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Causal relationship between immunophenotypes and rheumatoid arthritis

A 2-sample Mendelian randomization study

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Abstract

As previous studies have demonstrated an association between immune inflammation and rheumatoid arthritis (RA), our study aimed to lend novel insight by exploring the potential causal association between RA and different immunophenotypes. Data were obtained from the genome-wide association study (GWAS) from Finn Gen. The dataset of GWAS contains a cohort of 6236 RA cases and 147,221 controls in European population. Data on immune cell traits are publicly available from the GWAS catalog. A total of 731 immunophenotypes were included in this study including absolute cell counts (ACs), median fluorescence intensity, morphological parameters, and relative cell counts. Mendelian randomization analysis was performed by several methods, and sensitivity analysis and visualization of the results were also carried out. After being adjusted by false discovery rate (FDR), 6 immune phenotypes were significantly and causally associated with the development of RA: CD16 on $CD14^+$ $CD16^+$ monocytes (adjusted odds ratio [OR]: 0.950, 95% confidence interval [CI]: 0.924–0.977, $P = 4.04 \times 10^{-4}$), $CD62L^-CD86^+$ myeloid DC %DC (adjusted OR: 1.048, 95% CI: 1.021–1.076, $P = 4.29 \times 10^{-4}$), $CD62L^-CD86^+$ myeloid DC AC (adjusted OR: 1.050, 95% CI: 1.024–1.076, $P = 1.11 \times 10^{-4}$), $CD62L^-$ myeloid DC AC (adjusted OR: 1.067, 95% CI: 1.033–1.101, $P = 8.35 \times 10^{-5}$), DC AC (adjusted OR: 1.105, 95% CI: 1.062–1.149, $P = 7.73 \times 10^{-7}$), myeloid DC AC (adjusted OR: 1.060, 95% CI: 1.029–1.091, $P = 9.96 \times 10^{-5}$). In addition, we found that $CD62L^-$ Dendritic cell % increases with the onset of RA (OR: 1.136, 95% CI: 1.064–1.213, $P = 1.36 \times 10^{-4}$, $P_{FDR} = 0.099$). This study explored the association between different immunophenotypes and RA, which may lend some novel insights into RA pathogenesis and facilitate the development of new treatments.

Abbreviations: AC = absolute cell counts, cDCs = conventional Dendritic cells, DC = Dendritic cells, FDR = false discovery rate, GWAS = genome-wide association study, IVs = instrumental variables, IVW = inverse variance weighted, moDCs = monocyte-derived Dendritic cells, MP = morphological parameters, MR = Mendelian randomization, pDCs = plasmacytoid Dendritic cells, RA = rheumatoid arthritis, SM = simple mode, SNP = single-nucleotide polymorphism, TBNK = T cells, B cells, natural killer cells.

Keywords: immunophenotypes, Mendelian randomization, rheumatoid arthritis

1. Introduction

Rheumatoid arthritis (RA) is a common autoimmune rheumatic disease that can cause synovial inflammation, bone erosion, cartilage degradation, and joint destruction.[1,2] Extra-articular involvements mainly include the lungs, heart, kidneys, eyes, skin, etc.[3] Although the etiology of RA is not completely understood, genetic, environmental, immune, and stochastic factors may play important roles.[4] Various factors, for example, smoking, dust, genetic factors, and microorganisms, may induce the production of exogenous and endogenous antigens. The breakdown of tissue tolerance allows the entrance of innate and adaptive immune cells into the synovial membrane.[5,6] Antigen-presenting cells (primarily Dendritic cells [DCs]) present antigens to $CD4^+$ T cells, and $CD4^+$ T cells can differentiate into different subsets of T cells, including Th1, Th2, and Th17.[7] These T cell groups cooperate with mast cells, macrophages, and monocytes. These cells produce multiple proinflammatory mediators that act on fibroblast-like synoviocytes and osteoclasts, forming a vicious cycle that results more cytokine production and leads to sustained synovitis and bone erosion.[8] The pathogenesis of RA is driven by a complex interplay between the adaptive immune system that involves T cells and autoantibodies as well as the innate

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All data generated or analyzed during this study are included in this published article (and its supplementary information files).

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Key Points

1) Our study demonstrated the association between different immunophenotypes and RA by using MR.
2) The study may help improve our understanding of RA pathogenesis, which may subsequently improve its RA prevention and treatment.
3) Our observations may keep clinicians in mind the genetic factors when managing RA.

immune components such as myeloid cells and proinflammatory cytokines.[9] The complex genetic regulation of immune cell levels impacting immune-related diseases also suggests that their therapeutic modulation may be just as complex.[10,11] Subtle differences may also exist in the underlying immunological pathologies.

Mendelian randomization (MR) is a tool to overcome limitations inherent to observational studies and leverage human genetics to inform prevention strategies in RA. MR follows Mendel's laws of inheritance and is a commonly used statistical analysis method for inferring epidemiological etiologies. It uses genetic variation as instrumental variables (IVs), which capitalizes on the inherent properties of genetic variation to study the causal relationship between exposure and outcome.[12] The advantage of this approach is that it reduces confounding factors and reverses causation. Two-sample MR analyses can utilize single-nucleotide polymorphism (SNP)-exposure and SNP-outcome associations from independent genome-wide association study (GWAS) and combine them into a single causal estimate.[13]

Improved understanding of underlying immunological processes in RA may help us find novel and effective treatments.[6] Therefore, we conducted a 2-sample MR analysis using summary statistics from GWAS as a way to investigate the causal relationship between 731 immune cell traits and RA. We aimed to further explore the potential causal association between RA and different immunophenotypes.

2. Methods

2.1. Study design

We assessed the causal relationship between 731 immune cell traits and RA using a 2-sample MR analysis. There are 3 key assumptions that must be consistently followed when conducting MR analyses: (I) Relevance assumption: IVs and exposures are highly correlated with each other. (II) Independence assumption: IVs are not associated with confounders. (III) Exclusion restriction assumption: IVs affect outcome only through exposure and are not directly related to outcome.[14]

2.2. Data source

To avoid bias caused by overlapping exposure and outcome samples, we obtained exposure and outcome samples from different databases. GWAS summary data for each immunological trait are available from the GWAS Catalog (ID numbers from GCST0001391 to GCST0002121).[10] The 731 immune cell trait types contained 118 absolute cell counts (ACs), 389 median fluorescence intensities of surface antigens, 32 morphological parameters (MPs), and 192 relative cell counts. Immune cells with median fluorescence intensity, AC, and relative cell count characteristics include B cells, conventional dendritic cells (CDCs), T cells in the maturation phase, monocytes, myeloid cells, TBNK (T cells, B cells, natural killer cells), and Treg cells. Characterized by MP are CDC and TBNK cells. GWAS summary data for RA were obtained from FinnGen (finn-b-M13_RHEUMA), this GWAS dataset has 153,457 European participants (Case/Control = 6236/147,221). This dataset can be downloaded from the IEU GWAS database (https://gwas.mrcieu.ac.uk/).

2.3. MR analysis

In this study, MR analyses were carried out through the following steps: First, SNPs strongly associated with exposure were extracted. According to previous studies,[10,15,16] we used $P < 1 \times 10^{-5}$ as the screening criterion for SNPs when immune cells were considered as exposure, and $P < 5 \times 10^{-8}$ when RA was used as exposure. Meanwhile, leader SNPs should meet the requirement of low association with other SNPs in the region ($r^2 < 0.1$ in 500 kb window, when $P < 1 \times 10^{-5}$; $r^2 < 0.001$ in 10,000 kb window, when $P < 5 \times 10^{-8}$). Second, effect SNPs were extracted from the outcome GWAS dataset with a filtering criterion of minor allele frequency >0.01. Next, valid IVs were obtained after harmonizing exposure and outcome data as well as removing SNPs with *F*-statistics <10 or those that failed to harmonize. For the calculation of the *F*-statistics, we use the latest and most accurate methodology: $F = R^2(\text{N-K-1})/\text{K}(1\text{-}R^2)$. R^2 is the cumulative variance of exposure, K equals the total number of IVs, and N represents the total number of samples included in the exposure GWAS.[17] In addition, we used Phenoscanner V2 (https://www.phenoscanner.medschl.cam.ac.uk) to eliminate SNPs directly associated with confounders and outcomes. Valid IVs were then analyzed for MR using inverse variance weighted (IVW), MR-Egger regression, weighted median, weighted mode, and simple mode (SM) methods. Statistical methods for controlling the false discovery rate (FDR) have become popular and powerful tools for controlling the error rate. We used the Benjamini–Hochberg method to adjust *P*-values for the analysis of the MR with multiple exposures. Finally, sensitivity analysis and visualization of the MR results were performed. All statistical analyses in this study were performed using R v4.3.1 and the Two-Sample MR software package.

2.4. Ethical approval

Ethical approval was not required for the project because the data analyzed were publicly available and each data source had already received ethical approval from their respective institutions.

3. Result

3.1. Exploring the causal effect of immunophenotypes on RA

Finally, 56 immunophenotypes with $P < .05$ were obtained (Fig. 1) and their MR results were presented in Supplemental Digital Content (Additional file 1, Supplemental Digital Content, https://links.lww.com/MD/O832). After FDR adjustment, a total of 12 immune phenotypes that were causally associated with RA ($P_{FDR} < 0.2$) were identified (Figure 2, Supplemental Digital Content (Additional file 1, Supplemental Digital Content, https://links.lww.com/MD/O832)). Among them, 6 immunophenotypes showed significant causal relationship with RA ($P_{FDR} < 0.05$), and CD16 on $CD14^+CD16^+$ monocytes (monocyte panel) was protective against RA, $CD62L^-CD86^+$ myeloid DC % DC (conventional DC [cDC] panel), $CD62L^-CD86^+$ myeloid DC AC (cDC panel), $CD62L^-$ myeloid DC AC (cDC panel), DC AC (cDC panel), myeloid DC AC (cDC panel) were risk factors for RA. The odds ratio (OR) of CD16 on $CD14^+$ $CD16^+$ monocyte on RA was estimated to be 0.950 (95% confidence interval [CI]: 0.924–0.977, $P = 4.04 \times 10^{-4}$, $P_{FDR} = 0.045$) using the IVW approach. The results were similar to IVW: MR-Egger (OR: 0.932, 95% CI: 0.883–0.984, $P = .013$); weighted median (OR: 0.914, 95% CI: 0.874–0.956, $P = 8.31 \times 10^{-5}$);

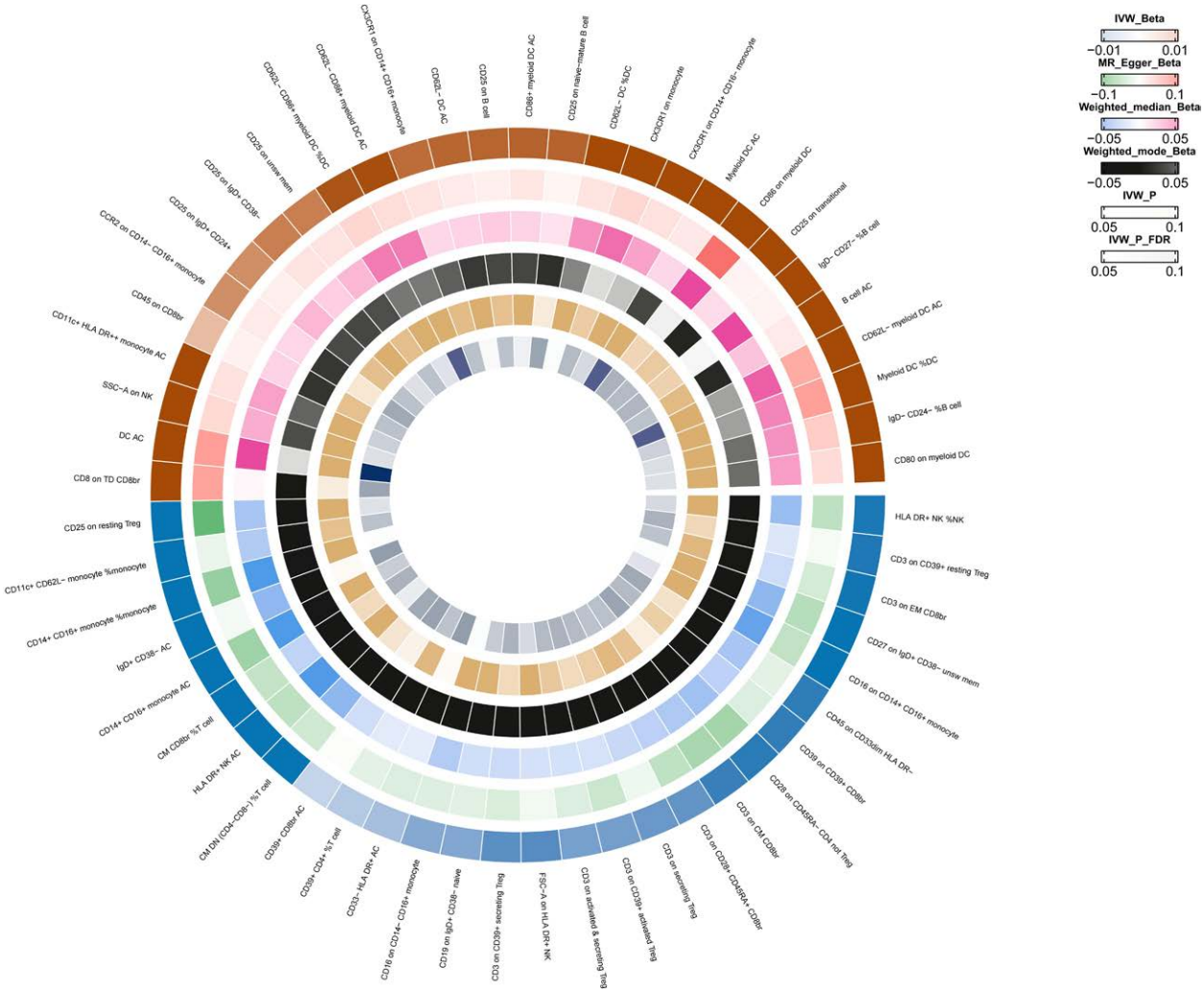

Figure 1. Heatmap for the causal effect of immune cell traits on RA. The outer circle represents beta of IVW, whereas the inner circle represents P_{FDR}. FDR = false discovery rate, IVW = inverse variance weighted, RA = rheumatoid arthritis.

weighted mode (OR: 0.921, 95% CI: 0.873–0.972, $P = .004$); SM (OR: 0.913, 95% CI: 0.835–0.997, $P = .047$). The OR for $CD62L^-CD86+$ myeloid DC % DC on RA was 1.048 (95% CI: 1.021–1.076, $P = 4.29 \times 10^{-4}$, $P_{FDR} = 0.045$) when using the IVW approach. The remaining 4 results were similar: MR-Egger (OR: 1.052, 95% CI: 1.004–1.102, $P = .037$); weighted median (OR: 1.079, 95% CI: 1.037–1.123, $P = 1.53 \times 10^{-4}$); weighted mode (OR: 1.067, 95% CI: 1.024–1.111, $P = .003$); SM (OR: 1.067, 95% CI: 0.992–1.146, $P = .084$). The OR of $CD62L^-CD86^+$myeloid DC AC on RA were as follows: IVW: (OR: 1.050, 95% CI: 1.024–1.076, $P = 1.11 \times 10^{-4}$, $P_{FDR} = 0.020$); MR-Egger (OR: 1.043, 95% CI: 0.997–1.090, $P = .069$); weighted median (OR: 1.079, 95% CI: 1.037–1.122, $P = 1.53 \times 10^{-4}$); weighted mode (OR: 1.067, 95% CI: 1.028–1.108, $P = 9.30 \times 10^{-4}$); SM (OR: 1.039, 95% CI: 0.962–1.121, $P = .332$). Despite the $P > .05$ of SM, it was directionally consistent with the other 4 methods. The 5 results are similar for $CD62L^-$ myeloid DC AC on RA: IVW: (OR: 1.067, 95% CI: 1.033–1.101, $P = 8.35 \times 10^{-5}$, $P_{FDR} = 0.020$); MR-Egger (OR: 1.109, 95% CI: 1.040–1.183, $P = .003$); weighted median (OR: 1.094, 95% CI: 1.046–1.145, $P = 8.70 \times 10^{-5}$); weighted mode (OR: 1.094, 95% CI: 1.045–1.145, $P = 2.87 \times 10^{-4}$); SM (OR: 1.061, 95% CI: 0.968–1.163, $P = .047$). The results of 5 methods were consistent for DC AC on RA: IVW (OR: 1.105, 95% CI: 1.062–1.149, $P = 7.73 \times 10^{-7}$, $P_{FDR} = 5.65 \times 10^{-4}$); MR-Egger (OR: 1.126, 95% CI: 1.037–1.222, $P = .007$); weighted median (OR: 1.143, 95% CI: 1.077–1.211, $P = 8.43 \times 10^{-6}$); weighted mode (OR: 1.128, 95% CI: 1.055–1.206, $P = 9.79 \times 10^{-4}$); SM (OR: 1.128, 95% CI: 1.021–1.247, $P = .022$). The results of the 5 methods of myeloid DC AC on RA were directionally consistent, although the 4 methods except IVW were not statistically significant: IVW (OR: 1.060, 95% CI: 1.029–1.091, $P = 9.96 \times 10^{-5}$, $P_{FDR} = 5.65 \times 10^{-4}$); MR-Egger (OR: 1.029, 95% CI: 0.986–1.073, $P = .196$); weighted median (OR: 1.024, 95% CI: 0.974–1.076, $P = .360$); weighted mode (OR: 1.033, 95% CI: 0.991–1.077, $P = .128$); SM (OR: 1.039, 95% CI: 0.952–1.135, $P = .395$). Furthermore, the MR-Egger intercept and the MR PRESSO Global Test excluded the possibility of horizontal pleiotropy. Detailed information from the sensitivity analysis demonstrated the robustness of the observed causal associations (Additional file 2, Supplemental Digital Content, https://links.lww.com/MD/O833). Scatter plots, funnel plots, and leave-one-out analyses also showed the stability of the results (Additional file 3, Supplemental Digital Content, https://links.lww.com/MD/O834).

3.2. Exploring the causal effect of RA on immunophenotypes

When exploring the causal effect of RA on immunophenotypes, we found 36 immunophenotypes with $P < .05$, and their MR results were presented in Supplemental Digital Content

(Additional file 4, Supplemental Digital Content, https://links.lww.com/MD/O835). After FDR adjustment, we found that RA was positively correlated only with CD62L⁻Dendritic Cell %Dendritic Cell (FDR < 0.2). The OR of RA on CD62L⁻ Dendritic Cell %Dendritic Cell was estimated to be 1.136 (95% CI: 1.064–1.213, $P = 1.36 \times 10^{-4}$, $P_{FDR} = 0.099$) by using the IVW method. The other 4 results were similar to IVW, albeit SM was not statistically significant: MR-Egger (OR: 1.145, 95% CI: 1.035–1.267, $P = .026$); weighted median (OR: 1.147, 95% CI: 1.057–1.244, $P = 9.97 \times 10^{-4}$); weighted mode (OR: 1.154, 95% CI: 1.064–1.252, $P = 5.28 \times 10^{-3}$); SM (OR: 1.151, 95% CI: 0.965–1.374, $P = .146$) (Table 1). The MR-Egger's intercept and the MR PRESSO Global Test excluded the possibility of horizontal pleiotropy (Table 2). Sensitivity analysis also proved the robustness of the results. Scatter plots (Fig. 3A), funnel plots (Fig. 3B), and leave-one-out analyses (Fig. 3C) also indicate the stability of the results. Figure 3D presents the forest plot of RA on CD62L⁻Dendritic Cell %Dendritic Cell.

4. Discussion

Based on a publicly available genetic database, we explored the causal relationship between 731 immune cells and RA using the MR approach. As far as we know, this is the first MR study to investigate the causal relationship between different immunophenotypes and RA. It revealed a statistically significant causal effect of 6 immunophenotypes on RA (FDR < 0.05), whereas

exposure	nsnp	method	pval	OR(95% CI)
CD14+ CD16+ monocyte %monocyte	66	Weighted median	**<0.001**	0.890 (0.848 to 0.934)
	66	Inverse variance weighted	**<0.001**	0.935 (0.900 to 0.972)
CD16 on CD14− CD16+ monocyte	173	Weighted median	**<0.001**	0.953 (0.926 to 0.980)
	173	Inverse variance weighted	**<0.001**	0.970 (0.953 to 0.988)
CD16 on CD14+ CD16+ monocyte	76	Weighted median	**<0.001**	0.914 (0.874 to 0.956)
	76	Inverse variance weighted	**<0.001**	0.950 (0.924 to 0.977)
CD25 on IgD+ CD38−	39	Weighted median	**0.039**	1.029 (1.001 to 1.058)
	39	Inverse variance weighted	**0.005**	1.035 (1.011 to 1.061)
CD25 on resting Treg	27	Weighted median	0.146	0.946 (0.877 to 1.020)
	27	Inverse variance weighted	**0.003**	0.827 (0.728 to 0.939)
CD27 on IgD+ CD38− unsw mem	60	Weighted median	**0.001**	0.932 (0.894 to 0.972)
	60	Inverse variance weighted	**<0.001**	0.950 (0.921 to 0.979)
CD62L− CD86+ myeloid DC %DC	87	Weighted median	**<0.001**	1.079 (1.037 to 1.123)
	87	Inverse variance weighted	**<0.001**	1.048 (1.021 to 1.076)
CD62L− CD86+ myeloid DC AC	85	Weighted median	**<0.001**	1.079 (1.037 to 1.122)
	85	Inverse variance weighted	**<0.001**	1.050 (1.024 to 1.076)
CD62L− DC %DC	62	Weighted median	**0.003**	1.067 (1.022 to 1.114)
	62	Inverse variance weighted	**<0.001**	1.055 (1.022 to 1.089)
CD62L− DC AC	62	Weighted median	0.192	1.026 (0.987 to 1.068)
	62	Inverse variance weighted	**0.001**	1.043 (1.017 to 1.070)
CD62L− myeloid DC AC	65	Weighted median	**<0.001**	1.094 (1.046 to 1.145)
	65	Inverse variance weighted	**<0.001**	1.067 (1.033 to 1.102)
CD80 on myeloid DC	38	Weighted median	**0.045**	1.059 (1.001 to 1.120)
	38	Inverse variance weighted	**0.004**	1.065 (1.020 to 1.113)
CD86+ myeloid DC AC	68	Weighted median	0.228	1.027 (0.983 to 1.073)
	68	Inverse variance weighted	**0.002**	1.044 (1.016 to 1.073)
DC AC	48	Weighted median	**<0.001**	1.143 (1.077 to 1.211)
	48	Inverse variance weighted	**<0.001**	1.105 (1.062 to 1.149)
HLA DR+ NK AC	61	Weighted median	**<0.001**	0.906 (0.854 to 0.960)
	61	Inverse variance weighted	**0.002**	0.941 (0.906 to 0.979)
IgD− CD24− %B cell	19	Weighted median	**0.038**	1.066 (1.004 to 1.133)
	19	Inverse variance weighted	**0.005**	1.065 (1.019 to 1.112)
Myeloid DC AC	54	Weighted median	0.360	1.024 (0.974 to 1.076)
	54	Inverse variance weighted	**<0.001**	1.060 (1.029 to 1.091)
SSC−A on NK	26	Weighted median	0.201	1.050 (0.975 to 1.131)
	26	Inverse variance weighted	**0.005**	1.081 (1.025 to 1.141)

Figure 2. Forest plots showed the causal associations between immune cell traits and RA by using different methods. CI = confidence interval; FDR = false discovery rate, RA = rheumatoid arthritis.

Table 1

MR results of the causal effect of rheumatoid arthritis (RA) on CD62L⁻ Dendritic cell %Dendritic cell.

Outcome	Exposure	Method	or	or_lci95	or_uci95	pval	FDR
CD62L⁻ Dendritic cell %Dendritic cell	RA	MR-Egger	1.145	1.035	1.267	0.026	
		Weighted median	1.147	1.057	1.244	9.97×10^{-4}	
		Inverse variance weighted	1.136	1.064	1.213	1.36×10^{-4}	0.099
		Simple mode	1.151	0.965	1.374	0.146	
		Weighted mode	1.154	1.064	1.252	5.28×10^{-3}	

Table 2

Pleiotropic and heterogeneous results of the causal effect of rheumatoid arthritis (RA) on CD62L⁻ Dendritic cell %Dendritic cell.

		Pleiotropy					Heterogeneity					
		MR-Egger_intercept			MR PRESSO Global Test		IVW			MR-Egger		
Outcome	Exposure	Intercept	SE	*P*	RSSobs	*P*	Q	Q_df	Q_pval	Q	Q_df	Q_pval
CD62L⁻ Dendritic cell absolute count	RA	0.005	0.015	.75	12.496	.571	10.284	11	0.505	10.174	10	0.425

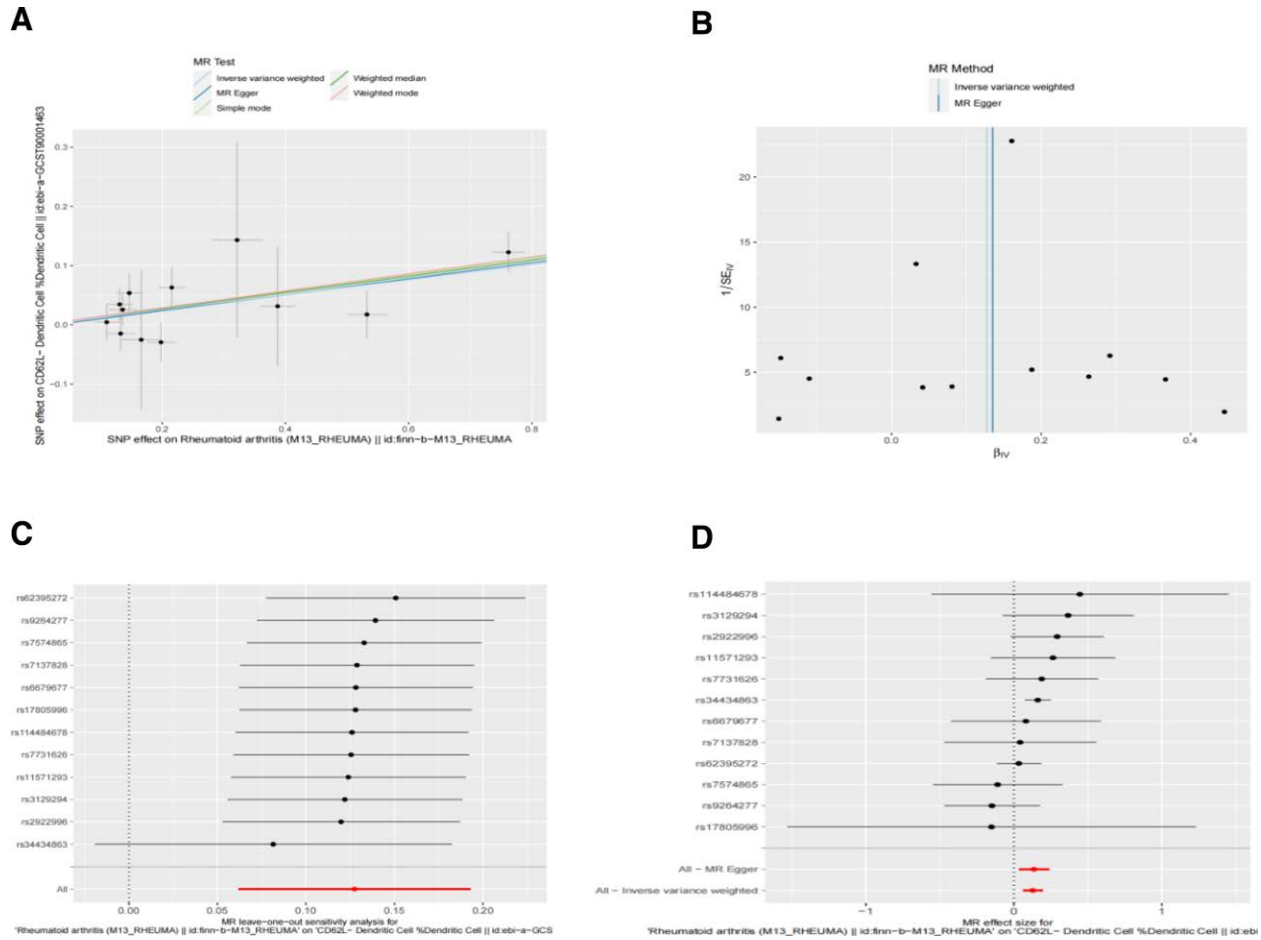

Figure 3. Visualization of the causal effects of RA on CD62L⁻ Dendritic cell %Dendritic cell. (A) Scatter plot. (B) Funnel plot. (C) Leave-one-out plot. (D) Forest plot. RA = rheumatoid arthritis.

RA was causally associated with only one immunophenotype (FDR < 0.2).

Monocytes are intrinsic immune cells of the mononuclear phagocyte system.[18] Monocytes were identified on the basis of MPs and HLA-DR positivity. They can be categorized as classical monocyte ($CD14^{+}CD16^{-}$), intermediate monocyte ($CD14^{++}CD16^{+}$), and nonclassical monocyte ($CD14^{+}CD16^{+}$).[19,20] Our study has shown that CD16 on $CD14^{+}CD16^{+}$ and CD16 on $CD16^{+}$ monocytes play a protective role for RA. Our MR analysis is contradictory to some of the existing observational studies that have proposed a negative correlation between immunophenotypes and RA, which is consistent with the observations by Tsukamoto et al[21] and Thomas et al[22] in that there was a positive correlation between intermediate monocytes and the risk of developing RA. Luo et al[23] found that CD64 on $CD14^{++}CD16^{+}$ monocytes are positively correlated with the development of RA. Increased levels of CD16 expression on $CD14^{++}$ monocytes in RA patients may lead to an intensified response of immune complexes and the excessive production of proinflammatory mediators.[24] However, more studies are warranted to confirm the association between monocytes and RA.

Myeloid cells are one of the major cellular subtypes that are primarily involved in the intrinsic immune responses mediated by monocytes, macrophages, granulocytes, and DCs.[25] DCs play a central role in immune response in that they can present antigens, stimulate naive T cells, and are involved in both innate and acquired immunities.[26,27] DCs can be categorized into classical or cDCs, plasmacytoid DCs (pDCs), and monocyte-derived DCs according to developmental origin.[28,29] Previous studies have demonstrated that high concentrations of both myeloid and plasmacytoid DCs are present in RA synovium.[30] We further identified 5 immunophenotypes among them: $CD62L^{-}CD86^{+}$myeloid DC %DC (cDC panel), $CD62L^{-}CD86^{+}$myeloid DC AC (cDC panel), $CD62L^{-}$myeloid DC AC (cDC panel), DC AC (cDC panel), myeloid DC AC (cDC panel). Overall, our findings are generally consistent with the above observational studies that cDCs may be associated with

an increased risk of developing RA. More studies are required to explore the underlying mechanisms underlying the role of different immunophenotypes in RA pathogenesis. In addition, we found that CD62L$^-$Dendritic Cell %Dendritic Cell increases with the onset of RA (FDR < 0.2).

We conducted a comprehensive 2-sample MR analysis based on a published GWAS database with a large sample size. It is the first MR study to evaluate the potential causal associations between different immunophenotypes and the development of RA. At the same time, we utilized multiple MR methods for causal inference and performed sensitivity analyses using a variety of methods, and the results are robust and not affected by pleiotropy. Our findings lend novel insight into the causal relationship between different immunophenotypes and RA.

However, this study has some limitations. First, the study was conducted on a European population and the results have not been verified on other populations. Secondly, we adopted a broader threshold, which may increase the likelihood of false positivity. Second, we searched only one GWAS database of RA and chose the cases of the most recent years. As the database are being updated continuously and the current conclusions are based on the existing data, the conclusions may change in the future to some extent. Third, some of the conclusions in our study were inconsistent with previous studies, which may explain the discordance between the observational studies and MR analysis.

In conclusion, we used a relatively novel statistical analysis to explore the relationship between 731 immune cell traits and the development of RA, which may significantly reduce the influence of confounding factors and reverse causality that is inevitable in traditional randomized controlled trials. This study may provide more valuable clues for future investigations into the pathogenesis, prevention, and treatment of RA.

Author contributions

Conceptualization: Hang Ma, Zhenyu Liu, Shuai Su, Xiangbiao He.
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Formal analysis: Hang Ma, Zhenyu Liu, Shuai Su, Xiangbiao He, Shuang Sun.
Investigation: Hang Ma, Zhenyu Liu.
Methodology: Hang Ma, Zhenyu Liu, Shuai Su, Xiangbiao He, Shuang Sun.
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Software: Hang Ma, Zhenyu Liu, Shuai Su, Xiangbiao He, Shuang Sun.
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Resources: Shuai Su, Xiangbiao He, Shuang Sun.
Writing – review & editing: Shuai Su, Xiangbiao He, Shuang Sun.

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