-03		RNA biosynthetic process	2.78E-06

RNApolyII, RNA polymerase II; R. Regulation; Pos. R., Positive regulation; Neg. R., Negative regulatio3.87e-06n; PCD, Programmed cell death; ROS, Reactive oxygen species

The induced gene ontologies clusters in challenged keratinocytes are presented in Table B.1a.

In 1st (ES 7.22) and 3rd (ES 5.83) clusters, gene ontology such as "RNA polymerase II regulation of transcription" was observed. We found that genes participating in nuclear transcription process e.g., helicases were upregulated. Some mitochondrial genes involved in gene expression were also induced. The genes for myeloid and lymphoid cells activation, development and differentiation were upregulated. The genes for immunity principally B and T-lymphocytes activation as well as antibody production were induced. The regulatory proteins for the immune cells were also induced. Negative regulators of apoptotic process were also found here, favoring stimulation of anti-apoptotic process in challenged cells. In addition, bacterial challenge response genes as TGF-β and TLR downstream signaling genes (FOS, JUN, IL-6, BCL3, NFKBIA and TNF-α) were induced; however, we did not find a single TLR gene in this cluster. Nuclear proteins such as receptors and transcription factors which participate in response to organic substance stimuli were induced. The growth factor VEGFA, important in angiogenesis, and associated proteins were also induced.

In 2nd (ES 5.85), 6th (ES 4.59), 7th (ES 3.98), 8th (ES 3.49), 9th (ES 3.26) and 10th (ES 3.16) clusters gene ontologies for the process of response to organic substance are presented. The bacterium and LPS response proteins which participate in effector responses as regulation of adhesion, internalization, migration of cells, secretion, defense response, NLR pathway (IκB/NF-κB) activation and activation of both innate and adaptive immunity processes were induced, however, genes for adaptive immunity were more prominent. The genes which participate in response to toxins or corticosterone hormone, such as FOS, JUN, CDKN1A, CYP1A1, NR4A2, PRKCA, IL-1β, IL-6 and IL-1R were induced. Furthermore, oxidative stress response genes MAPK signaling pathway and responses to cAMP were induced. The positive regulators of cell cycle and positive as well as negative regulators of apoptosis as caspases and p53 signaling pathway were also upregulated.

The top ten gene ontologies and clusters downregulated in *S. aureus* challenged cells are given in Table B.1b. The suppressed gene clusters including clusters 1 (ES 41.69), 2 (ES 26.78) and 7 (ES 5.91) presented the genes for processes including ribonucleoprotein complex biogenesis and splicing of mRNA specifically rRNA. In addition genes important in transcription process as regulators, histone acetyl-transferases, helicases and transcription terminators were suppressed. The genes for RNA export from cell as well as for tRNA metabolism were presented in these clusters. The cytoskeletal proteins such as actin, microtubules formation and cell migration were also downregulated. As well, genes for DNA repair, cell cycle progression and apoptosis repression were downregulated. Moreover, oxidative stress response genes involving signaling pathways such as MAPK and NGF were suppressed.

In 3rd (ES 10.77), 5th (ES 6.47), 6th (ES 6.20) and 8th (ES 4.55) clusters; gene ontologies such as cell cycle and chromosomes were presented. The gene sets for cell cycle processes including spindle fiber formation, chromosomal organization, mitotic interphase, cell cycle check point and cell cycle regulation were found. The processes that involve ribonucleoprotein complex formation in response to DNA damage were downregulated. In addition, genes for ubiquitin mediated protein degradation were suppressed, positive regulators of cell death, BAX mediated catabolic processes were downregulated. In addition, the repressors of zinc binding transcriptional proteins were also downregulated. In 4th cluster most of the processes which participate in "RNA processing" and "RNA splicing" were found (ES 8.49). The genes for post-transcriptional gene silencing; tRNA, mRNA and rRNA splicing via spliceosome were downregulated.

The 9th gene cluster presented ontological categories "regulation of apoptosis" and "regulation of PCD" (ES 4.52). The innate immunity specific signal transducers for positive regulation of TLR, RLR and NLR pathways specifically IκB/NF-κB cascade were represented here. In addition, adaptive immunity pathway genes as B and T-cells signaling including defense, immune, inflammation, adhesion, vesicle formation and wound healing processes were also downregulated. We found that Rho GTPases, PDGF signaling, positive regulators of taxis, immune

response to organic substance and peptides were suppressed. In 10th cluster ontological category "transcription" and "RNA biosynthesis process" are presented (ES 4.46). Study in detail revealed that mitochondrial promoter based RNA polymerase II-dependent transcription process was principally reduced in keratinocytes challenged with secreted factors from *S. aureus* cultures.

In summary we found that adaptive immunity was dominantly induced. Moreover, responses to molecule of bacterial origin, cell survival and anti-apoptosis processes were upregulated. In addition, apoptosis mediators for example caspase9 as well as p-53 mediated mitochondrial apoptosis were induced. While gene expression, mitotic cell cycle and apoptotic process genes were suppressed.

Comparison of genes induced in keratinocytes challenged with secreted factors from *S. aureus* biofilms *vs.* planktonic cultures

The clusters of gene ontologies induced particularly in keratinocytes challenged with secreted factors from biofilms over those challenged with secreted factors from planktonic cultures are presented in Table B.2a.

Table B.2: Top ontological cate gories induced in biofilm *vs.* planktonic cultures treated keratinocytes

	a) Biofilm Challenged: Induced			b) Planktonic cultures Challenged : Induced	
Sr	**Gene Ontologies**	***P* Value**	**Sr**	**Gene Ontologies**	***P* Value**
1	13.91		1	9.66	
	Nuclear lumen	1.19E-17		transciption	8.97E-14
	Nucleolus	2.40E-09		R. of transciption	1.04E-13
2	8.13		2	6.21	
	Nucleolus	2.40E-09		Neg. R. of cellular biosynthesis	1.95E-08
	non-membrane-boind organelle	1.29E-08		Neg. R. of gene expression	5.26E-08
3	6.51		3	5.54	
	Blood Vessel morphogenesis	1.32E-07		Non membrane-bounded organelle	9.07E-08
	angiogenesis	2.76E-07		microtule cytoskeleton	1.58E-06
4	6.11		4	5.04	
	R. of opoptosis	6.16E-09		nuclear lumen	5.98E-07
	anti-apoptosis	1.68E-06		nucleoplasm part	7.52E-07
5	4.82		5	4.65	
	R. of opoptosis	6.16E-09		RNA polyII transcription R.	4.65E-07
	Pos.R. of opoptosis	1.40E-04		pos. R. of biosynthesis	3.92E-06
6	4.2		6	4.03	
	R. of mRNA transcription	5.48E-09		chromatin organization	3.14E-05
	neg. R. of macromolecule synthesis	7.36E-04		chromatin modification	5.32E-05
7	3.89		7	3.85	
	apoptosis	3.88E-05		transcription cofactor activity	1.84E-06

	PCD	5.20E-05		transcription corepressor activity	3.72E-04
8	3.88		8	3.29	
	nucleoplasm part	2.19E-05		conjugating protein ligase activity	1.80E-05
	nuclear body	6.63E-04		ubiquitin-protein ligase activity	3.43E-05
9	3.65		9	2.86	
	RNA Processing	2.82E-08		zinc ion binding	6.34E-06
	mRNA metabolic process	1.25E-06		transition metal ion binding	4.89E-05
10	2.71		10	2.74	
	R. of binding	6.26E-04		chromosomal part	2.08E-04
	Neg. R. of transcription factor	9.98E-04		nuclear chromosome part	7.04E-03

R. Regulation; Pos. R., Positive regulation; Neg. R., Negative regulation; RNApolyII, RNA polymerase II; PCD, Programmed cell death

In 1st (ES 13.91), 2nd (ES 8.13), 6th(ES 4.20), 8th (ES 3.88), 9th (ES 3.65) and 10th (ES 2.71) clusters ontological categories related to nuclear lumen and nucleolus are presented. These ontologies contained genes for transcription process inside nucleus and specific to thymus. The genes involved in the process of transcription of messenger and ribosomal RNA were induced. The positive regulators of cell and DNA damage response mediated apoptosis were induced. TGF-β signaling pathway, cell cycle, DNA replication and DNA repair proteins were also induced. However, some negative regulators of the transcription were also induced. The signaling genes for innate immune specific cell surface and intracellular receptors were induced. We also found that adhesion and low oxygen response apoptotic stimulators were induced.

In 3rd cluster we found ontological categories such as "blood vessel morphogenesis" and "angiogenesis" (ES 6.51). These categories mainly harbored genes which play role in aggregation of platelets and membrane repair in response to growth factors activity. Similarly, the genes which regulate hemostasis, signaling in wound healing and injury response immuno-attractants (IL-8) were upregulated.

In 4th (ES 6.11), 5th (ES 4.82) and 7th (ES 3.89) clusters the genes for the apoptosis regulation were induced. The positive and negative regulators of macromolecule biosynthesis, apoptosis and immune system processes including myeloid cell differentiation, chemotaxis, adhesion, TLR/ NLR pathway, signaling molecules (MAP kinases), hemopoiesis and wound healing genes were upregulated.

The genes induced in keratinocytes challenged with secreted factors from *S. aureus* planktonic cultures are given in the Table B.2b.

The top ten clusters presented two main processes gene expression and chromosomes. In addition, fewer genes for protein degradation, cytoskeleton formation and ion transport were also induced. The 1st (ES 9.66), 2nd (ES 6.21), 4th (ES 5.04), 5th (ES 4.65), 7th (ES 3.85), 8th (ES 3.29) and 9th (ES 2.86) clusters presented ontological categories directly related to the nucleus confined process of transcription regulation. The first cluster largely presented the genes for the transcription, its regulators, post-translational modifiers and zinc finger transcription factors. In addition, DNA helicases and histone modification proteins were induced. The genes required to maintain structure of nucleolus and events in initiation of transcription processes during cell cycle were also found in these clusters. Ankyrin and KH domain containing proteins which inhibit the process of translation by preventing eIF4F complex formation and thus functioning as anti-apoptosis process were induced. The genes for lymphoid cells

formation were also seen in this cluster.

In 3rd (ES 5.54) and 6th (ES 4.03) clusters ontological categories "intra cellular non-membrane bound organelles", "microtubule cytoskeleton", "chromatin organization" and "chromatin modification" were found. The genes for chromosomal arrangement on spindle fibers were induced. We also found that proteins interacting selectively and non-covalently with Rho protein, and member of the Rho subfamily of the Ras superfamily of monomeric GTPases were induced. Moreover, the genes for transcription inhibition, DNA repair and Wnt signaling pathway were induced. In 10th cluster the gene ontologies such as "chromosomal part" and "nuclear chromosomal part" were presented (ES 2.74). These gene ontologies included the genes for process of histone modification and transcription repression by DNA packaging. On the contrary, microtubule organization proteins participating in cell cycle were also induced.

In summary, we have found that secreted factors from *S. aureus* biofilms induced angiogenesis and anti-apoptosis genes whereas secreted factors from *S. aureus* planktonic cultures induced mitotic cell cycle and protein catabolism genes in keratinocytes.

Comparison of differential transcription in *S. aureus* Superantigens challenged *vs.* control cells

Superantigens are *S. aureus* toxins that cause toxic shock syndrome and septic shock. The superantigens stimulate T-cells much more efficiently than typical antigens.[3] The clusters of ontological categories upregulated in cells challenged with *S. aureus* super-antigens are given in Table B.3a.

Table B.3: Clusters of ontological categories induc ed or suppressed by *S. aureus* super-antigens

	a) Super-antigen Challenged : induced			b) Super-antigen Challenged : Suppressed	
Sr	**Gene Ontologies**	***P* Value**	**Sr**	**Gene Ontologies**	***P* Value**
1	16.18		1	4.81	
	immune response	1.71E-32		response to wounding	1.80E-07
	cytokine activity	9.72E-25		inflammatory response	1.75E-04
				defense response	1.80E-04
2	2.89		2	2.52	
	defense response	4.72E-12			
	inflammatory response	1.91E-10		Carbohydrate binding	4.87E-05
	response to wounding	2.34E-09		polysaccharide binding	3.63E-03
3	8.21		3	1.92	
	Pos. reg. of macromolecule metabolism	2.67E-10		extracellular space	4.76E-03
	Pos.R. of cell proliferation	8.98E-09		extracellular region part	7.43E-03
4	6.59		4	1.89	
	Asthma	3.03E-14		locomotory behavior	5.04E-04
	Signaling in Immune System	1.36E-08		taxis	7.69E-04
5	5.6		5	1.63	
	pos. reg. of macromolecule metabolism	2.70E-10		Pos. R. of locomotion	1.93E-04
	Pos. R . of gene expression	1.42E-06		R. of locomotion	5.79E-04

6	4.68		6	1.59	
	R. of immune effector process	7.14E-10		plasma membrane part	1.93E-04
	reg. of lymphocyte mediated immunity	3.01E-07		integral to plasma membrane	3.56E-02
7	4.62		7	1.52	
	Chemokine activity	8.16E-08		sugar binding	3.59E-03
	chemotaxis	8.82E-09		cell adhesion	8.63E-02
8	4.49		8	1.5	
	R. phosphorylation of STAT	1.24E-10		Pos. R. of locomotion	1.93E-04
	Pos. R. of cell proliferation	8.98E-09		R. of Pos. chemotaxis	2.21E-02
9	4.04		9	1.48	
	R. of immune effector process	7.14E-10		R. of gene-specic transcription	5.98E-03
	R. of lg production	5.97E-08		Neg. reg. RNA polyII transcription	2.21E-02
10	3.58		10	1.41	
	R. of leukocyte activation	1.35E-07		R. of cell proliferation	5.91E-04
	R. of lymphocyte activation	4.03E-07		pos.R. of signal transduction	3.35E-03

R. Regulation; Pos. R., Positive regulation; Neg. R., Negative regulation; RNApolyII, RNA polymerase II

In 1st (ES 16.18), 2nd (ES 9.89), 4th (ES 6.59), 7th (ES 4.62), 8th (ES 4.49), 9th (ES 4.40) and 10th (ES 3.58) clusters genes representing adaptive immune system were found. The genes for adaptive immune process including MHCII complex involved in antigen presentation and processing, were induced. A number of T-lymphocytes specific chemokines (C-C and C-X-C motifs, CXCL) and costimulatory molecules (CD40, CD40LG), serpin peptidase inhibitor were also upregulated. In addition, Th1 and Th2 specific interleukin genes, including IFNγ, IL-2 IL-4, IL-9, IL-12 and IL-27, were induced. The signaling molecules of JAK-STAT cascade were found in this cluster. GM-CSF cytokines which controls production and differentiation of granulocytes and monocytes-macrophages were found in this cluster. Few innate immune response genes, including IgG receptors, complement component and lipoprotein transporters, were also found in these clusters. Some genes for apoptosis stimulation were also upregulated. The negative regulators of myeloid cells differentiation were also upregulated. Immune cells mediated negative regulation of cell death and wounding response was also activated.

In 3rd (ES 8.21) and 5th (ES 5.60) clusters gene ontologies "positive regulation of macromolecule metabolism" and "positive regulation of cell proliferation" were induced. These ontologies contained nuclear receptors and proteins which participate in regulation of immune cells transcription processes. The regulators of adaptive immunity, which also negatively regulate the innate immunity, were induced. Moreover, cell surface and intracellular receptors (but not TLRs) which transduce signal to activate immune response were induced. In 6th cluster we found that ontological categories such as "regulator of immune effector responses" and "regulation of lymphocyte mediated immunity" were presented (ES 4.68). These genes function in positive regulation of NK cells-mediated toxicity process. Platelet specific proteins which represent activation of wound healing process were also upregulated.

The clusters of gene ontologies suppressed in *S. aureus* super-antigen challenged cells are given in Table B.3b. Surprisingly, like the induced one (B.3a)

the ontological categories "inflammatory response", "defense response" and "immune response" were found in the top cluster suppressed in super-antigen challenged cells (ES 4.81). Further study of the genes presented in this cluster showed that receptors, transcriptional modulators, chemotactic factors specifically which participate downstream TLR4 (the Gram negative receptors) and provide defense against bacterial infection were reduced. In addition, the innate immunity-specific genes driving the processes such as polysaccharide binding, wound healing, chemotaxis, regulation of cytokine production, immunity mediated cell death and regulation of cell proliferation were downregulated.

In 2nd cluster the gene ontologies such as "carbohydrate binding" and "polysaccharide binding" were found (ES 2.52). The genes included integral membrane proteins as sialic acid binding immunoglobulin like lectin proteins that are found in NK cells. In addition, lectins as well as lipoprotein binding proteins which are present in dendritic cells were repressed. In 3rd cluster ontological categories such as "extracellular space" and "extracellular region part" were found (ES 1.92). The LPS-binding immune activator proteins were downregulated. The innate immune specific chemo-attractants (CXL-2, -3, -5, -6 and CC-23) and extracellular matrix proteins such as MMP9, LPL, IGF1, TNXB, VEGFA and WNTA5 were also suppressed. In 4th (ES 1.89) and 5th (ES 1.63) clusters genes principally involved in chemo-attraction of innate immunocytes and GPCR pathways were found. Moreover, membrane-bound receptors (cholinergic, formyl peptide and complement component), specific either for interaction with molecule of Gram negative bacterial origin or involved in innate immunity were suppressed.

In 6th (ES 1.59) and 8th clusters (ES 1.50), the genes presented were largely integral membrane proteins. The adherence genes (TNS, SHROOM and NEURL) that function in formation of junctions between cell membrane and actin filaments were downregulated. In 7th cluster membrane integral adhesion proteins mostly which mediate sialic-acid dependent binding to cells are principally presented (ES 1.52). Additionally, myeloid cells activators and dendritic cells-specific proteins were also downregulated. In 9th cluster the targeted transcription process proteins which might be involved in the process of hemopoiesis, genes for IGF and its receptor were repressed (ES 1.48). In 10th cluster genes for growth factor response processes involved in transcription and chemical homeostasis inside the cells were suppressed (ES 1.41).

Collectively, these results indicated that *S. aureus* super-antigens induce activators of adaptive immunity, cell proliferation, gene expression and anti-apoptosis while suppressing the innate immunity and molecule of bacterial origin binding and processing process in host cells.

Comparison of differential transcription in *S. aureus* PVL and iPVL challenged *vs.* control cells

The emergence of hospital acquired or community associated MRSA gives a major problem to pharmaceutical industry and human health. PVL is a myeloid cells specific cytolytic toxin and produced by many community associated MRSA strains. In *S. aureus* infection, PVL is required for pore formation in membrane of myeloid cells leading to cell lysis.[2] We analyzed differential expression in PVL and iPVL challenged versus control PMNL cells using Meta-analysis procedure. Gene ontologies induced and suppressed in cells in response to active and inactivated PVL-treated *vs.* control cells are presented in Table B.4.

Table B.4: Comparison of differential transcription in *S. aureus* PVL and iPVL challenged *vs.* control cells

	a) PVL and iPVL channenged : induced			b) PVL and iPVL channenged : Suppressed	
Sr	**Gene Ontologies**	***P* Value**	**Sr**	**Gene Ontologies**	***P* Value**
1	2.86		1	3.37	
	non-membrane-bounded organelle	3.50E-04		integral to plasma membrane	2.63E-04

	cytoskeleton	2.43-03		plasma membrane	4.56E-04
2	2.15		2	2.32	
	sequence-specific DNA binding	3.50E-04		plasma membrane	4.56E-04
	R. of transcription	2.43E-03		intrinsic to membrane	1.29E-02
3	1.64		3	1.48	
	DNA repair	4.56E-03		cell fraction	2.55E-02
	cellular response to stress	7.29E-02		membrane fraction	4.20E-02
4	1.64		4	1.48	
	nuclear lumen	9.18E-03		fatty acid metabolism	4.97E-03
	intracellular organelle lumen	1.45E-02		lipid biosynthesis	6.43E-03
5	1.33		5	1.18	
	ATPase activity	2.01E-02		response to wounding	4.86E-02
	helicase activity	4.81E-02		defense response	7.47E-02
				inflammatory response	8.20E-02
6	1.28		6	1.06	
	cell junction	2.48E-02		actin binding	6.58E-03
	cell-substrate adherens junction	4.04E-02		cytoskeleton	3.69E-01
7	1.24		7	1.05	
	microtubule cytoskeleton	3.65E-02		sarcomere	6.38E-02
	centrosome	5.67E-02		myofibril	9.11E-02
8	0.98		8	1.02	
	response to organic substance	5.53E-02		purinergic receptor activity, GPC	7.89E-02
	response to glucocorticoid	8.45E-02		nucleotide receptor activity	1.14E-01
9	0.93		9	1.01	
	response to nutrient levels	4.71E-02		sterol metabolic process	2.41E-02
	response to nutrient acid	7.04E-02		steroid metabolic process	1.10E-01
10	0.89		10	0.99	
	response to gamma radiation	3.67E-02		oxidoreductase activity	9.02E-02
	response to hypoxia	1.02E-01		iron ion binding	1.25E-01

The top seven clusters in cells challenged with PVL and iPVL *S. aureus* toxins included the genes for the processes of stress-induced DNA repair and gene transcription (B.4a). In addition, the cytoskeleton genes which are mainly involved in cell cycle and DNA replication process were induced. In 8th, 9th and 10th clusters responses to the organic substance and hypoxia were induced. The genes for processes

involved in transcription regulation, cell death initiation, and growth regulation were induced. Moreover, chemo-attractant for lymphocytes, cytoplasmic cell to cell interaction transducers and iron transferrin receptors were induced. Some death regulators as (DUSP, SOCS and SOD) were also upregulated.

The top three clusters of suppressed processes in PVL and iPVL-challenged cells included the genes confined to membrane part of cells (B.4b).

The membrane receptors involved in signaling process, especially GPCR and associated affecters proteins as adenylate cyclase were suppressed. GPCRs are also involved in immune-modulation and directly involved in suppression of TLR-induced immune responses from T-cells. They also bind inflammatory mediators and engage target cell types in the inflammatory response. In addition, the adhesion proteins involving integrin's were also induced. The 4th and 5th cluster represented the genes for identification and activation of immune system. The last five clusters again presented the cytoskeleton genes and GPCR-mediated activities. There is growing evidence that GPCRs act as signal transducers in signaling pathways including integrin's, receptor tyrosine kinases and cytokines receptors, such as (JAK/STAT). Although less prominently it was again observed that genes for cytoskeleton, DNA repair and adaptive immune processes were induced whereas lipid metabolism and innate immunity genes were suppressed in *S. aureus* PVL and iPVL challenged cells.

The Meta-analysis of local and systemic responses to *S. aureus* challenges in humans helped us to understand the immune strategies adopted by *S. aureus* to resist host defense mechanisms.

Global comparison of *S. aureus* and its components challenged versus control cells

In global comparison, containing microarrays from all the studies, of cellular and systemic responses to *S. aureus* challenged versus control cells, we found that ontological categories such as “defense response”, “response to wounding” and “inflammatory response” were presented in top upregulated cluster (ES: 22.56; Table A.2 and B.5a).

Table B.5: Clusters of ontological categories induced or suppressed in global comparison of *S. aureus* and *S. aureus* components challenged *vs.* control cells

	a) S. aureus Challenged: Induced			b) S. aureus Challenged : Induced	
Sr	**Gene Ontologies**	***P* Value**	**Sr**	**Gene Ontologies**	***P* Value**
1	22.56		1	10.05	
	defense response	6.54E-25		intracellular organelle lumen	3.07E-13
	response to wounding	2.01E-23		nuclear lumen	1.10E-10
	inflammatory response	1.61E-20			
2	9.38		2	8.73	
	response to organic substance	5.47E-17		lymphocyte activation	3.18E-15
	response to endogenous stimulus	6.74E-09		leukocyte activation	1.48E-14
3	9.08		3	4.52	
	response to bacterium	5.47E-17		ribonucleoprotein complex	2.28E-08
	response to LPS	6.47E-09		Metabolism of proteins	3.69E-06
4	7.42		4	4.35	
	extracellular space	5.31E-10		T cell receptor complex	2.19E-08
	extracellular region part	5.33E-08		pos. R. of Ca-mediated signaling	2.16E-05

5	7.22		5	4.31	
	intrinsic to plasma membrane	4.86E-09		chromosome organization	6.79E-06
	plasma membrane part	6.17E-09		chromation modification	8.25E-05
6	7.12		6	3.53	
	R. of apoptosis	4.86E-09		T cell differentiation in the thymus	1.07E-05
	anti-apoptosis	6.17E-09		positive thymic T cell selection	1.10E-05
7	6.23		7	3.46	
	R. of cytokine production	4.36E-08		non-membrane-bounded organelle	9.08E-06
	neg. R. of multicellular process	7.28E-07		cytoskeleton	4.88E-01
8	5.64		8	3.43	
	protein dimerization activity	6.35E-09		T cell activation	6.19E-11
	protein homodimerization activity	3.28E-04		Pos. R. of immune system	5.01E-10
9	5.4		9	3.33	
	R. of cytokine production	4.36E-08		Intestinal lgA production	3.05E-06
	Pos. R. of cytokine production	5.03E-05		MHC protein binding	4.57E-06
				Antigen processing and presentation	9.25E-06
10	5.38		10	3.07	
	response to oxidative stress	2.09E-07		Pos.R. of immune response	6.72E-05
	response to ROS	8.48E-05		TCR signaling pathway	8.28E-03

LPS, Lipopolysaccharide; R. Regulation; Pos. R., Positive regulation; ROS, Reactive oxygen species; Ca, Calcium; MHC, Major histocompatibility complex; TCR, T cell receptor

Detailed study of the genes presents in the mentioned categories showed that majority of the innate immune specific genes including pattern recognition receptors (PRR's) for example TLRs (TLR 2, 5 and 8) and TLR induced cytokines including IL-1, IL-2, IL-18 and TNF-α were upregulated in *S. aureus* challenged cells. The genes involved in cellular response to infection were found in this cluster including primarily extracellular and membrane receptor genes. The upregulated genes included adhesion molecules, inflammatory cytokines, chemotactic cytokines, interferon stimulated cytokines, interleukin receptors, cytokine receptors and TNF receptors and some intracellular antigen processing genes were also found in this cluster. The MyD88 independent pathway genes were induced. Similarly, in 2nd (ES: 9.38), 3rd (ES: 9.08), 4th (ES: 7.42) and 5th (ES: 7.22) clusters extracellular and membrane receptors genes involved in host responses to bacteria and bacterial components were presented.

Interestingly in addition to the activation of defense and immune response against *S. aureus*, the anti-apoptosis process was also activated in response to *S. aureus* treatment and shown in the 6th cluster (ES: 7.12). The ontological categories including “regulation of apoptosis” and “anti-apoptosis” were presented in this cluster including genes for cell survival/death and apoptosis/anti-apoptosis processes. The upregulation of cell survival/cell death processes suggests that during infection, pathogen-specific immune cells are targeted for

killing without affecting the death of S. aureus-infected cells. This adaptation supports S. aureus survival within infected cells.

The genes for cellular processes for example cytokines expression were also upregulated and presented in 7th (ES: 6.23), 8th (ES: 5.64) and 9th clusters (ES: 5.40). The 10th cluster (ES: 5.38) presented ontological categories "response to oxidative stress" and "response to reactive oxygen species". These categories included critical genes responsive for cellular antioxidant, heavy metal and mitochondrial DNA damage stress.

In contrast to the multiple upregulated processes, only two critical processes were suppressed by the *S. aureus* and its components in host cells including the cell cycle and importantly, adaptive immunity (Table B.5b). We found that the top most *S. aureus*-suppressed cluster of the genes (ES: 10.05) presented the ontological categories such as "intracellular organelle lumen" and "nuclear lumen". The genes involved in processes mainly occurring inside the mitochondria and nucleus for example protein expression and cell cycle were down regulated. These processes are also shown in 3rd (ES: 4.52), 5th (ES: 4.31) and 7th (ES: 3.46) clusters.

The most striking and consistent process suppressed by the *S. aureus* components was adaptive immunity, specifically T-cells synthesis and maturation as well as antibody production was downregulated. These processes are represented in the six clusters out of top ten *S. aureus*-suppressed gene clusters. The adaptive immune system gene ontologies are presented in 2nd (ES: 8.73), 4th (ES: 4.35), 6th (ES: 3.53), 8th (ES: 3.43), 9th (ES: 3.33) and 10th (ES: 3.07) clusters. The most surprising result was upregulation of innate immunity and anti-apoptotic processes. Whereas adaptive immunity pathways such lymphocyte activation, T cells and T cell receptor (TCR) genes were suppressed.

Discussion

S. aureus is known to form surface associated complex communities recognized as biofilms and secretes polymers which differentiate it from relevant planktonic cultures. *S. aureus* biofilms are resistant to antibiotics and delay the process of chronic wound healing. The evidence that genes participating in biofilm formation and those which determine virulence are regulated by common determinants propose that biofilms and planktonic cultures might have different effect on cellular immunity.[3,9] Interestingly, the Meta-analysis of microarray data for secreted factors from biofilm and planktonic cultures treated cells showed different results from live- and inactivated-*S. aureus* treated cell lines (Table B.1 and B.2). While live- and inactivated-*S. aureus* induced innate immune response and cell cycle, secreted factors from biofilm and planktonic cultures predominantly induced adaptive immune process and suppressed mitotic cell cycle. Interestingly, comparison of differential expression in keratinocytes challenged with secreted factors from biofilm versus planktonic cultures revealed different cyto-protective strategies used by cells infected with biofilm cultures- or planktonic cultures-conditioned media. The biofilms conditioned media treated keratinocytes upregulated anti-apoptosis genes and immunity while planktonic cultures conditioned media treated keratinocytes upregulated cell cycle as major process. This analysis reveals that secreted factors from *S. aureus* biofilms stimulate different cell survival strategies and immune responses than their planktonic equivalents. The differential gene expression might have effects on chronic complications in keratinocytes. Therefore, biofilm formation must be considered in studies investigating cause or cure of the bacterial infections.[3,8]

Hierarchal cluster analysis showed that differentially expressed genes in SEI- and SEB-challenged cells, cluster together (Figure A.2, Table B.3). The SEI and SEB induced similar gene expression, inflammatory mediators and T-cell mitogenic effects. Similarly, in current study the comparison of differentially expressed genes in super-antigens (SEI and SEB) challenged *vs.* non-challenged (control) cells using Meta-analysis technique produced comparable results. Similar to the secreted factors from *S. aureus* cultures, super-antigens induced adaptive immunity and suppressed innate immunity in challenged cells. This finding is in parallel with previous results, where super-antigens challenged cells upregulated adaptive immunity specific genes.[1]

PVL is a *S. aureus* pore-forming cytolytic toxin for

lymphoid cells and expressed by many CA-MRSA strains.[10,11] However, specific cytolytic concentrations of PVL are required to kill cells that may not be reached *in vivo.*[12,13] Meta-analysis in current study revealed that PMNL cells exposed to PVL and iPVL induce weak adaptive immune response whereas suppress innate immune response (Table B.4). This favors the observation that *S. aureus* components induce adaptive immune system as a defensive mechanism. However, in contrast to super-antigens and *S. aureus* culture secreted factors, stimulation of weak immune response by PVL and iPVL challenged cells may point out their diminished potential to cause disease. Importantly, these *S. aureus* components increased microbiocidal activity in infected cells.[14] Together these properties propose that PVL could be a potential priming agent for myeloid cells against virulent *S. aureus* infections.[2] Further investigations into bactericidal ability of PVL will provide efficient therapy against CA-MRSA infections.

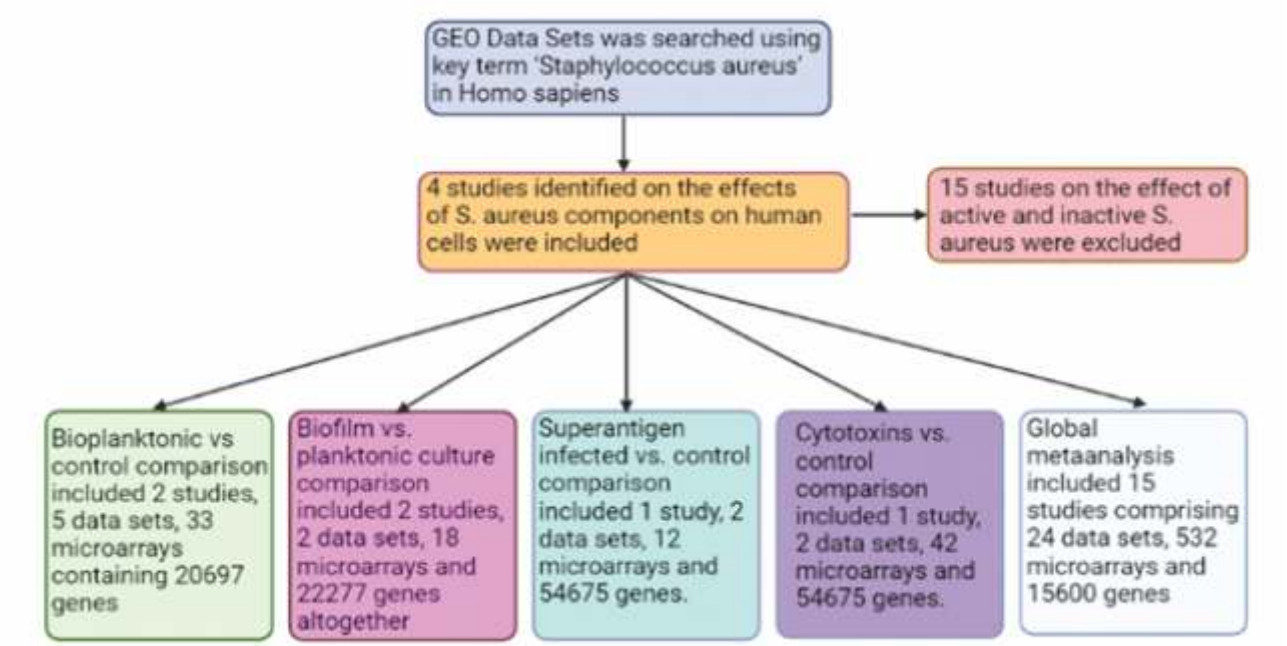
The global Meta-analysis of transcriptional profile from *S. aureus* challenged cells revealed that *S. aureus* infection intensely stimulates immunity process (Table B.5). This observation is in parallel with previous studies where neutrophils treatment with *S. aureus* produced robust expression of immune modulators specified by cytokines, chemokines and bacterial processing genes.[15,16] Same research group reported that *S. aureus* induced necrosis while inhibited apoptosis pathway, conversely the current Meta-analysis indicated that both cell death pathways were induced in infected cells. Interestingly, the upregulation of large number of negative regulators of cell death pathways especially those modulated by TNF-α and Fas and associated molecules argue that *S. aureus* is capable to stimulate cyto-protective effects which can prevent infected host cells removal and consequently ensure intracellular survival and systemic distribution.[17] Collectively, activation of robust immune response characterized by acute inflammation and cyto-protective mechanisms make *S. aureus* more pathogenic than other cutaneous microbes.

Interestingly, second most prominent finding of current Global Meta-analysis of *S. aureus* infected *vs.* controls microarrays was strong upregulation of innate immune process while downregulation of adaptive immune process. Similarly, Stark and coworker reported upregulation of innate immunity in endothelial cells by *S. aureus* isolated from blood and anterior nares.[18] Furthermore, supporting results of the current Meta-analysis, Ardura *et al.* published that innate immunity especially defined by monocytes was induced while adaptive immunity primarily CD4 and CD8 T-cells were downregulated in children infected with invasive *S. aureus* strains.[19,20] Further downregulation of cell cycle genes, enzymes involved in multiple cellular processes, gene expression and maturation processes proposed that during *S. aureus* infection, normal cell processes are suppressed and all host resources are used for *S. aureus* mediated immune and apoptotic responses. This study increased our knowledge about effects of *S. aureus* components on host immunity. Further research is needed to confirm the findings and design alternative drugs for *S. aureus* infections.

The strength of the current study is that it analyzed data from different research papers thus increasing the statistical power of the results as compared to individual studies. However, further *in vivo* studies are required.

Conclusion

Meta-analysis of microarray data from *S. aureus*- and *S. aureus* components-challenged cells indicated that innate immune process genes were induced while adaptive immunity genes were suppressed in the *S. aureus* challenged cells. Conversely, *S. aureus* components induced adaptive immunity genes and suppressed innate immunity genes. In addition, this Meta-analysis revealed different cyto-protective strategies adopted by *S. aureus* to evade host immune system mediated bactericidal activity. In this context, conditioned media from planktonic cultures of *S. aureus* induced cellular processes. However, *S. aureus* biofilms conditioned media induced anti-apoptotic genes as cyto-protective strategy. Confirmatory studies will assist in designing drugs against the *S. aureus* infections.



Prisma Statement for Components Metanalysis. Illustration created with BioRender.com

Acknowledgments

We acknowledge financial support from Higher Education Commission, Islamabad, Pakistan, and from the R.O. Perelman Department of Dermatology, NYU Medical School, New York, USA.

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