IMMUNOMODULATORY EFFECTS OF TLR-4 RECEPTORS AND ENDOTOXIN IN ALLERGIC CONDITION

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Abstract:
Allergic diseases encompassing asthma, allergic rhinitis, atopic dermatitis, and food allergies, pose a significant global health burden. Toll-like receptor 4 (TLR-4) activation, endotoxin exposure, monocyte chemoattractant protein (MCP-1), thymus and activation-regulated chemokine (TARC), and interleukin-5 (IL-5) have emerged as key factors implicated in the pathogenesis of allergic diseases. This study aimed to explore the roles of these factors in allergic diseases by analyzing their correlations and associations. IgE, IL-5, and TARC levels were categorized into two groups (Group 1 and Group 2),based on high and lowigE levels and their correlations with TLR-4, endotoxin, and MCP-1 were examined. The results showed a significant difference in IgE levels between the groups, but no significant correlations were observed between IgE and the other factors. IL-5 levels exhibited a significant difference between groups, and positive correlations were found with TLR-4, endotoxin, and MCP-1 in Group 1. TARC levels also differed significantly between groups, with positive correlations observed in Group 2. These findings highlight the complexity of allergic diseases and suggest the involvement of TLR-4, endotoxin, MCP-1, TARC, and IL-5 in different contexts. Further investigations are warranted to elucidate the underlying mechanisms and explore the therapeutic potential of targeting these molecules in allergic diseases

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Introduction:

There has been a rise of non-communicable diseases in recent years, and allergy is one of them. Allergic disorders could be food allergies, hay fever, atopic dermatitis, allergic asthma, anaphylaxis etc. The development of allergic disease is complex and not fully understood, with both environmental and genetic components. Environmental changes are thought to have contributed to the increased incidence of allergy in recent years with evidence for a role for tobacco smoke exposure, respiratory viral infections, use of antibiotics, diet and exposure to allergens to name a few.

Allergic diseases, characterized by an aberrant immune response to harmless substances, continue to pose a significant burden on global health. These conditions encompass a broad spectrum of disorders, including asthma, allergic rhinitis, atopic dermatitis, and food allergies, affecting millions of individuals worldwide. The intricate interplay between genetic predisposition and environmental factors has been recognized as a key contributor to the development and progression of allergic diseases. Among the factors that play a crucial role in allergic diseases, Toll-like receptor 4 (TLR-4) activation, endotoxin exposure, monocyte chemoattractant protein (MCP), thymus and activation-regulated chemokine (TARC), and interleukin-5 (IL-5) have gained considerable attention in recent research. Understanding the involvement and interactions of these factors can provide valuable insights into the underlying mechanisms driving allergic disease pathogenesis.

Allergic inflammation is a result of a complex interplay amongst structural tissue cells and inflammatory cells, including mast cells, basophils, lymphocytes, dendritic cells, eosinophils, and sometimes, neutrophils. Cytokine secretion is one of the major allergic inflammatory response. Cytokines include interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 6 (IL-6), interleukin 8(IL-8), interleukin 10 (IL-10), monocyte chemoattractant protein (MCP)-1/CCL2, and thymus and activation-regulated chemokine (TARC). This cytokine release can be triggered by toll like receptor (TLR)and proteinase activating receptor (PAR) (1-3).

TLRs are pattern recognition receptors that serve a very important role in innate and acquired immune responses (4,5,). TLRs participate in the innate immune response but also affect the type and intensity of the acquired immune response, stimulate immune cells to synthesize immune factors and regulate the differentiation of T cells (6). TLR-4 is a member of the toll-like receptor family. TLR-4 is a transmembrane protein that in humans is encoded by the *TLR4* genome. TLR-4 on activation leads to a signaling pathway resulting in inflammatory cytokine production. TLR4 is expressed on the cell surface on both hematopoietic and non-hematopoietic cells, including endothelial cells and cells of the central nervous system (7,8). Upon TLRs recognition and binding to their ligands, they undergo conformational changes, dimerization as well as interaction with adaptor molecules passing series of intracellular signal transduction pathways that involve transcription factors NF-κB, IRFs, and mitogen-activated protein kinase (MAPK) activation. These pathways finally resulting in the secretion of pro-inflammatory mediators including nitric oxide (NO), CK- like tumour necrosis factor-alpha (TNF-α), IL-6 and IL-1β, chemokines (CC), and type I interferon (IFN) (9, 10).

TLR-4, a member of the pattern recognition receptor family, acts as a crucial mediator in innate immunity. Its activation by various ligands, including endotoxins derived from bacteria, triggers a cascade of immune responses that can contribute to the development and exacerbation of allergic diseases. Endotoxins, such as lipopolysaccharides (LPS), are potent immune activators that have been linked to airway inflammation, bronchial hyperresponsiveness, and increased allergic sensitization (11). Exposure to low or moderate doses of LPS results in a state of "endotoxin tolerance", which will render the host hypo responsive to further stimulation Endotoxin tolerance is characterized by attenuated production of pro-inflammatory cytokines such as TNFα, IL-6 and IFN γ, and increased production of anti-inflammatory mediators such as IL-10, TGFβ and IL-1 receptor antagonist (IL-1Ra) in response to a second endotoxin challenge (12). Those alterations allow the endotoxin tolerant host to survive a normally lethal secondary challenge with endotoxin. Thus, endotoxin tolerance is thought to be an adaptive mechanism designed to protect the host from inflammatory injury caused by repeated or excessive exposure to LPS or Gram-negative infection (13).

TLR-2 and TLR-4 are widely expressed on various innate immune cells, including decidual macrophages and dendritic cells. Along with these immune cells, TLR-4 is reported to express in decidual cells during the first trimester, EVTs, villous cytotrophoblasts, and hofbauer cells, though not in syncytiotrophoblasts. These cells

protect the fetus from various microbes and infectious agents, which indicates their critical role in placenta. Activation of innate immune PRR through TLR4 at the materno–fetal interface ensures that the developing fetus is protected from invading pathogens at early stage of pregnancy. TLR4, the first identified human Toll-like receptor, is the only TLR that can signal via an MyD88-dependent as well as an MyD88-independent manner. It acts as a specific receptor for gram-negative bacterial lipopolysaccharide (LPS) and can also bind DAMPs, such as hyaluronic acid and β-defensin 2, fibrinogen, and heat shock proteins hsp60 and hsp70 (14). High serum concentrations of thymus and activation-regulated chemokine (TARC) are observed in allergic diseases such as atopic dermatitis and bronchial asthma. Thymus and Activation-Regulated Chemokine (TARC)/CCL17 is transiently expressed in Phyto hemagglutinin-stimulated peripheral blood mononuclear cells and constitutively and selectively in the thymus (15). The TARC receptor CCR4 is selectively expressed in T helper cells (16).

IL-5, a cytokine primarily produced by Th2 lymphocytes and eosinophils, plays a pivotal role in eosinophil recruitment, activation, and survival. Elevated levels of IL-5 have been associated with eosinophilic inflammation observed in asthma, allergic rhinitis, and atopic dermatitis. Understanding the mechanisms underlying the dysregulated production and signaling of IL-5 can provide novel therapeutic targets for the treatment of allergic diseases (17).

MCP, a chemokine involved in the recruitment and activation of monocytes and T lymphocytes, has been implicated in the pathogenesis of allergic diseases. Monocyte/macrophages have a remarkable multipotency in response to various inflammatory environments and the ability to infiltrate inflammatory tissues, where they influence the inflammatory process. Blood monocytes are derived from precursors in the bone marrow, adhere to the vascular endothelial surface, and migrate into tissue following chemotactic gradients and inflammatory signals in response to tissue inflammation (19). MCP-1/CCL2 is a member of the C-C chemokine family, and a potent chemotactic factor for monocytes. MCP-1 plays an important role in the development of allergic inflammatory reactions by recruiting various immune cells (20). Elevated levels of MCP have been found in the bronchoalveolar lavage fluid and serum of asthmatic patients, suggesting its role in promoting airway inflammation and allergic responses.

TARC, also known as CCL17, is a chemokine produced by activated dendritic cells and T lymphocytes. It plays a crucial role in the recruitment and activation of Th2 lymphocytes, which are central to allergic inflammation and atopic diseases (21).

This study aims to understand the intricacies of the roles played by TLR-4, endotoxin, MCP, TARC, and IL-5, and to evaluate the interplay of these factors in the pathogenesis of allergic conditions. Furthermore, we will explore the potential of targeting these molecules as therapeutic interventions to alleviate allergic symptoms and prevent allergic reactions.

Material and methods

Study setting: This study was conducted at the Centre for Basic Sciences, Bejai, Mangalore

Study design: Prospective study conducted based on patient laboratory reports of IgE, TARC and IL-5 and estimation of TLR4, endotoxin and MCP. Levels in left over samples o. TLR4, endotoxin, TARC , IL5 and MCP were compared among serumsamples with high IgE (>100 IU/ml and normal IgE (<100 IU/ml.).Study was conducted after getting instituitional clearance

- Inclusion criteria: Suspected cases of allergic diseases
- Exclusion criteria: Known case of malignancy, infections, neurodegenerative disorders
- Sample size: 82 (males or females or mixed population? Ages?)

Sampling method: Random sampling - Samples from patients who are advised allergy profile analysis (IgE, TARC, IL-5)

Tool for data collection: Independent sample-t-test followed by ANOVA

Test procedure:

IgE estimated by ECLIA in ROCHE COBAS 6000

TARC and IL-5 estimated using ELISA method using kit procured from Genex Bio

TLR4, endotoxin, and MCP are estimated by ELISA method using kit procured from elabscience/ELK Biotech

Results

1. Quantification of IgE levels in serum

IgE was divided into 2 groups: Group 1: <100IU/ml ; Group2: >100 IU/ml *p* value as per Wilcoxon signed rank test= <0.00001

Group 1	IgE	TLR-4	Endotoxin	MCP-1
Mean	47.2	83.58793	0.060733	153.2768
SD	23.97	108.2719	0.072623	137.2185
Median	52	40.76	0.019	70.48
Q1	31	18.955	0.008	52.623
Q3	64	114.6935	0.103	247.787
Spearman's rank correlation coefficient		-0.282	0.279	0.1
p value		0.308	0.313	0.724

Group 2	IgE	TLR-4	Endotoxin	MCP-1
Mean	1112.5	161.629	0.045753	128.682
SD	1401.2	233.13	0.087802	92.4047
Median	528	63.7	0.008	98.71
Q1	226	30.63	0.007	60.15
Q3	1300	150	0.028	187.969
Spearman's rank correlation coefficient		-0.116	0.119	-0.156
p value		0.32	0.31	0.18

2. Quantification of IL-5 levels in serum

IL-5 was divided into 2 groups. Group 1: <10pg/ml ; Group 2: >10pg/ml *p* value as per Wilcoxon-signed rank test = <0.0001

Group 1	IL-5	TLR-4	Endotoxin	MCP-1
Mean	2.81578	148.32	0.046711	123.5217
SD	2.55765	216.00	0.08395	89.6779
Median	2.15	58.23	0.009	85.465
Q1	1.1	28.455	0.007	53.4525
Q3	4.55	150	0.0295	188.798
Spearman's rank correlation coefficient		0.019	0.167	0.184
p value		0.866	0.149	0.111

Group 2	IL-5	TLR-4	Endotoxin	MCP-1
Mean	65.275	148.314	0.05841	192.110
SD	78.555	243.801	0.096103	145.8509
Median	35.9	51.3405	0.008	138.594
Q1	11.85	29.7275	0.007	97.16
Q3	83.025	90.1275	0.083	233.8105
Spearman's rank correlation coefficient		-0.165	0.008	-0.221
p value		0.609	0.978	0.491

3 Quantification of .TARC: was divided into 2 groups. Group 1<100pg/ml Group 2: >100 pg/ml p value as per Wilcoxan- Signed rank test = <0.00001

Group 1	TARC	TLR-4	Endotoxin	MCP-1
Mean	60.9	172.5962	0.0274	113.8302
SD	21.18936	258.475606	0.040714	72.57623
Median	59.5	48.455	0.0105	84.513
Q1	44	22.0975	0.007	62.442
Q3	78	154.704	0.02975	173.7375
Spearman's rank correlation coefficient		0.078	0.189	0.321
p value		0.838	0.601	0.368

Group 2	**TARC**	**TLR-4**	**Endotoxin**	**MCP-1**
Mean	439.0769	145.2156923	0.0507	135.3164
SD	374.0236	214.5741461	0.08914	104.0988
Median	316	58.23	0.009	100.61
Q1	224.5	30.225	0.007	59.445
Q3	479.5	126.8125	0.0305	189.4848
Spearman's rank correlation coefficient		0.0351	0.0233	0.196
p value		0.76	0.83	0.08

Discussion:

The present study aimed to investigate the role of TLR-4, endotoxin, MCP-1, TARC, and IL-5 in allergic diseases. The results showed distinct patterns and correlations between these factors in different groups, shedding light on their potential involvement in the pathogenesis of allergic conditions.

Elevated levels of IgE are a hallmark of allergic diseases, and in this study, IgE levels were divided into two groups: Group 1 (<100Iu/ml) and Group 2 (>100IU/ml). The analysis revealed a significant difference in IgE levels between the two groups (p<0.00001).TLR-4 decreased with increasing IgE levels endotoxin and MCP1 increased with IgE levels in both groupst However, there was no significance in correlation within the groups c

IL-5 is a critical cytokine involved in eosinophilic inflammation, and its dysregulation has been implicated in various allergic diseases. The study divided IL-5 levels into two groups: Group 1 (<10pg/ml) and Group 2 (>10pg/ml) based on refrence ranges in kit insert The analysis showed a significant difference in IL-5 levels between the two groups (p<0.0001).With an increasing IL_5 there was an increase in TLR-4,endotoxin and MCP-1in group-1.Interstingly there was adecrease in TLR_4 and MCP in group-2.However there was no significance in correlation

TARC, also known as CCL17, is a chemokine associated with the recruitment and activation of Th2 lymphocytes, which play a central role in allergic inflammation. TARC levels were categorized into two groups: Group 1 (<100) and Group 2 (>100). There was significant difference been the two groupsp<0.00001).There was an elevation of TLR4,endotoxin and MCP-1 with an increase in TARC values suggesting their potential inviolvement in aller suggesting their potential inviolvement in allergi mediated responses

The activation of several pro-inflammatory transcription factors and the stimulation of pro-inflammatory cytokine production through TLR-4 signaling suggest that it can participate in several inflammatory conditions such as allergic disease. Some studies demonstrated that expression levels of TLR4 increased in allergic rhinitis. For instance, Fransson et al. have studied the relationship between the expression of TLR-4 in allergic rhinitis and reported a significant upregulation of TLR-4, at both the mRNA and protein levels, in the nasal mucosa of the patients (**22).** Lin and Ming demonstrated that expression levels of TLR-4 and its corresponding transcription factor, NF-κ□, increased in rat nasal mucosa in and experimental allergic rhinitis model **(**23). Another study reported that up-regulation of TLR-4 can lead to lipopolysaccharide-induced augmentation of nasal cytokine release in allergic rhinitis (24).

These results suggest that TLR-4 may participate significantly in the pathogenesis of allergy. However, it seems that this is not all of the story and negative as well as non-association of TLR-4 with allergy have been reported repeatedly. For example, in contrast to the aforementioned studies, Lauriello et al. reported that TLR-4 expression was decreased significantly in allergic rhinitis patients (25). Vanhinsbergh et al. have also revealed that TLR-4 was down-regulated during allergic rhinitis (26). Interestingly, Ryu et al. also showed that LPS is not essential to activate innate immunity in the nasal mucosa of patients with allergic rhinitis (27) and it may be related to down-regulation of TLR-4 in these patients. It has also been documented that mRNA levels of TLR4 decreased in allergic rhinitis. In addition to the data reviewed here, several investigations demonstrated the controversial roles of genetic variations within the TLR-4 gene in the pathogenesis of allergic rhinitis. For instance, Fuertes et al. revealed that the rs 1927911 polymorphism within the TLR4 gene is associated with a higher risk of allergic rhinitis **(28).** However, Hussein et al. reported that there is not an association between TLR4 polymorphisms and allergic rhinitis but they have suggested that the polymorphism can be significantly associated with disease severity (29). Negative associations of TLR4 with allergic rhinitis were also reported by Kurowski et al., who demonstrated that CD14/-159CC and CD14/-159TT genotypes were associated with reduced incidence of allergic rhinitis in children (30).

There is hypothesis that an increase in prevalence of allergic disorders could be due to a change in exposur**e.** Braun-Fahrlander et al. subsequently reported high environmental endotoxin levels on

livestock farms and that endotoxin levels in the house were inversely correlated with subsequent sensitization and allergic rhinitis (AR) (31). Research on Amish and Hutterite communities has also reported higher levels of endotoxin and lower frequencies of sensitization and asthma from dust (32). Cross-sectional studies, particularly those of children raised in rural European communities, suggest that early endotoxin exposure may protect against the development of allergic sensitization and atopic asthma. However, endotoxin exposure may also contribute to other non-atopic respiratory diseases and may exacerbate disease in individuals with preexisting asthma (33).These findings highlight the complexity and heterogeneity of allergic diseases and the diverse interplay between TLR-4, endotoxin, MCP-1, TARC, and IL-5 in different patient groups. The observed correlations provide insights into potential mechanisms underlying allergic inflammation, particularly in relation to IL-5 production and TARC-mediated allergic responses. Studies have shown that TLR-4 activation by endotoxins can promote allergic inflammation and airway hyperresponsiveness in asthma (1; 34). Elevated levels of MCP-1 have been observed in the bronchoalveolar lavage fluid and serum of asthmatic patients, implicating its role in airway inflammation (35). TARC has been associated with the recruitment and activation of Th2 lymphocytes, contributing to allergic inflammation and atopic diseases IL-5 has been recognized for its involvement in eosinophil recruitment and activation in asthma, allergic rhinitis, and atopic dermatitis.

Conclusion

The results of this study are consistent with previous research that has demonstrated the involvement of TLR-4, endotoxin, MCP-1, TARC, and IL-5 in allergic diseases. However, it is important to note that the correlations observed in this study do not imply causation and warrant further investigation to elucidate the underlying mechanisms.

It is important to acknowledge the limitations of this study, including its cross-sectional design and relatively small sample size. Future studies with larger cohorts and longitudinal designs are needed to validate and expand upon these findings. Additionally, investigating other factors and their interactions within the context of allergic diseases would provide a more comprehensive understanding of the underlying mechanisms.

In conclusion, this study provides valuable insights into the roles of TLR-4, endotoxin, MCP-1, TARC, and IL-5 in allergic diseases. The findings suggest complex interactions and correlations between these factors, highlighting their potential involvement in the pathogenesis of allergic conditions. Further research is warranted to elucidate the underlying mechanisms and explore the therapeutic potential of targeting these molecules for the treatment of allergic diseases.

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