Prediction of immune factors and signaling pathways in lung injury induced by LPS based on network analysis

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ABSTRACT

Objective: To construct a regulatory network involved in acute lung injury, so as to provide a new theoretical basis and research ideas for studying the relationship between inflammatory factors and immune proteins to collectively regulate the occurrence of acute lung injury.
Method: By using Meta-analysis, GO, KEGG and other methods notarized and constructed the regulatory network pathways of cytokine cascade and lung injury induced by LPS.
Results: The result of Meta-analysis showed that the correlation between CD14, TNF-α, IL-6 gene and acute lung injury was statistically significant. GO analysis and KEGG analysis showed that acute lung injury contained CD14, TNF-α, IL-6 and other involved factors in the induced process of LPS, these inflammatory factors and immune proteins jointly regulate the process of disease development.
Conclusion: CD14 receptor is an important receptor involved in mediating LPS-activated cells, and is a high-affinity LPS receptor. LPS stimulates inflammatory effector cells to bind to LPS receptor- CD14 to activate intracellular signal cascade. Direct or indirect involvement of pathogenic factors enable cytokine caused by induction form a particularly complex network of cytokine regulatory pathways, of which the inflammatory factors TNF-α and IL-6 are simultaneously involved in LPS-mediated and CD14-mediated cytokine cascades.

1. Introduction

Acute lung injury (ALI) is an inflammatory reaction produced by various pathogenic factors (such as infection, trauma, etc.) in lung tissue through direct or indirect action. Severe ALI is prone to develop into acute respiratory distress syndrome (ARDS) (Wang, 2014). Diffuse alveolar damage, pulmonary vascular endothelial cells (PVEC) and extensive alveolar epithelial damage are the main pathological features of ALI (Ma et al., 2013). With the people's

deeper understanding of the regulation of acute lung injury on immune factors, more experimental studies have shown that CD3+, CD4+, CD8+, NK cells, B cells and other immune and inflammation-related factors play a very important role in the pathogenesis of the disease (Shi and Ren, 2013). The scholars at home and abroad tends to study the regulatory network of immune cytokines, and how immune cytokines play a regulatory role in lung injury, as the result, immune dysfunction has become a hot spot for medical researchers.

This study intended to explore the relationship between various immune cytokines such as CD3+, CD4+, CD8+, NK cells and B cells and lung injury through meta-analysis (meta-analysis), combined with bioinformatics methods such as GO and KEGG database, it systematically comprehensively analyzed the related regulatory networks of the above-mentioned immune cytokines in the development of lung injury, which provided research direction and important theoretical basis for further exploring the molecular mechanism of immune and inflammation of acute lung injury and its clinical diagnosis and treatment.

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They are co-lead first authors of this paper, and their contributions to this study are equally significant.

2. Material method

2.1. Meta analysis

The first is the literature search work related to the study of acute lung injury. We searched for the keywords "acute lung injury", "inflammation-related factors", and "immune-related factors" in CNKI (the China Integrated Knowledge Resources Database) and the pubmed public database. According to conditions such as the year of research, completeness of result data and similarity of mechanism pathways were read and screened for the articles needed for this study.

Among them, the literature screening criteria were: (1) the content of the study was about the immune factors or inflammatory factors related to acute lung injury and should be in English or Chinese; (2) the type of literature was retrospective study; (3) in all the included literature, the standard method was ELISA test of plasma samples, the experimental group was the group with acute lung injury induced by lipopolysaccharide, the control group was in normal condition; (4) According to the literature results, the number of samples and the expression levels of the relevant factors of the experimental group and control group were obtained from the experiment, they were expressed in the form of mean ± standard deviation; (5) the research method was rigorous, the idea was clear and standard; (6) the literatures that were selected all were full text, and the language was in Chinese or English, only published literature were selected, all the data were obtained from original articles.

Exclusion criteria: (1) literature data is not examined by using standard methods; (2) reviews were excluded; (3) data cannot copy from the literature searched in previous step.

Meta-analysis of selected literature was performed by RevMan 5.3. Heterogeneity was analyzed by using the I^2 test of Q statistic. $P > 0.05$ was considered to have no significant heterogeneity between studies, the data was combined through a fixed effect model; if there was significant heterogeneity between studies ($P < 0.05$), the data was combined by a random effect model. For the measurement data, the SMD value was used as the effect statistic, and the effect index was expressed by the combined SMD value and the 95% confidence interval (95%CI). Z-test was performed on the combined statistic, $P \geq 0.05$ showed that the combined statistic of several studies was not statistically significant, $P < 0.05$ represented that the combined statistic was statistically significant.

2.2. GO analysis

The Gene Ontology Consortium is an online site for GO analysis. We performed GO ontology analysis on encoding related factors' gene. On the home page of the Gene Ontology Consortium website, the searching keywords were "gene name" and "Homo sapiens", then GO annotation analysis was conducted. After that, we focused on the genetic annotation information involved in the regulatory network of acute lung injury based on the correlation between the annotation information of GO and acute lung injury.

2.3. KEGG network analysis

The online website "KEGG PATHWAY Database" can analyze the regulatory network of genes. After entering the KEGG PATHWAY's home page, the search conditions were: organism was "hsa", key words was "gene name" (Wilby and Nasr, 2016). By searching the network pathway of the gene in inflammation and immune response, the regulatory network of the gene was regarded as a large network, we collected and organized the pathways and nodes involved in the regulation of each factor, and there was a cross between the pathways, so it was integrated into a primary regulatory network involving related factors of acute lung injury.

3. Results

3.1. Meta-analysis of factors involved in acute lung injury

3.1.1. Situation of literature screening

A total of 210 articles on acute lung injury-related factors were searched in the database, the methods section and results section of the literatures focused on the study of acute lung injury caused by lipopolysaccharide, and the research methods were consistent, the same measurement method was adopted to obtain the result data. Based on the above three aspects, we screened the articles for meta-analysis, including 6 articles on immune factor CD14 and acute lung injury; 7 articles on inflammatory factor TNF-α and acute lung injury; 5 articles on inflammatory factor IL-6 and acute lung injury. The results of the literature screening are shown in Tables 1, 2, and 3.

3.1.2. Meta-analysis results of CD14 correlation with acute lung injury

In this study, a meta-analysis of six literatures related to CD14 and acute lung injury was performed (Srivalli and Mishra, 2016). The results are shown in Fig. 1. It can be seen that the heterogeneity test result $I^2 = 0\%$, indicating that there was no heterogeneity between the literatures, and a random effect model was adopted; the combined effect SMD value was 3.81, 95%CI was 2.87–4.74, the upper and lower limits of combined SMD value and 95%CI were all greater than 1, indicating that the correlation between CD14 gene and acute lung injury was statistically significant.

3.1.3. Meta-analysis results of the correlation between TNF-α and acute lung injury

We performed a meta-analysis of seven articles related to TNF-α and acute lung injury. The results of the analysis are shown in

Table 1
Literature search results of CD14 gene.

| Literature cited	Title	Source
Guo et al. (2004)	Relationship between expression of endotoxin receptor CD14 gene and acute lung injury in lung tissue of multiple organ dysfunction syndrome model induced by cerebral ischemia septal defects	Journal of Clinical Neurology
Ma (2009)	Effects of intravenous anesthetics on CD14 and TLR4 receptors in lung tissue of rats with acute lung injury induced by lipopolysaccharide	Doctoral thesis of China Medical University
Su et al. (2012)	Effect of Xuanbai Chengqi Decoction on Expression of CD14 and NF-κB mRNA in Lung Tissue of Rats with Acute Lung Injury	Chinese Journal of Experimental Traditional Medical Formulae
Wang (2005)	The mechanism of action of CD14 and TLR4 receptor susceptibility in lung tissue in multiple organ dysfunction syndrome in aged rats	Doctoral thesis of the Third Military Medical University
Wu et al. (2015)	Effects of valnemulin on the expression of TLR4 and CD14 in mice with lipopolysaccharide-induced acute lung injury	Master's thesis of Heilongjiang Bayi Agricultural University
Yang et al. (2011)	Expression of TOLL-like receptor 4 and CD14 mRNA in lung tissue of rats with acute lung injury	Acta Laboratorium Animalis Scientia Sinica

Table 2
Literature search results of TNF-α gene.

Literature cited	Title	Source
Zhang et al. (2019a,b)	Effect of thoracic epidural block on inflammatory factors in rats with acute hypoxic lung injury	Journal of Xinxiang Medical College
Meng et al. (2019)	Protective effect of simvastatin on lipopolysaccharide-induced acute lung injury in mice and its effect on HMGB1/TLR4/NF-κB signaling pathway	Occupation and Health
Zhang et al. (2019a,b)	Changes of Notch signaling pathway in acute lung injury in septic rats	Journal of Qiqihar Medical College
Wang and Shi (2019)	Protective effect and mechanism of bone marrow mesenchymal stem cells on acute lung injury after hip fracture in aged rats	Chinese Journal of Gerontology
Wu et al. (2019)	Experimental study of lung extracellular matrix hydrogel in the treatment of rats with radiation-induced lung injury	China Journal of Modern Medicine
Liu et al. (2019)	Effect of Shenfu Injection on Lung Tissue Inflammation in Rats with Endotoxin Shock and Anti-inflammatory Mechanism	China Pharmacy
Zheng et al. (2018)	Effects of Ghrelin on Akt, NF-κB and iNOS in inflammatory signaling pathways of alveolar macrophages in septic rats	Journal of Sun Yat-sen University (Medical Sciences)

Table 3
Literature search results of IL-6 gene.

Literature cited	Title	Source
Wu et al. (2019)	Experimental study of lung extracellular matrix hydrogel in the treatment of rats with radiation-induced lung injury	China Journal of Modern Medicine
Zheng et al. (2018)	Effects of Ghrelin on Akt, NF-κB and iNOS in inflammatory signaling pathways of alveolar macrophages in septic rats	Journal of Sun Yat-sen University (Medical Sciences)
Wang and Shi (2019)	Protective effect and mechanism of bone marrow mesenchymal stem cells on acute lung injury after hip fracture in aged rats	Chinese Journal of Gerontology
Chepurnova et al. (2018)	Compounds of IL-6 Receptor Complex during Acute Lung Injury	Bull Exp Biol Med
Fu et al. (2017)	Evaluation of LPS-Induced Acute Lung Injury Attenuation in Rats by Aminothiazole-Paeonol Derivatives	Molecules
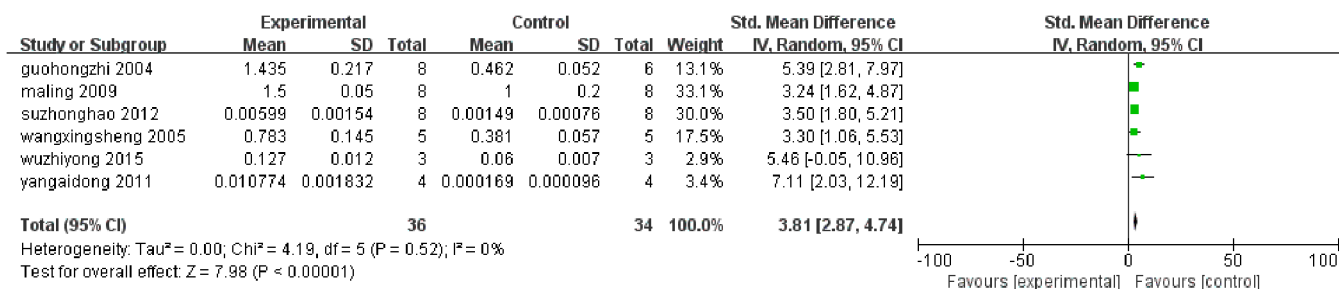
Fig. 1. Meta-analysis forest map of CD14 gene and acute lung injury.
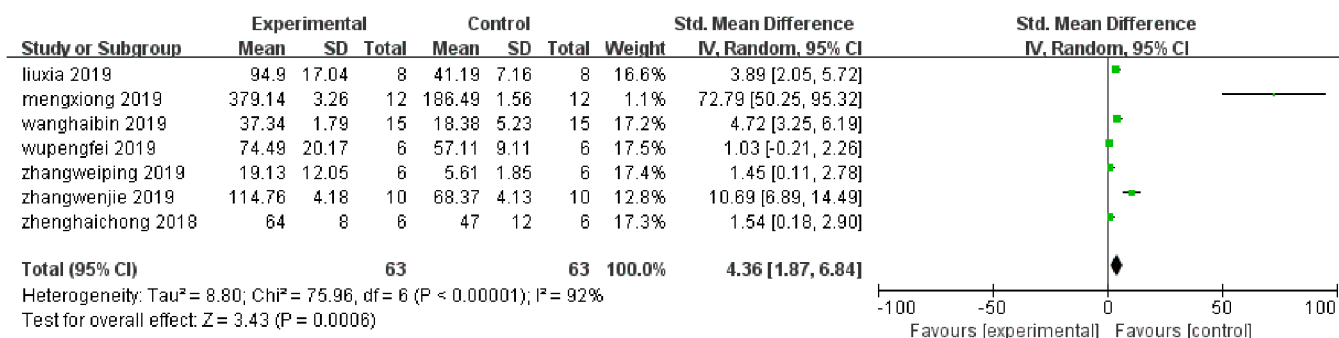
Fig. 2. Meta-analysis forest map of TNF-α gene and acute lung injury.

Fig. 2. Among them, the heterogeneity test results was $P < 0.05$, indicating that there was heterogeneity among the literatures, the random effects model was used; combined effect SMD value was 4.36, 95%CI was 1.87–6.84, the upper and lower limits of combined SMD value and 95%CI all were higher than 1, representing that the correlation between TNF-α gene and acute lung injury was statistically significant.

3.1.4. Meta-analysis results of correlation between IL-6 and acute lung injury

This paper conducted a meta-analysis of five articles related to acute lung injury caused by IL-6. The results of the analysis are shown in Fig. 3. It showed that from the figure that the heterogeneity test results was $P < 0.05$, demonstrating that there is heterogeneity between the articles, a random effect model was
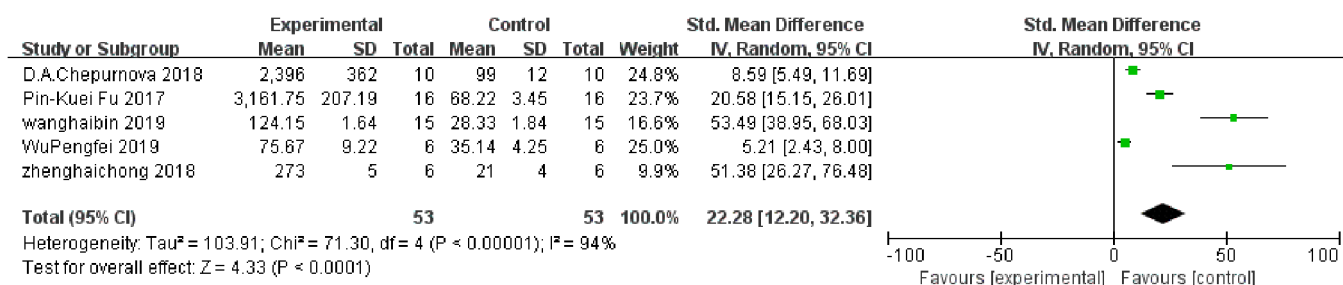
Study or Subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
D.A.Chepurnova 2018	2,396	362	10	99	12	10	24.8%	8.59 [5.49, 11.69]
Pin-Kuei Fu 2017	3,161.75	207.19	16	68.22	3.45	16	23.7%	20.58 [15.15, 26.01]
wanghaibin 2019	124.15	1.64	15	28.33	1.84	15	16.6%	53.49 [38.95, 68.03]
WuPengfei 2019	75.67	9.22	6	35.14	4.25	6	25.0%	5.21 [2.43, 8.00]
zhenghaichong 2018	273	5	6	21	4	6	9.9%	51.38 [26.27, 76.48]
Total (95% CI)			**53**			**53**	**100.0%**	**22.28 [12.20, 32.36]**

Heterogeneity: Tau² = 103.91; Chi² = 71.30, df = 4 (P < 0.00001); I² = 94%
Test for overall effect: Z = 4.33 (P < 0.0001)

Fig. 3. Meta-analysis forest map of IL-6 gene and acute lung injury.

employed; the combined effect SMD value was 22.28, 95%CI was 12.2–32.36, and the upper and lower limits of combined SMD value and 95%CI were all greater than 1, indicating that the correlation between IL-6 gene and acute lung injury was statistically significant.

3.2. GO analysis of factors involved in acute lung injury

Functional annotation information of CD14, TNF-α and IL-6 was obtained by using GO online database (Rajiah et al., 2016). As shown in Table 4, the molecular function of the CD14 gene involves the binding of lipopolysaccharide and the activation of the opsin receptor, while biological processes include the toll-like receptor signaling pathway, the MyD88-dependent toll-like receptor pathway, and the positive regulation of interleukin-8 secretion. These functions are all involved in the regulation of immune response and are closely related to the occurrence of acute lung injury.

The functional annotation results of the TNF-α gene are shown in Table 5. The biological processes include lipopolysaccharide-regulated signaling pathway, IkB kinase or NF-kB signaling pathway, positive and negative regulation of interleukin-6, and the biosynthesis process of positive regulation of interleukin-8. Table 6

shows the results of GO analysis of IL-6 gene, and the biological processes involved include positive regulation of acute inflammatory response, cellular response of lipopolysaccharide, positive regulation of NF-kB transcription factor activity, and the regulation process of the signaling pathwayinterleukin-1. Molecular function and cellular composition are involved in the binding of the interleukin-6 receptor and the interleukin-6 receptor complex.

3.3. KEGG pathway analysis of factors involved in acute lung injury

By analyzing the regulatory network of CD14, TNF-α and IL-6 genes in cellular immune responses in the KEGG database, a signaling pathway of the regulation of acute lung injury process that immune and inflammatory factors involved was constructed, as shown in Fig. 4. The green boxes in the figure represent the closely-watched genes mainly studied in this paper.

4. Discussion

Acute lung injury is an acute hypoxic respiratory failure disease characterized by acute respiratory distress syndrome (ARDS),

Table 4
GO function analysis of CD14 gene.

Gene	Accession	GO class	Ontology	Reference
CD14	GO:0001530	Lipopolysaccharide binding	molecular_function	PMID:12594207
	GO:0002224	Toll-like receptor signaling pathway	biological_process	Reactome:R-HSA-168898
	GO:0001847	Opsonin receptor activity	molecular_function	PMID:2402637
	GO:0002755	MyD88-dependent toll-like receptor signaling pathway	biological_process	Reactome:R-HSA-166058
	GO:0002756	MyD88-independent toll-like receptor signaling pathway	biological_process	Reactome:R-HSA-166166
	GO:0007249	I-kappaB kinase/NF-kappaB signaling	biological_process	Reactome:R-HSA-937072
	GO:2000484	Positive regulation of interleukin-8 secretion	biological_process	PMID:15039339

Table 5
GO function analysis of TNF-α gene.

Gene	Accession	GO class	Ontology	Reference
TNF-α	GO:0043123	I-kappaB kinase/NF-kappaB signaling	biological_process	PMID:21873635
	GO:0031663	Lipopolysaccharide-mediated signaling pathway	biological_process	PMID:21147091
	GO:0032715	Negative regulation of interleukin-6 production	biological_process	PMID:10443688
	GO:0045416	Positive regulation of interleukin-8 biosynthetic process	biological_process	PMID:20551324
	GO:2000778	Positive regulation of interleukin-6 secretion	biological_process	PMID:29702085

Table 6
GO function analysis of IL-6 gene.

| Gene	Accession	GO class	Ontology	Reference
IL-6	GO:0002675	Positive regulation of acute inflammatory response	biological_process	PMID:2444978
	GO:0005138	Interleukin-6 receptor binding	molecular_function	PMID:12829785
	GO:0005896	Interleukin-6 receptor complex	cellular_component	PMID:12829785
	GO:0071222	Cellular response to lipopolysaccharide	biological_process	PMID:23776175
	GO:0051092	Positive regulation of NF-kappaB transcription factor activity	biological_process	PMID:12419823
	GO:2000660	Negative regulation of interleukin-1-mediated signaling pathway	biological_process	GO_REF:0000024
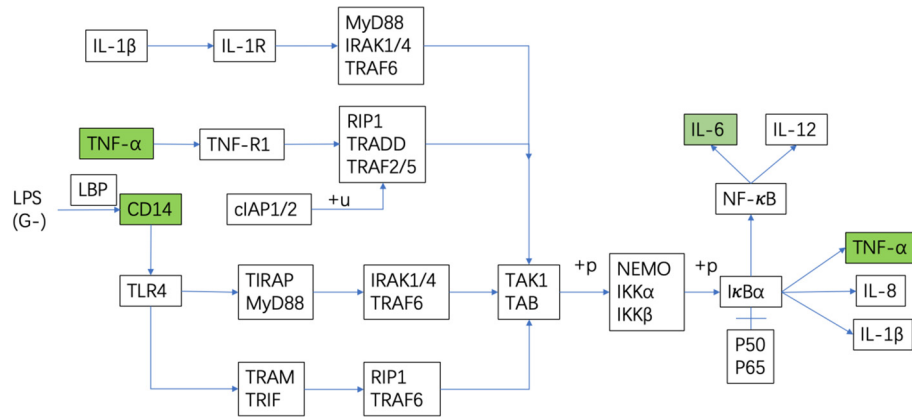
Fig. 4. Regulation network of acute lung injury related factors.

which is a common disease with high mortality rate in medicine. Animal studies have shown that after one hour of lipopolysaccharide injection or organ reperfusion after blood loss, the experimental animals will show a variety of morbidity of lung function and organ, such as dyspnea, decrease of arterial oxygen partial pressure, lung enlargement, increase of the coefficient of pulmonary edema, lobar hemorrhage; alveolar edema thickening, bronchial epithelial cell death, pulmonary interstitial and alveolar hemorrhage accompanied by edema, a large number of inflammatory cell infiltration (Wu, 2015), this phenomenon changes with time obviously. Numerous studies have shown that lipopolysaccharide is one of the leading causes of ALI and ARDS and ultimately lead to death caused by infection. The mechanism of lipopolysaccharide-induced lung injury is most commonly caused by induction of uncontrolled inflammatory response, which causes activated inflammatory cells and effector cells to release a large amount of inflammatory mediators or cellular mediators. Excessive or uncontrolled inflammatory factors can lead to complications, then resulting in lung injury (Li et al., 2010). Lipopolysaccharide can induce the activation of monocytes/macrophages to generate inflammatory factors such as tumor necrosis factor TNF-α, interleukin IL-1, IL-6, IL-8 and IL-12, etc. (Gouda and Bhandary, 2019). When generated inflammatory factors are excessive or are out of control, they can cause a variety of complications, such as microcirculatory disorders, tissue damage, septic shock, and multiple organ damage. Lipopolysaccharide first stimulates effector cells to induce transcription factors to generate a large number of inflammatory factors and inflammatory mediators, including cell nucleus factor kappaB (NF-kB) and activated protein 1 (AP-1). LPS requires the recognition of LPS receptor complex and combines effector cells to transduce signals (Zheng et al., 2018). The LPS receptor complex consists of three receptor proteins: the CD14 receptor, the TLR4 receptor, and the MD-2 receptor. A variety of lipopolysaccharide receptor complexes are present on the surface of both monocytes and macrophages, which are essential for the body to recognize and initiate inflammatory responses (Wang, 2005). The CD14 receptor is also an important receptor mediating LPS-activated cells and has high affinity and sensitivity to LPS receptors.

Numerous studies have shown that LPS-induced lung tissue neutrophil aggregation and pulmonary microvascular endothelial cell response in mice are CD14-dependent. LPS stimulates inflammatory effector cells to bind to LPS receptor CD14 as a LPS complex, thereby activating intracellular cascade signaling and cell nucleus factor NF-kB, then nuclear translocation occurs. It specifically binds to the promoter of the target gene and the enhancer region, which in turn initiates regulation of a series of inflammatory factor responses, such as the expression and release of TNF-α. In addition,

they are further involved in the activation of NF-kB factor in effector cells with LPS, and subsequently initiate the expression of more cytokines (e.g., interleukin IL- 1, IL-6, etc.) (Gouda and Bhandary, 2019). NF-kB is a kind of eukaryotic transcription factor, of which p50/p65 was founded earliest, and it has the widest distribution and effect (Pan et al., 2012). Studies have shown that proteins of the NF-kB family often bind to the IkB family of inhibitory proteins in the form of homomultimer or heterodimers and exist in inactive forms within the cell. Increasing studies have found proteins related to immunity and inflammation such as TNF-α, IL-1, IL-6, IL-8, monocyte chemotactic protein 1 (MCP-1), ICAM-1, iNOS, etc., they all contain a binding site for NF-kB (Chepurnova et al., 2018). In the case of trauma, infection, etc., NF-kB in the cytoplasm of inflammatory cells is induced to activate, causing excessive production of inflammatory mediators such as inflammatory cytokines, adhesion molecules, chemotactic molecules and biologically active enzymes, thereby triggering the systemic inflammatory response characterized by cell self-destruction. Interaction between cytokines forms an extremely complex cytokine regulatory networks that are involved in mediating and regulating immune as well as inflammatory processes. Due to the interaction and synergy between the cytokines, a cascade of amplification of the inflammatory mediators will be triggered, resulting in a large number of mediators involved in the induction of tissue cell damage, ultimately leading to acute lung injury.

In this paper, 210 related literatures such as LPS, CD14, TNF-a and IL-6 were included, and the pathway regulatory network of LPS-induced cytokine cascade involved in lung injury was initially screened and constructed by meta-analysis, GO and KEGG. The effect of LPS on the expression of inflammatory factors such as CD14, TNF-α and IL-6 in the pathway was confirmed. According to the results of meta-analysis, the correlation between CD14, TNF-α, IL-6 gene and acute lung injury was statistically significant. GO analysis and KEGG analysis showed that the process of acute lung injury induced by LPS contained CD14, TNF-α, IL-6 and other factors, these inflammatory factors and immune proteins collectively regulate the process of disease occurrence.

5. Conclusion

In summary, this paper adopted Meta, GO and KEGG analysis to identify three important cytokines involved in the process of acute lung injury, and to construct a network of regulatory pathways for LPS-induced ALI. However, there are still some shortcomings in this study. Among them, the number of high-cited literature and top journal articles is relatively small, and most of the selected literatures are animal test sample data, and there are fewer experi-

mental samples, which may cause difference in clinical symptoms and therapeutic effects to some extent. At the same time, most of the relevant researches at home and abroad lack clinical sample data, or there is a small number of observational cohort studies, in addition, the complete research system has not been established yet, so the support strength of this study is not enough. It is hoped that the results of this paper could guide the development of similar research and provide reference for researchers.

The establishment of immune system related factors involved in the regulation of acute lung injury network provides a new theoretical basis and research ideas for clinical acute lung injury inflammatory factors and immune proteins to jointly regulate the process of disease occurrence and early clinical screening. In the follow-up work, we will conduct more in-depth and detailed studies, as well as experimental science and clinical science on the signaling pathway of immune inflammatory response to lung injury.

Declaration of Competing Interest

The author states that there is no conflict of interest in the content of this article.

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