Article

J. Biol. Regul. Homeost. Agents. 2024; 38(6): 5105–5115
https://doi.org/10.23812/j.biol.regul.homeost.agents.20243806.407

Mendelian Randomization Analysis of Immune Cells and Asthma

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Submitted: 11 March 2024 Revised: 27 April 2024 Accepted: 29 April 2024 Published: 1 June 2024

Background: The immune cells play a substantial role in the development and advancement of asthma. Therefore, we utilized mendelian randomization (MR) analysis to investigate the correlation between immune cells and asthma.
Objective: Given that immune cells play a crucial role in the onset and progression of the condition, this study aimed to elucidate the unclear links between immune cells and asthma.
Methods: The publicly available genetic data regarding asthma were obtained from the IEU database, and genetic variation points were selected as instrumental variables (IV_S). Moreover, genetic information concerning immune cells was obtained from published literature. We used five different methods for dual sample mendelian randomization (MR) analysis, including inverse variance weighted (IVW), weighted median (WMI), MR-Egger regression, simple mode (SM), and weighted mode (WM). Furthermore, sensitivity analysis was utilized to examine the heterogeneity, horizontal pleiotropy, and stability of the outcomes.
Results: IVW results showed that B-cell Activating factor of the TNF family receptor (BAFF–R) on B cell, BAFF–R on IgD$^-$ CD27$^-$ B cell, BAFF–R on IgD$^+$ CD24$^-$ B cell, BAFF–R on IgD$^+$ CD38^{dim} B cell, CD33^{br} HLA DR$^+$ CD14^{dim} myeloid cell, CD25 on B cell, CD25 on IgD$^+$ CD24$^-$ B cell, CD25 on IgD$^+$ CD38$^-$ naive B cell, CD25 on naive-mature B cell, CD25 on transitional B cell, CD33 on basophil, CD33 on CD14$^+$ monocyte, CD33^{dim} HLA DR$^+$ CD11b$^-$ myeloid cell, CD33 on CD66b$^+$ myeloid cell, CD38 on IgD$^-$ CD38^{dim} B cell, CD86 on myeloid dendritic cells (DC), HLA DR on CD14$^-$ CD16$^+$ monocyte, IgD$^+$ CD38^{br} lymphocyte, and transitional lymphocyte may be the risk factors of asthma. Moreover, CD11b on CD14$^+$ monocyte, CD24 on IgD$^+$ CD38^{br} B cell, CD28 on CD45RA$^+$ CD4$^+$ Treg, CD45 on NK, HLA DR$^+$ CD3$^-$ NK, HLA DR$^+$ NK cell, IgD$^-$ CD38$^-$ B cell, PDL–1 on CD14$^+$ CD16$^-$ monocyte, and plasmacytoid dendritic cells (DC) were identified as protective factors for asthma.
Conclusion: This study explored the causal relationship between asthma and immune cells and identified immune cells correlated with asthma development. These immune cells may become new biomarkers or therapeutic targets, provide better treatment options for the prevention and treatment of asthma, and promote the understanding of asthma.

Keywords: asthma; immunity; causal inference; mendelian randomization; MR

Introduction

Asthma is a heterogeneous disease, usually characterized by chronic airway inflammation and variable expiratory airflow limitation. Common clinical symptoms include recurrent wheezing, shortness of breath, chest tightness, and coughing [1]. Asthma imposes a considerable global burden on patients and society, affecting over 300 million people worldwide, including 25 million in the United States [2]. Globally, asthma contributes to productivity losses due to absence from school and work. The adjusted life span for disability caused by asthma is approximately 15.3 years, ranking it 22nd globally in terms of impact, similar to other chronic diseases such as diabetes or Alzheimer's disease [3]. Presently, the primary method of treating asthma involves bronchodilators. Alternatively, treatment may also involve inhibition of inflammation, in which glucocorticoids are the main choice [1].

Asthma is a multifactorial complex disorder influenced by genetic predisposition and environmental elements. Genetic factors determine individual susceptibility to asthma [4]. The pathophysiological mechanism of asthma involves the complex involvement of multiple cells from both innate and adaptive immunity [5]. Th2 lymphocytes are crucial in this process as they secrete interleukins, such as IL-4, IL-5, and IL-9, which are critical factors in driving allergic asthma development [6]. Furthermore, dendritic cells (DC), specialized antigen-presenting cells within the immune system, play an essential role in regulating Th2 response in the upstream of Th2 lymphocytes [7]. Research has found that dendritic cells (DC) can use p38 α and Fas signaling pathway to regulate Th2 cell-mediated allergic asthma [8]. On the other hand, B lymphocytes play a vital role in immune function, such as antibody production, antigen presentation, and cytokines secretion, and they participate in immune regulation and inflam-

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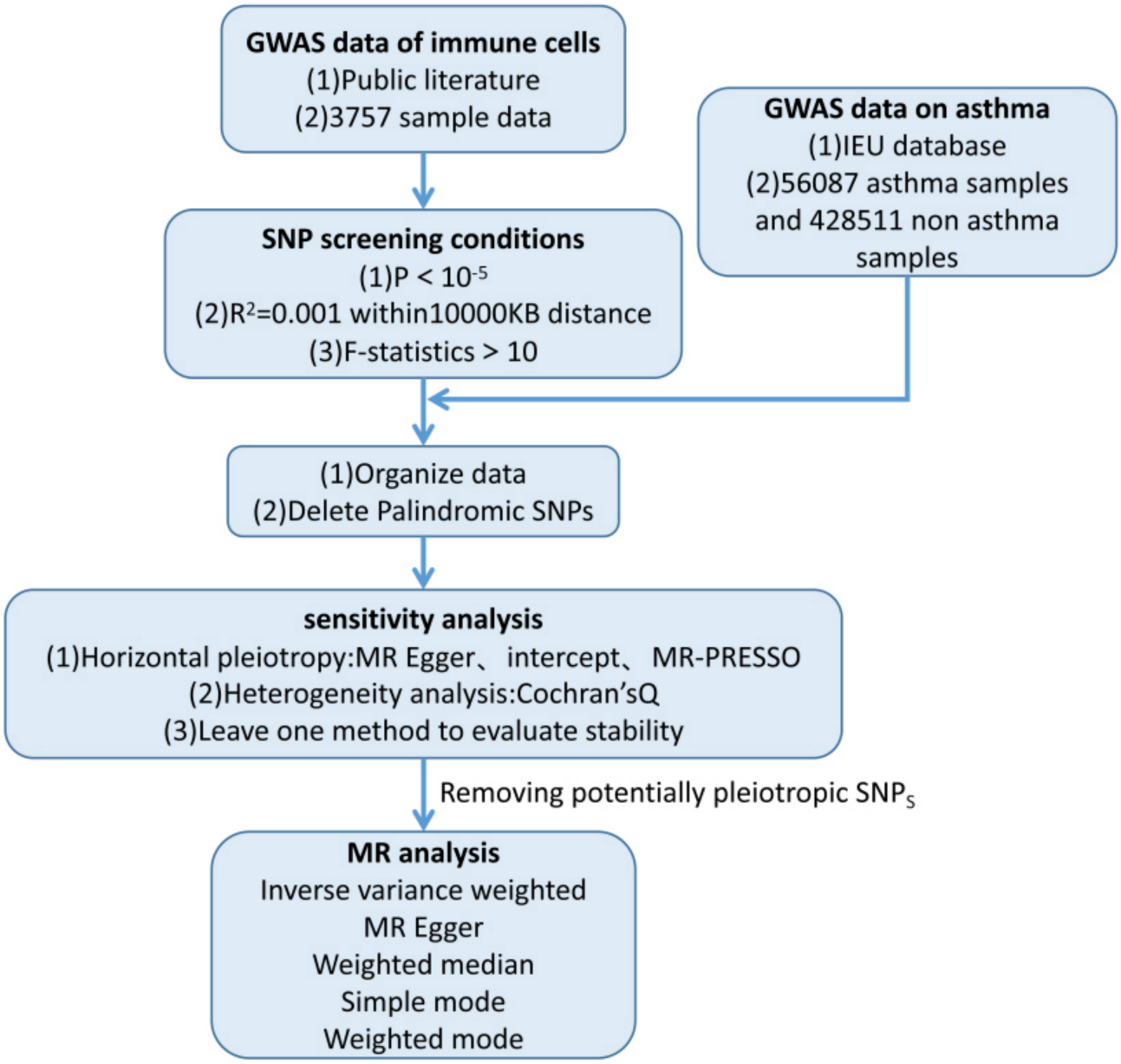

Fig. 1. A flowchart of IV_S screening. IV_S, instrumental variables; GWAS, genome-wide association studies; MR-PRESSO, mendelian randomization pleiotropy residual sum and outlier; SNPs, single nucleotide polymorphisms.

matory responses. Recently, Bcl-3 has been found to prevent house dust mite-induced asthma by inhibiting the production of IL-10 from B cell [9]. The correlation between asthma and immune cells is determined by complex regulatory networks, with various immune cells significantly related to asthma pathology. However, the correlation between asthma and immune cells is affected by various interfering factors and reverse causal associations, which limits its causal inference in traditional epidemiological study [10]. Consequently, assessing which immune cell phenotypes have a causal relationship with asthma is yet to be explored.

Mendelian randomization, as a new epidemiological method to reveal causality, can effectively avoid the problems of interference factors, reverse causality, representativeness, and feasibility of RCT studies in observational studies [11]. Nonetheless, there is a lack of substantial research investigating the causal relationship between asthma and immune cells utilizing the mendelian randomization (MR) method. Consequently, we employed the mendelian randomization (MR) approach using single nucleotide polymorphisms (SNPs) as instrumental variables (IV_S) to investigate the potential causal link between immune cells and asthma. This study aims to offer novel insights into the prevention and management of asthma.

Table 1. The outcomes of mendelian randomization.

Exposure	nsnp	Method	*p*	OR (95% CI)	OR_lci95	OR_uci95
BAFF−R on B cell	20	Weighted median	0.014	1.002	1.000	1.003
	20	Inverse variance weighted	0.040	1.001	1.000	1.002
BAFF−R on IgD$^-$ CD27$^-$ B cell	19	Weighted median	0.008	1.002	1.001	1.003
	19	Inverse variance weighted	0.005	1.002	1.001	1.003
BAFF−R on IgD$^+$ CD24$^-$ B cell	21	Weighted median	0.013	1.002	1.000	1.003
	21	Inverse variance weighted	0.023	1.001	1.000	1.002
BAFF−R on IgD$^+$ CD38^{dim} B cell	24	Weighted median	0.005	1.002	1.001	1.003
	24	Inverse variance weighted	0.012	1.001	1.000	1.002
CD11b on CD14$^+$ monocyte B cell	20	Weighted median	0.071	0.999	0.997	1.000
	20	Inverse variance weighted	0.044	0.999	0.998	1.000
CD33^{br} HLA DR$^+$ CD14^{dim} Myeloid cell	18	Weighted median	0.004	1.003	1.001	1.005
	18	Inverse variance weighted	0.001	1.003	1.001	1.004
CD24 on IgD$^+$ CD38^{br} B cell	24	Weighted median	0.068	0.998	0.996	1.000
	24	Inverse variance weighted	0.001	0.998	0.996	0.999
CD25 on B cell	24	Weighted median	0.142	1.001	1.000	1.003
	24	Inverse variance weighted	0.043	1.001	1.000	1.002
CD25 on IgD$^+$ CD24$^-$ B cell	25	Weighted median	0.073	1.001	1.000	1.002
	25	Inverse variance weighted	0.010	1.001	1.000	1.002
CD25 on IgD$^+$ CD38$^-$ naive B cell	25	Weighted median	0.069	1.002	1.000	1.004
	25	Inverse variance weighted	0.036	1.002	1.000	1.003
CD25 on naive−mature B cell	24	Weighted median	0.299	1.001	0.999	1.003
	24	Inverse variance weighted	0.002	1.002	1.001	1.003
CD25 on transitional B cell	21	Weighted median	0.247	1.002	0.999	1.005
	21	Inverse variance weighted	0.004	1.003	1.001	1.005
CD28 on CD45RA$^+$ CD4$^+$ Treg	11	Weighted median	0.012	0.996	0.993	0.999
	11	Inverse variance weighted	0.003	0.997	0.994	0.999
CD33 on basophil Myeloid cell	21	Weighted median	0.118	1.001	1.000	1.002
	21	Inverse variance weighted	0.019	1.001	1.000	1.001
CD33 on CD14$^+$ monocyte	22	Weighted median	0.001	1.002	1.001	1.003
	22	Inverse variance weighted	0.024	1.001	1.000	1.002
CD33^{dim} HLA DR$^+$ CD11b$^-$ Myeloid cell	23	Weighted median	0.002	1.002	1.001	1.003
	23	Inverse variance weighted	0.035	1.001	1.000	1.002
CD33 on CD66b$^+$ myeloid cell	18	Weighted median	0.003	1.002	1.001	1.003
	18	Inverse variance weighted	0.026	1.001	1.000	1.002
CD38 on IgD$^-$ CD38^{dim}	18	Weighted median	0.363	1.001	0.999	1.002
	18	Inverse variance weighted	0.023	1.001	1.000	1.002
CD45 on NK cell	24	Weighted median	0.086	0.998	0.995	1.000
	24	Inverse variance weighted	0.001	0.996	0.994	0.998
CD86 on myeloid DC	21	Weighted median	0.013	1.004	1.001	1.007
	21	Inverse variance weighted	0.014	1.003	1.001	1.005
HLA DR on CD14$^-$ CD16$^+$ monocyte	10	Weighted median	0.013	1.006	1.001	1.010
	10	Inverse variance weighted	0.000	1.008	1.004	1.012
HLA DR$^+$ CD3$^-$ NK cell	25	Weighted median	0.003	0.996	0.994	0.999
	25	Inverse variance weighted	0.001	0.997	0.995	0.999
HLA DR$^+$ NK cell	19	Weighted median	0.000	0.995	0.993	0.998
	19	Inverse variance weighted	0.000	0.996	0.994	0.997
IgD$^-$ CD38$^-$ B cell	11	Weighted median	0.047	0.996	0.992	1.000
	11	Inverse variance weighted	0.011	0.997	0.994	0.999
IgD$^+$ CD38^{br} lymphocyte	26	Weighted median	0.025	1.003	1.000	1.005
	26	Inverse variance weighted	0.014	1.002	1.000	1.004
PDL−1 on CD14$^+$ CD16$^-$ monocyte	15	Weighted median	0.162	0.998	0.994	1.001
	15	Inverse variance weighted	0.002	0.996	0.994	0.999

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Table 1. Continued.

Exposure	nsnp	Method	*p*	OR (95% CI)	OR_lci95	OR_uci95
Plasmacytoid DC	24	Weighted median	0.025	0.998	0.995	1.000
	24	Inverse variance weighted	0.011	0.998	0.996	0.999
Transitional lymphocyte	25	Weighted median	0.000	1.007	1.003	1.010
	25	Inverse variance weighted	0.000	1.006	1.003	1.009

BAFF–R, B-cell Activating factor of the TNF family receptor; DC, dendritic cells; OR, odds ratio; CI, confidence interval.

Table 2. Specific information on immune cells.

Trait	Panel	Trait_type	Effect
BAFF−R on B cell	B cell	MFI	Risk factor
BAFF−R on IgD^- $CD27^-$ B cell	B cell	MFI	Risk factor
BAFF−R on IgD^+ $CD24^-$ B cell	B cell	MFI	Risk factor
BAFF−R on IgD^+ $CD38^{dim}$ B cell	B cell	MFI	Risk factor
CD24 on IgD^+ $CD38^{br}$ B cell	B cell	MFI	down
CD25 on B cell	B cell	MFI	Risk factor
CD25 on IgD^+ $CD24^-$ B cell	B cell	MFI	Risk factor
CD25 on IgD^+ $CD38^-$ naive B cell	B cell	MFI	Risk factor
CD25 on naive−mature B cell	B cell	MFI	Risk factor
CD25 on transitional B cell	B cell	MFI	Risk factor
CD38 on IgD^- $CD38^{dim}$ B cell	B cell	MFI	Risk factor
IgD^- $CD38^-$ B cell	B cell	Relative count	Protective factor
IgD^+ $CD38^{br}$ lymphocyte B cell	B cell	Relative count	Risk factor
Transitional lymphocyte B cell	B cell	Relative count	Risk factor
CD86 on myeloid DC	cDC	MFI	Risk factor
Plasmacytoid DC	cDC	Relative count	Protective factor
HLA DR on $CD14^-$ $CD16^+$ monocyte	Monocyte	MFI	Risk factor
PDL−1 on $CD14^+$ $CD16^-$ monocyte	Monocyte	MFI	Protective factor
CD11b on $CD14^+$ monocyte	Monocyte	MFI	Protective factor
$CD33^{br}$ HLA DR^+ $CD14^{dim}$ Myeloid cell	Myeloid cell	MFI	Risk factor
CD33 on basophil	Myeloid cell	MFI	Risk factor
CD33 on $CD14^+$ monocyte	Myeloid cell	MFI	Risk factor
$CD33^{dim}$ HLA DR^+ $CD11b^-$ Myeloid cell	Myeloid cell	MFI	Risk factor
CD33 on $CD66b^+$ myeloid cell	Myeloid cell	MFI	Risk factor
CD45 on NK cell	TBNK	MFI	Protective factor
HLA DR^+ $CD3^-$ NK	TBNK	Relative count	Protective factor
HLA DR^+ NK cell	TBNK	Absolute count	Protective factor
CD28 on $CD45RA^+$ $CD4^+$ Treg	Treg	MFI	Protective factor

MFI, median fluorescence intensity; TBNK, T cells, B cells, and natural killer cells.

Materials and Methods

Data Sources

Genetic data regarding asthma were retrieved from the IEU database (https://gwas.mrcieu.ac.uk/) accessed on December 5, 2023. The dataset included 56,087 asthma patients and 428,511 control subjects, all of whom are European origin, with a total of 9,587,836 SNPs. Moreover, genetic information concerning immune cells was obtained from published literature. The study included the effects of about 22 million variants in a cohort of 3757 subjects from Sardinians, focusing on 731 immune phenotypes, including 118 absolute cell counts (AC), 192 Relative cell counts (RC), median fluorescence intensity (MFI) of 389 surface antigens, and 32 morphological parameters [12]. Details about the data are given in **Supplementary Material 1**. As the study data used were from public databases, no further ethical approval was necessary.

IV_S Selection

To ensure the authenticity and accuracy of the causal association between immune cells and asthma, the following screening criteria were used to select the most suitable IV_S: ① To obtain more comprehensive data, the SNP_S most relevant to cellular immunity were selected from the genome-wide association studies (GWAS) data of immune

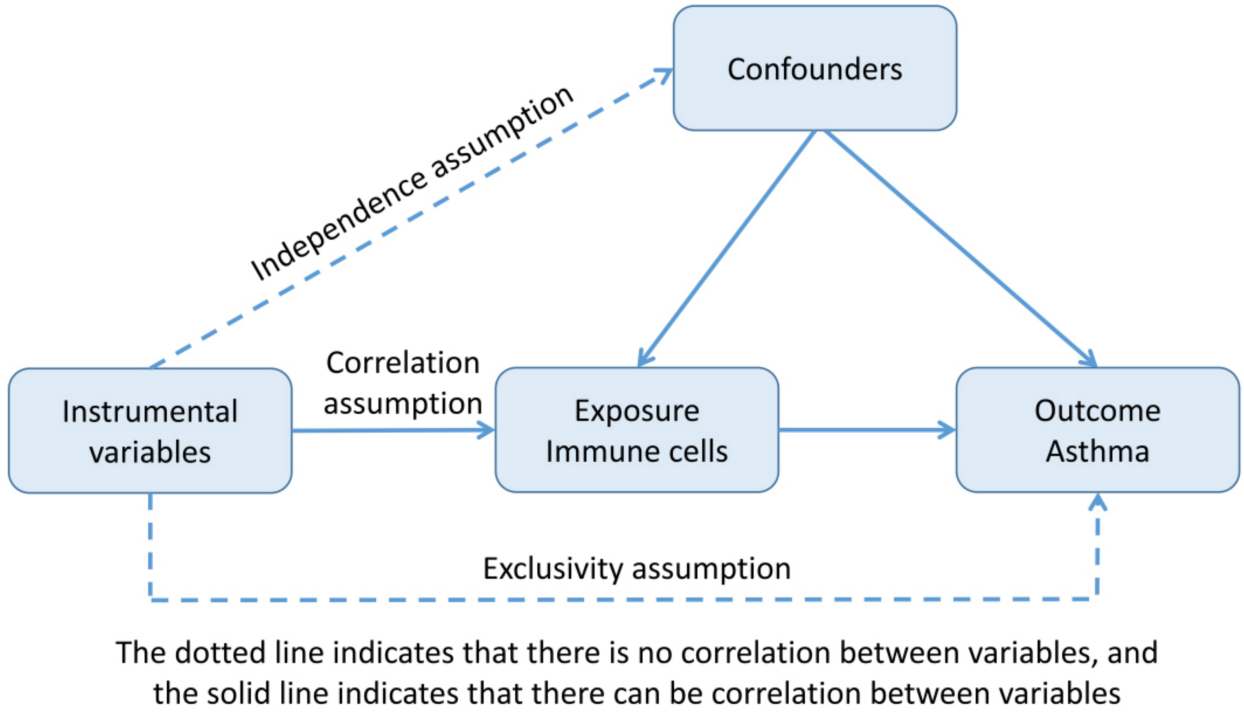

Fig. 2. Core assumptions regarding the selection of variations.

cells ($p < 5 \times 10^{-5}$). ② Based on the independence assumption of MR, each SNP must be independent of one another. To avoid linkage disequilibrium (LD) between individual IV_S, we established an R2 threshold of 0.001 and a domain width of 10,000 KB. ③ Additionally, to avoid the bias caused by weak IV_S, we considered IV with F statistic >10 as strong and reserved it for subsequent analysis. ④ Furthermore, to ensure that the same SNP has the same allele in the immune cell group and asthma group, the palindrome SNP was removed. The process of IV_S screening is shown in Fig. 1.

MR Assumptions

According to the requirements of MR analysis, genetic variation as IV_S must adhere to the following three core assumptions. ① Independence assumption: The selected IV_S were independent of the confounding factors affecting immune cells and asthma; ② Correlation assumption: There was a strong correlation between IV_S and asthma. The correlation strength between IV_S and exposure factors was evaluated using F statistics, where F >10 indicates fulfilment of the correlation assumption; ③ Exclusive assumption: The IV_S affect asthma through immune cells, without involvement through other ways. There should be no horizontal pleiotropy between IV_S and asthma (Fig. 2).

Statistical Methods

This study used R 4.3.2 software (http://www.Rproject.org) for MR analysis and sensitivity analysis. The original codes are given in **Supplementary Material 2.**

MR Analysis

In this study, inverse variance weighted (IVW), MR-Egger regression, weighted median (WMI), simple mode (SM), and weighted mode (WM), were used for MR analysis to investigate a causal correlation between immune cells and asthma. In case of no horizontal pleiotropy, the IVW result serves as the main result. This approach does not consider the intercept term in regression analysis and uses the reciprocal of the result variance as a weight for fitting. Based on the presence of heterogeneity, IVW selects either fixed or random effects models. The MR-Egger regression gives the advantage of yielding consistent estimates even when all IV_S show genetic pleiotropy. The main advantage of the WMI method lies in its ability to consistently estimate causal relationship even in the presence of over 50% invalid IV_S. SM is not as powerful as other methods, but its advantage lies in providing robustness against gene pleiotropy [13]. WM is sensitive to the difficult bandwidth selection for mode estimation [14]. Therefore, MR-Egger regression, WMI, SM, and WM were used as supplementary methods. A p-value < 0.05 was utilized to indicate statistically significant differences.

Sensitivity Analysis

Heterogeneity was assessed using Cochran's Q statistic, while outliers were identified through the mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) test and subsequently excluded. The MR analysis was then re-conducted. Horizontal pleiotropy was evaluated employing MR-Egger, where statistically significant

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differences in intercept terms indicated significant horizontal pleiotropy. The stability of MR results was assessed through the leave-one-out method, scatter plot, and funnel plot. The statistically significant difference was determined at $p < 0.05$.

Results

IV_S Selection Results

A total of 18,621 SNPs were selected as IV_S through screening of immune cell GWAS data, with each IV meeting F >10.

MR Analysis

IVW results showed that: B-cell Activating factor of the TNF family receptor (BAFF–R) on B cell (odds ratio (OR) = 1.001, 95% confidence interval (CI) 1.000~1.002, $p < 0.05$), BAFF–R on IgD^- $CD27^-$ B cell (OR = 1.002, 95% CI 1.001~1.003, $p < 0.05$), BAFF–R on IgD^+ $CD24^-$ B cell (OR = 1.001, 95% CI 1.000~1.002, $p < 0.05$), BAFF–R on IgD^+ $CD38^{dim}$ B cell (OR = 1.001, 95% CI 1.000~1.002, $p < 0.05$), $CD33^{br}$ HLA DR^+ $CD14^{dim}$ Myeloid cell (OR = 1.003, 95% CI 1.001~1.004, $p < 0.05$), CD25 on B cell (OR = 1.001, 95% CI 1.000~1.002, $p < 0.05$), CD25 on IgD^+ $CD24^-$ B cell (OR = 1.001, 95% CI 1.000~1.002, $p < 0.05$), CD25 on IgD^+ $CD38^-$ naive B cell (OR = 1.002, 95% CI 1.000~1.003, $p < 0.05$), CD25 on naive-mature B cell (OR = 1.002, 95% CI 1.001~1.003, $p < 0.05$), CD25 on transitional B cell (OR = 1.003, 95% CI 1.001~1.005, $p < 0.05$), CD33 on basophil (OR = 1.001, 95% CI 1.000~1.001, $p < 0.05$), CD33 on $CD14^+$ monocyte (OR = 1.001, 95% CI 1.000~1.002, $p < 0.05$), $CD33^{dim}$ HLA DR^+ $CD11b^-$ Myeloid cell (OR = 1.001, 95% CI 1.000~1.002, $p < 0.05$), CD33 on $CD66b^+$ myeloid cell (OR = 1.001, 95% CI 1.000~1.002, $p < 0.05$), CD38 on IgD^- $CD38^{dim}$ B cell (OR = 1.001, 95% CI 1.000~1.002, $p < 0.05$), CD86 on myeloid DC (OR = 1.003, 95% CI 1.001~1.005, $p < 0.05$), HLA DR on $CD14^-$ $CD16^+$ monocyte (OR = 1.008, 95% CI 1.004~1.012, $p < 0.05$), IgD^+ $CD38^{br}$ lymphocyte (OR = 1.002, 95% CI 1.000~1.004, $p < 0.05$), and transitional lymphocyte (OR = 1.006, 95% CI 1.003~1.009, $p < 0.05$) may be potential risk factors for asthma. Furthermore, CD11b on $CD14^+$ monocyte (OR = 0.999, 95% CI 0.998~1.000, $p < 0.05$), CD24 on IgD^+ $CD38^{br}$ B cell (OR = 0.998, 95% CI 0.996~0.999, $p < 0.05$), CD28 on $CD45RA^+$ $CD4^+$Treg (OR = 0.997, 95% CI 0.994~0.999, $p < 0.05$), CD45 on NK cell (OR = 0.998, 95% CI 0.996~0.999, $p < 0.05$), HLA DR^+ $CD3^-$ NK cell (OR = 0.997, 95% CI 0.995~0.999, $p < 0.05$), HLA DR^+ NK cell (OR = 0.996, 95% CI 0.994~0.997, $p < 0.05$), IgD^- $CD38^-$ B cell (OR = 0.997, 95% CI 0.994~0.999, $p < 0.05$), PDL–1 on $CD14^+$ $CD16^-$ monocyte (OR = 0.996, 95% CI 0.994~0.999, $p < 0.05$), and plasmacytoid DC (OR = 0.998, 95% CI 0.996~0.999, $p < 0.05$) were observed as protective factors for the development of asthma. The findings of mendelian randomization are shown in Table 1. The specific information on immune cells is shown in Table 2.

Sensitivity Analysis

Heterogeneity was assessed using Cochran's Q statistic. The results indicated that there was no significant heterogeneity between 5 merged IV_S ($p < 0.05$), and the fixed effect model was used for IVW analysis. Heterogeneity was found among 23 merged IV_S ($p > 0.05$), and a random effects model was used for IVW analysis. Outliers were identified using the MR-PRESSO method, with results suggesting the absence of outlier values ($p > 0.05$). We assessed horizontal pleiotropy through MR-Egger, and the findings did not show the presence of horizontal pleiotropy ($p > 0.05$, Table 3). Moreover, the stability of MR results was evaluated by the leave-one-out method, scatter plot, and funnel plot. The results indicated no significant impact of a single SNP on the stability of the study results (**Supplementary Material 3**, **Supplementary Material 4**, and **Supplementary Material 5**). Therefore, it is believed that the MR analysis results concerning immune cells and asthma demonstrate stability.

Discussion

We utilized publicly available genetic data to investigate the causal connection between 731 immune cell characteristics and asthma. To our knowledge, this is the first mendelian randomized analysis of the causal association between numerous immune cell phenotypes and asthma. Among the four identified immune characteristics (MFI, RC, AC, and MP), 28 immune cell phenotypes exhibited significant causal effects on asthma.

Our study revealed a positive correlation between the proportion of BAFF–R on B cells and the risk of asthma. BAFF–R, encoded by the TNFRSF13C gene, is one of the primary receptors for promoting the survival of B cells. BAFF is essential for the growth and maintenance of B cells, enhancing their longevity. A previous study has demonstrated a significant role of BAFF in promoting the growth and maturation of progenitor B cells in the mouse lung [15]. Furthermore, three distinct B cell immunophenotypes expressing BAFF–R (BAFF–R on IgD^- $CD27^-$B cell, BAFF–R on IgD^+ $CD24^-$ B cell, BAFF–R on IgD^+ $CD38^{dim}$) were identified as risk factors for the development of asthma.

Our study demonstrated that CD24 expression on IgD^+ CD38br B cells acts as a protective factor against asthma. This effect might be associated with the presence of CD24 molecules on the cell membrane surface. It suggests that $CD24^+$ could be a protective factor against asthma. A study has indicated that $CD24^{hi}CD27^+$B cells produce IL-10 to alleviate allergic and autoimmune inflammation [16].

Table 3. Mendelian sensitivity analysis.

Immune cells	Cochran's Q statistic			MR-Egger regression			MR-PRESSO	
	Method	Cochran's Q	*p*	Egger intercept	SE	*p*	RSSobs	*p*
BAFF−R on B cell	MR-Egger	1.59 × 10	5.96×10^{-1}	1.51×10^{-4}	3.34×10^{-4}	6.56×10^{-1}	2.00×10^{1}	5.70×10^{-1}
	IVW	1.62 × 10	6.47×10^{-1}					
BAFF−R on IgD^{-} $CD27^{-}$ B cell	MR-Egger	2.11 × 10	2.20×10^{-1}	-4.21×10^{-5}	3.94×10^{-4}	9.16×10^{-1}	2.21×10^{1}	3.98×10^{-1}
	IVW	2.12 × 10	2.71×10^{-1}					
BAFF−R on IgD^{+} $CD24^{-}$ B cell	MR-Egger	1.86 × 10	4.83×10^{-1}	1.87×10^{-4}	2.96×10^{-4}	5.35×10^{-1}	2.21×10^{1}	5.00×10^{-1}
	IVW	1.90 × 10	5.22×10^{-1}					
BAFF−R on IgD^{+} $CD38^{dim}$ B cell	MR-Egger	1.86 × 10	4.83×10^{-1}	1.87×10^{-4}	2.96×10^{-4}	5.35×10^{-1}	2.21×10^{1}	5.00×10^{-1}
	IVW	1.90 × 10	5.22×10^{-1}					
CD11b on $CD14^{+}$ monocyte B cell	MR-Egger	2.12 × 10	2.71×10^{-1}	-8.96×10^{-5}	3.28×10^{-4}	7.88×10^{-1}	2.25×10^{1}	4.24×10^{-1}
	IVW	2.13 × 10	3.23×10^{-1}					
$CD33^{br}$ HLA DR^{+} $CD14^{dim}$ Myeloid cell	MR-Egger	2.06 × 10	1.95×10^{-1}	3.49×10^{-5}	5.64×10^{-4}	9.51×10^{-1}	2.32×10^{1}	2.85×10^{-1}
	IVW	2.06 × 10	2.45×10^{-1}					
CD24 on IgD^{+} $CD38^{br}$ B cell	MR-Egger	2.97 × 10	1.26×10^{-1}	9.02×10^{-5}	3.58×10^{-4}	8.03×10^{-1}	3.17×10^{1}	2.09×10^{-1}
	IVW	2.98 × 10	1.56×10^{-1}					
CD25 on B cell	MR-Egger	1.69 × 10	7.70×10^{-1}	-1.16×10^{-4}	2.79×10^{-4}	6.82×10^{-1}	1.79×10^{1}	8.20×10^{-1}
	IVW	1.71 × 10	8.06×10^{-1}					
CD25 on IgD^{+} $CD24^{-}$ B cell	MR-Egger	1.94 × 10	6.81×10^{-1}	9.41×10^{-5}	2.90×10^{-4}	7.48×10^{-1}	2.14×10^{1}	7.25×10^{-1}
	IVW	1.95 × 10	7.27×10^{-1}					
CD25 on IgD^{+} $CD38^{-}$ naive B cell	MR-Egger	3.43 × 10	6.13×10^{-2}	-1.82×10^{-4}	4.18×10^{-4}	6.67×10^{-1}	3.71×10^{1}	9.00×10^{-2}
	IVW	3.46 × 10	7.52×10^{-2}					
CD25 on naive−mature B cell	MR-Egger	2.32 × 10	3.91×10^{-1}	-2.20×10^{-4}	2.77×10^{-4}	4.34×10^{-1}	2.82×10^{1}	4.07×10^{-1}
	IVW	2.39 × 10	4.12×10^{-1}					
CD25 on transitional B cell	MR-Egger	2.47 × 10	1.72×10^{-1}	7.63×10^{-5}	4.06×10^{-4}	8.53×10^{-1}	2.89×10^{1}	1.83×10^{-1}
	IVW	2.47 × 10	2.13×10^{-1}					
CD28 on $CD45RA^{+}$ $CD4^{+}$	MR-Egger	8.52	4.83×10^{-1}	-2.74×10^{-4}	5.24×10^{-4}	6.14×10^{-1}	1.06×10^{1}	6.08×10^{-1}
	IVW	8.79	5.52×10^{-1}					
CD33 on basophil	MR-Egger	1.81 × 10	5.14×10^{-1}	-2.15×10^{-4}	2.87×10^{-4}	4.64×10^{-1}	2.13×10^{1}	5.69×10^{-1}
	IVW	1.87 × 10	5.42×10^{-1}					
CD33 on $CD14^{+}$ monocyte	MR-Egger	3.08 × 10	5.78×10^{-2}	-4.05×10^{-4}	4.10×10^{-4}	3.35×10^{-1}	3.88×10^{1}	1.11×10^{-1}
	IVW	3.23 × 10	5.44×10^{-2}					
$CD33^{dim}$ HLA DR^{+} $CD11b^{-}$ Myeloid cell	MR- Egger	2.63 × 10	1.97×10^{-1}	-4.87×10^{-4}	3.74×10^{-4}	2.07×10^{-1}	3.82×10^{1}	1.56×10^{-1}
	IVW	2.84 × 10	1.64×10^{-1}					
CD33 on $CD66b^{+}$ myeloid cell	MR-Egger	1.54 × 10	4.98×10^{-1}	-5.02×10^{-4}	4.49×10^{-4}	2.81×10^{-1}	2.54×10^{1}	2.99×10^{-1}
	IVW	1.66 × 10	4.81×10^{-1}					

Table 3. Continued.

Immune cells	Cochran's Q statistic			MR-Egger regression			MR-PRESSO	
	Method	Cochran's Q	*p*	Egger intercept	SE	*p*	RSSobs	*p*
CD38 on IgD^{-} $CD38^{dim}$	MR-Egger	1.98 × 10	2.27×10^{-1}	-4.30×10^{-5}	3.94×10^{-4}	9.14×10^{-1}	2.27×10^{1}	3.29×10^{-1}
	IVW	1.99 × 10	2.81×10^{-1}					
CD45 on NK cell	MR-Egger	3.19 × 10	7.89×10^{-2}	-6.05×10^{-4}	3.95×10^{-4}	1.40×10^{-1}	3.81×10^{1}	7.10×10^{-2}
	IVW	3.53 × 10	4.84×10^{-2}					
CD86 on myeloid DC	MR-Egger	2.77 × 10	9.00×10^{-2}	1.43×10^{-4}	5.10×10^{-4}	7.82×10^{-1}	3.21×10^{1}	8.90×10^{-2}
	IVW	2.78 × 10	1.15×10^{-1}					
HLA DR on $CD14^{-}$ $CD16^{+}$ monocyte	MR-Egger	2.31 × 10	3.23×10^{-3}	-2.68×10^{-4}	8.91×10^{-4}	7.71×10^{-1}	4.35×10^{1}	5.90×10^{-2}
	IVW	2.34 × 10	5.42×10^{-3}					
HLA DR^{+} $CD3^{-}$ NK cell	MR-Egger	2.92 × 10	1.74×10^{-1}	8.02×10^{-5}	3.77×10^{-4}	8.33×10^{-1}	3.31×10^{1}	1.85×10^{-1}
	IVW	2.93 × 10	2.11×10^{-1}					
HLA DR^{+} NK cell	MR-Egger	1.93 × 10	3.12×10^{-1}	8.76×10^{-5}	4.41×10^{-4}	8.45×10^{-1}	2.07×10^{1}	4.36×10^{-1}
	IVW	1.93 × 10	3.72×10^{-1}					
IgD^{-} $CD38^{-}$ B cell	MR-Egger	9.42	3.99×10^{-1}	-1.29×10^{-4}	5.70×10^{-4}	8.26×10^{-1}	1.08×10^{1}	5.91×10^{-1}
	IVW	9.47	4.88×10^{-1}					
IgD^{+} $CD38^{br}$ lymphocyte	MR-Egger	3.39 × 10	8.57×10^{-2}	-3.86×10^{-4}	3.24×10^{-4}	2.45×10^{-1}	3.83×10^{1}	1.19×10^{-1}
	IVW	3.60 × 10	7.23×10^{-2}					
PDL−1 on $CD14^{+}$ $CD16^{-}$ monocyte	MR-Egger	1.14 × 10	5.76×10^{-1}	-5.86×10^{-4}	4.70×10^{-4}	2.34×10^{-1}	1.55×10^{1}	5.44×10^{-1}
	IVW	1.30 × 10	5.28×10^{-1}					
Plasmacytoid DC	MR-Egger	4.78 × 10	1.17×10^{-3}	-1.00×10^{-4}	3.94×10^{-4}	8.02×10^{-1}	5.12×10^{1}	2.00×10^{-3}
	IVW	4.79 × 10	1.73×10^{-3}					
Transitional lymphocyte	MR-Egger	3.13 × 10	1.16×10^{-1}	-2.54×10^{-4}	5.14×10^{-4}	6.26×10^{-1}	3.45×10^{1}	1.68×10^{-1}
	IVW	3.16 × 10	1.37×10^{-1}					

IVW, inverse variance weighted.

Concurrently, our study identified two B cell immunophenotypes (BAFF–R on IgD$^+$ CD24$^-$ B cell, CD25 on IgD$^+$ CD24$^-$) lacking expression of CD24 as risk factors for asthma. This provides additional evidence that the CD24 molecule plays a crucial role in suppressing the onset and progression of asthma.

Our study identified multiple B cell immunophenotypes expressing CD25 molecules as risk factors for asthma, including CD25 on B cell, CD25 on IgD$^+$ CD24$^-$, CD25 on IgD$^+$ CD38$^-$ naive, CD25 on naive-mature B cell, and CD25 on transitional, involving the immature, transitional, and mature stages of B cells. These findings are consistent with the existing research. CD25 positive is a marker of B cell activation [17]. CD25$^+$B cells exhibit increased capability in effective antigen presentation and show more mature phenotypes [18].

Our study revealed contradictions in some results regarding CD38 molecules. Notably, one immunophenotype of B cells expressing CD38 (CD24 on IgD$^+$CD38^{br}) was found to be a protective factor for asthma, whereas another immunophenotype of B cells expressing CD38 (IgD$^+$CD38^{br}%lymphocyte) was identified as a risk factor against asthma. Similarly, an immunophenotype of B cells lacking expression of CD38 (IgD$^-$ CD38$^-$ B cell) was recognized as a protective factor for asthma, while another immunophenotype of B cells lacking expression of CD38 (CD25 on IgD$^+$CD38$^-$ naive) was found as a risk factor for asthma. Moreover, two B cell immunophenotypes (BAFF–R on IgD$^+$ CD38^{dim}, CD38 on IgD$^-$ CD38^{dim}) with weak expression of CD38 were also identified as risk factors for asthma. This observation presents an interesting phenomenon. Previous study suggests that CD38 may alleviate the progress of asthma by restoring Th1/Th2 balance [19]. These contradictions could be caused by the methodological defects of our study or by the complex interactions between the immune system and asthma, warranting further study.

Our study found that CD86 expression on myoid DC was observed as a risk factor for asthma, consistent with previous studies. Some studies suggest that mature dendritic cells expressing CD86 can activate Th2 cells, which is closely associated with airway inflammation [20].

Our study indicated the percentage of plasmacytoid as a protective factor against the development of asthma, which aligns with previous research results. Plasmacytoid DCs exhibit lower immunostimulatory capabilities compared to traditional DCs. However, they possess the capacity to modulate immunity and facilitate the development of tolerance, contributing to their protective role against asthma [21].

Our study revealed HLA DR expression on CD14$^-$ CD16$^+$ monocytes as a risk factor and PDL–1 on CD14$^+$ CD16$^-$ monocytes as a protective factor for asthma. HLA DR belongs to class II human major histocompatibility antigen, responsible for recognizing and presenting exogenous antigens. Interestingly, our results demonstrated that HLA DR expression was observed not only in "risk" immune cells (HLA DR on CD14$^-$ CD16$^+$ monocyte) but also in "protective" immune cells (HLA DR$^+$ CD3$^-$ NK cell), indicating a potential association with HLA-DR polymorphism [22]. Furthermore, our study implies that CD14 and PDL–1 may act as protective factors against asthma development, while CD16 could serve as a risk factor, consistent with previous studies [23–25].

We found that CD11b expression on CD14$^+$ monocytes act as a protective factor for asthma. CD11b regulates various biological functions, and studies have shown that CD11b deficient mice exhibit increased susceptibility to inflammatory and autoimmune diseases [26–30]. On the contrary, other studies have indicated that CD11b promotes inflammatory damage, and blocking CD11b or its deficiency can reduce inflammation and tissue damage [31–33]. This contradictory phenomenon needs further study. Mendelian randomization results suggested that CD11b expressed on CD14$^+$ monocytes serve as a protective factor for asthma.

Our study demonstrated that three immune cells expressing CD33 were observed as risk factors for asthma, including CD33 on basophil, CD33 on CD14$^+$ monocyte, CD33^{dim} HLA DR$^+$ CD11b$^-$ myeloid cell, as well as CD33 on CD66b$^+$ myeloid cell and CD33^{br} HLA DR$^+$ CD14^{dim} myeloid cell.

Previous research has revealed that CD33 is typically expressed in most myeloid cells and exerts immunosuppressive effects [34], which is contradictory to our results, suggesting a discrepancy. This inconsistency may be caused by the methodological limitations in our study or the complex interactions between the immune system and asthma, which needs further investigation.

We identified three immune phenotypes as protective factors for the development of asthma: CD45 on NK cells, HLA DR$^+$ CD3$^-$ NK cells, and HLA DR$^+$ NK cells. In previous mouse models of allergic asthma, conflicting results regarding the role of NK cells have been obtained depending on allergen sensitization strategies and the tools used to deplete or inhibit NK cells [35]. NK cells either possess pro-inflammatory properties [36–38], have no detectable effect [39], or even promote the resolution or inhibition of allergic inflammation [40–42]. This variability may be caused by the heterogeneity of NK cell phenotypes or subsets across various asthma endotypes.

Furthermore, we identified CD28 expression on CD45RA$^+$CD4$^+$Treg as a protective factor for the development of asthma. Treg cells, a subgroup of CD4$^+$T cells, possess anti-inflammatory properties. Treg cells primarily inhibit autoreactive T cells and play an essential role in preventing autoimmune diseases in humans [43]. Mice lacking CD28 have a decrease in Treg cells in both the thymus and periphery, making them more susceptible to autoimmune diseases [44], which is consistent with our study. Additionally, CD45 is an essential protein on the surface of hema-

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

tological and immune system cells. However, its precise function and structure have not been widely understood. It exhibits negative and positive regulatory effects on T and B cell activation. Moreover, CD45 plays an essential role in regulating innate immune signaling, but its specificity in regulation needs further research [45].

Conclusion

In summary, we demonstrated the causal relationship between several immune phenotypes and asthma using mendelian randomization analysis, highlighting the complex interaction patterns between the immune system and asthma. Furthermore, our study significantly reduced the impact of inevitable confounding factors and other variables. These findings provide a novel pathway for researchers to explore the biological mechanisms underlying asthma and may lead to the exploration of early intervention and treatment.

Abbreviations

BAFF–R, B-cell Activating factor of the TNF family receptor; DC, dendritic cells; GWAS, genome-wide association studies; IV_S, instrumental variables; IVW, inverse variance weighted; MR-PRESSO, mendelian randomization pleiotropy residual sum and outlier; OR, odds ratio; SNPs, single nucleotide polymorphisms.

Availability of Data and Materials

The data supporting the current study are available from the corresponding author upon request.

Author Contributions

YSP, NNQ contributed to conceptualization, supervision and writing-review and editing. YSP, YBQ, NNQ contributed to formal analysis and writing-original draft. YSP and YBQ contributed to acquisition of data. YSP contributed to statistical analysis. All authors have been involved in revising it critically for important intellectual content. All authors gave final approval of the version to be published. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

The authors would like to thank the medical staff and participants for their contributions to this study.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.23812/j.biol.regul.homeost.agents.20243806.407.

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