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Original Article

Causal Relationships between Circulating Immune Cell Traits and the Risk of Rheumatoid Arthritis and Osteoarthritis: A Bidirectional Two-Sample Mendelian Randomization Study

*#Dujuan Mao [1], #Shan Li [1], Xiufang Li [1], Lijuan You [1], Jiaqi Yu [1], Yaohua Wu [1], *Quanshui Hao [1], *Heng Du [2]*

1. *Department of Anesthesiology, Huanggang Central Hospital Affiliated to Yangtze University, Huanggang 438000, Hubei, China*
2. *Department of Gastrointestinal Surgery, Huanggang Central Hospital Affiliated to Yangtze University, Huanggang, 438000, Hubei, China*

***Corresponding Authors:** Email: haoquanshui@163.com, duheng_hg@yeah.net
# These authors contributed equally to this work

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Abstract

Background: Rheumatoid arthritis (RA) and osteoarthritis (OA) are prevalent chronic joint disorders with immunological pathogenesis. However, the causal relationships between circulating immune cells and them remain largely unknown. Therefore, we conducted a bidirectional two-sample Mendelian randomization (MR) study to determine their causal relationship.

Methods: Genome-wide association study summary statistics were extracted from publicly available databases regarding immune cell phenotypes, RA, and OA. MR analysis was conducted using five MR methods, with inverse-variance-weighted (IVW) as the primary analysis method. False discovery rate correction (FDR) was used to reduce the likelihood of type 1 errors. We also conducted MR-Egger intercept tests to evaluate horizontal pleiotropy.

Results: After FDR adjustment of the *P* values for the IVW method, the CD27 expression on memory B cells was negatively related to the risk of RA ($P < 0.001$), and the human leukocyte antigen (HLA)--DR expression on CD14+ monocytes was negatively related to the risk of OA ($P < 0.001$). We also found that RA was negatively associated with the expression of HLA-DR on myeloid dendritic cells ($P < 0.001$), but significant horizontal pleiotropy was observed.

Conclusion: Our study demonstrates a causal relationship between specific immune cell traits and RA as well as OA, providing further insight into the role of immune cells in the pathogenesis of these disorders.

Keywords: Immune cells; Rheumatoid arthritis; Osteoarthritis; Mendelian randomization; Causal association

Introduction

Rheumatoid arthritis (RA) and osteoarthritis (OA) are two of the most common chronic joint disorders. As of 2020, 17.6 million people had RA and 595 million had OA (1, 2). RA is an autoimmune disease that primarily affects the synovial joints, causing inflammation, pain, and joint de-

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struction (3), while OA is a degenerative condition characterized by the progressive loss of articular cartilage, subchondral bone remodeling, and synovial inflammation (4). Both diseases lead to significant disability, reduced quality of life, and substantial economic burden (5). Despite advances in understanding the pathogenesis of RA and OA, current treatment options remain limited, focusing mainly on symptom management and slowing disease progression (6, 7).

The pathogenesis of RA and OA involves a complex interplay between genetic, environmental, and immunological factors (8, 9). In RA, the immune system mistakenly attacks the body's own tissues, leading to chronic inflammation in the synovial membrane. This inflammation is driven by the infiltration of various immune cells, such as T cells, B cells, and macrophages, which produce pro-inflammatory cytokines and chemokines (10). Similarly, in OA, low-grade inflammation in the synovium contributes to the progression of the disease, with the involvement of innate and adaptive immune cells (11). Although immunotherapies targeting specific cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-6, have shown promise in the treatment of RA, their efficacy is limited, and many patients do not respond adequately (12). Moreover, the causal relationships between specific immune cell subpopulations in the circulation and the development of RA and OA remain largely unknown.

Mendelian randomization (MR) is a powerful genetic epidemiological approach that uses genetic variants as instrumental variants (IVs) to assess the causal effects of modifiable risk factors on disease outcomes (13). By leveraging the random assignment of genetic variants at conception, MR minimizes confounding factors and avoids reverse causality, providing robust evidence for causal inference (14, 15). MR studies have been increasingly applied to investigate potential risk factors and underlying mechanisms for RA and OA, such as lifestyle-related risk factors, nutrients, metabolites, and inflammatory biomarkers (16-20). However, the causal role of specific circulating immune cell subpopulations in the development of these diseases has not been comprehensively examined using MR analysis.

Therefore, we aimed to investigate the causal relationships between various circulating immune cell traits and the risk of developing RA and OA using a bidirectional two-sample MR approach.

Methods

Study design

This was a bidirectional two-sample MR study. A forward MR analysis considered immune cell traits as exposures, and RA as well as OA as outcomes, while a reverse MR analysis considered RA and OA as exposures, and immune cell traits as outcomes. Single nucleotide polymorphisms (SNPs) are used as IVs in MR, and valid IVs must meet the following three assumptions (21-23): the relevance assumption requires IVs to be robustly associated with exposure; the independence assumption demands that IVs be independent of any confounding factors; the exclusion limitation assumption states that IVs only affect outcomes through the risk factor, not other pathways.

Data sources

Genome-wide association study (GWAS)-summary statistics for each immune trait are obtained through the GWAS Catalog (accession numbers GCST90001391 to GCST90002121) (24). We analyzed a total of 731 immune cell phenotypes, which included absolute counts (n = 118), relative counts (n = 192), fluorescence intensities (n = 389), and morphological parameters (n = 32). In the GWAS on immune traits, 3,757 European individuals were analyzed using 20,143,392 SNPs and the associations were examined after adjusting for covariates such as sex, age, and age^2 (25). GWAS-summary statistics on RA and OA are available through the GWAS catalog (accession numbers: GCST90013534 and GCST007092). There are 14,361 cases and 43,923 controls for RA (26), 39,427 cases and 378,169 controls for OA (27). The GWAS statistics were all derived from European individuals.

Ethical approval for this project was not required as the data analyzed were publicly available, and each source of data had already obtained ethical approval from their respective institutions.

Selection of IVs

In accordance with previous studies (28, 29), we extracted significant SNPs at a significance level of 1×10^{-5} for each immune trait. With the PLINK clumping procedure, we identified independent SNPs based on linkage imbalance (LD) r^2 threshold < 0.1 with a window size of 500 kb (28), where LD r^2 was calculated based on the 1000 Genomes Project reference panel (30). For GWAS data of RA and OA, we set a significance level of 5×10^{-8}, and a LD $r^2 < 0.0001$ with a window size of 1000 kb. An F statistic was calculated for each SNP to evaluate its strength. To avoid weak instrumental bias, only SNPs with F > 10 were included in the analysis as IVs (31). Additionally, we used the Phenoscanner V2 to eliminate SNPs that were directly associated with confounders and outcomes (32, 33).

Statistical analysis

Five MR analyses were conducted to examine causal relationships between various immune cell phenotypes and RA and OA traits: inverse variance weighting (IVW), MR Egger, weighted median, simple mode, and weighted mode, with IVW (random effects) being the primary analysis (34). To minimize the likelihood of type 1 errors in this study, we used the false discovery rate (FDR) correction, and a $P_{FDR} < 0.1$ was considered significant. Cochran's Q statistic was employed to assess heterogeneity among selected IVs, and a P value < 0.05 indicates the existence of heterogeneity (34). We evaluated horizontal pleiotropy using the MR-Egger intercept test. If the MR-Egger intercept was significantly ($P < 0.05$), it suggested that association results may be influenced by horizontal pleiotropic effects of other traits (35). In order to visualize our results, forest plots, heatmaps, and scatter plots were generated. Statistical analyses were conducted using R version 4.3.1 (http://www.Rproject.org).

Results

The causal effects of immune cell phenotypes on RA

We first examined the causal effects of immune cell phenotypes on RA, and the IVW method was used as the primary analysis method. A total of 57 immune cell phenotypes were found to be associated with RA ($P < 0.05$) (Fig. 1A). However, following FDR adjustment, only one was found to be causally associated with RA ($P_{FDR} < 0.1$), i.e., CD27 on memory B cells. As shown in Fig. 1B, the expression of CD27 on memory B cells was negatively related to the risk of RA (odds ratio [OR] = 0.92; 95% confidence interval [CI], 0.88-0.95; $P < 0.001$; $P_{FDR} = 0.009$). In addition, we performed MR analysis using four other methods, and the results showed that the MR Egger and weighted median methods yielded comparable results to the IVW method (Fig. 1B). Regardless, the causal relationships suggested by the various MR methods are consistent with those suggested by the IVW method. Fig. 1C illustrate the scatter plots for the MR analysis of the immune cell phenotype.

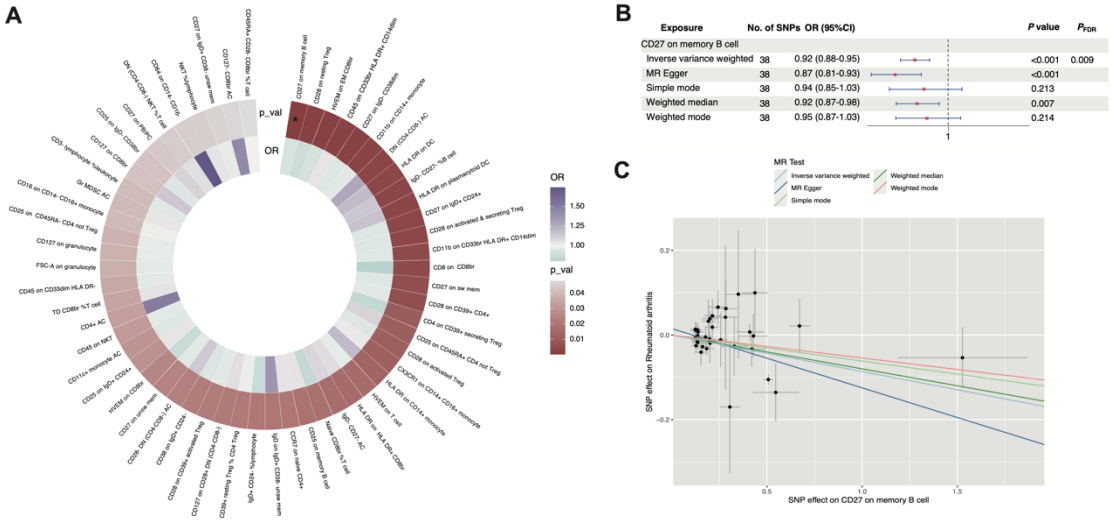

Fig. 1: The causal effect of circulating immune cells on rheumatoid arthritis. (A) Heatmap for the causal effect of circulating immune cells on rheumatoid arthritis using the inverse variance weighting (IVW) method. The outer circle represents P values, whereas the inner circle represents ORs. * indicates the FDR corrected *P* values < 0.1. (B) Forest plot and (C) scatter plot for the causal effect of CD27 expression on circulating memory B cells on rheumatoid arthritis. MR, mendelian randomization; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; FDR, false discovery rate

While conducting MR analysis on the above immune cell phenotype, significant heterogeneity (Cochran's Q statistic, $P < 0.05$) was observed (Table 1). Based on the MR-Egger-intercept analysis, there was no significant horizontal pleiotropy (Table 1), suggesting that the SNPs did not influence the outcome via factors unrelated to the exposure factors.

Table 1: Heterogeneity and horizontal pleiotropy results for the Mendelian randomization analysis

Exposure	*Outcome*	*P value (Q-Egger)*	*P value (Q-IVW)*	*Egger-Intercept*	*P value (Egger-Intercept)*
CD27 on memory B cell	RA	0.001	0.002	0.015	0.087
RA	HLA DR on myeloid dendritic cell	0.025	0.050	0.019	0.035
HLA DR on CD14+ monocyte	OA	0.365	0.357	-0.003	0.390

RA, rheumatoid arthritis; OA, osteoarthritis; HLA, human leukocyte antigen; IVW, inverse variance weighted

The causal effects of RA on immune cell phenotype

The IVW method was first used to conduct MR analysis. Following FDR adjustment, RA showed causal association with one immune cell phenotypes, i.e., human leukocyte antigen (HLA)-DR on myeloid dendritic cells (mDCs) (beta = -0.14; 95% CI = -0.20 to -0.07; $P < 0.001$; P_{FDR} = 0.067) (Fig. 2A). The other four MR methods were mostly consistent with those suggested by the IVW method (Fig. 2A). However, we observed significant heterogeneity (Cochran's Q statistic, $P < 0.05$) and significant horizontal pleiotropy ($P = 0.035$) (Table 1), which suggested that the SNPs may influence the outcome via factors unrelated to the exposure factors. Fig. 2B illustrate the scatter plots for the MR analysis of the immune cell phenotype.

We also found that RA did not have a causal effect on the expression of CD27 on memory B cells, which was also demonstrated by the four other methods (MR Egger, weighted median, simple mode, and weighted mode) (Fig. 2C).

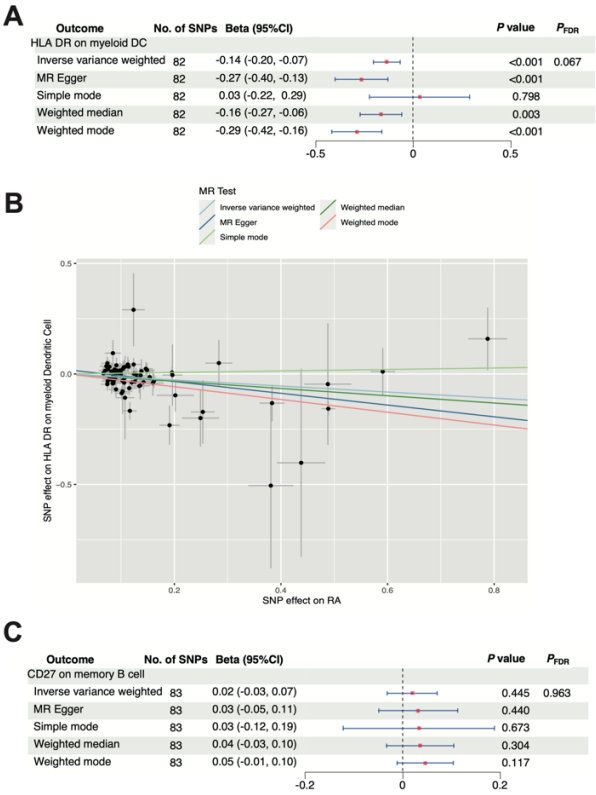

Fig. 2: Forest plot and scatter plot for the causal effect of rheumatoid arthritis on circulating immune cells. (A) forest plot and (B) scatter plot for the causal effect of rheumatoid arthritis on HLA-DR expression on myeloid DC. (C) forest plot for the causal effect of rheumatoid arthritis on CD27 expression on memory B cells. MR, mendelian randomization; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; FDR, false discovery rate, HLA, human leukocyte antigen, DC, dendritic cell.

The causal effects of immune cell phenotypes on OA

First, we examined the causal effects of immune cell phenotypes on OA. Based on the MR analysis using the IVW method, 48 immune cell phenotypes were associated with OA ($P < 0.05$) (Fig. 3A). A causal association was determined between one immune phenotype and OA after adjustment for FDR ($P_{FDR} < 0.1$), namely: HLA-DR expression on CD14+ monocyte. As shown in Fig. 3B, the expression of HLA-DR on CD14+ monocytes was negatively related to the risk of OA (OR = 0.98; 95% CI = 0.96-0.99; $P < 0.001$; $P_{FDR} = 0.054$). However, the other four MR methods showed that there was no association between HLA-DR on CD14+ monocytes and OA (Fig. 3B). Fig. 3C illustrate the scatter plots for the MR analysis of the immune cell phenotype.

While conducting MR analysis on the above immune cell phenotype, no significant heterogeneity (Cochran's Q statistic, $P > 0.05$) was observed (Table 1). Based on the MR-Egger-intercept analysis, there was no significant horizontal pleiotropy (Table 1), suggesting that the SNPs did not influence the outcome via factors unrelated to the exposure factors.

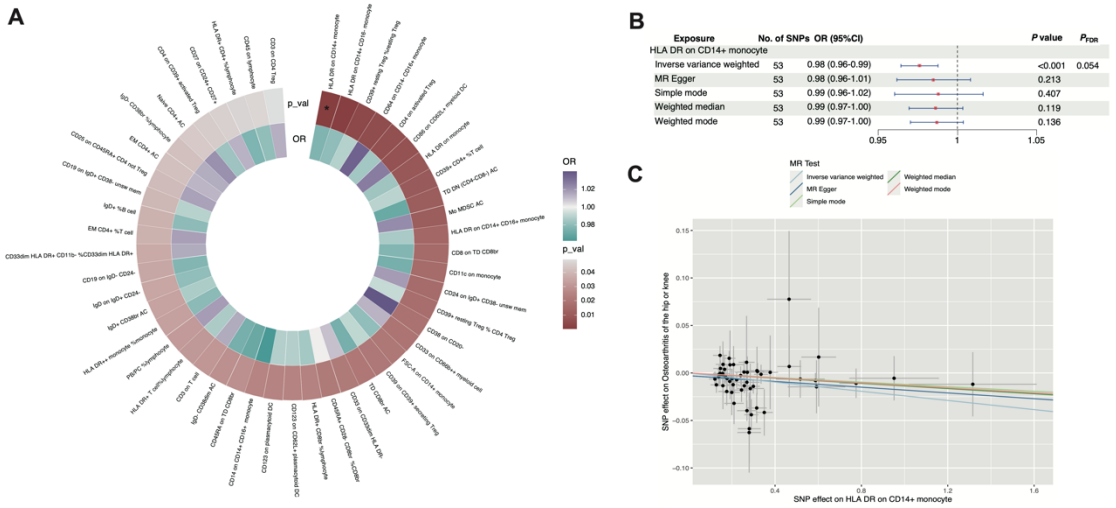

Fig. 3: The causal effect of circulating immune cells on osteoarthritis. (A) Heatmap for the causal effect of circulating immune cells on osteoarthritis using the inverse variance weighting (IVW) method. The outer circle represents P values, whereas the inner circle represents ORs. * indicates the FDR corrected P values < 0.1. Forest plot (B) and scatter plot (C) for the causal effect of HLA-DR expression on circulating CD14+ monocyte on osteoarthritis. MR, mendelian randomization; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; FDR, false discovery rate; HLA, human leukocyte antigen

The causal effects of OA on immune cell phenotype

The IVW method was first used to conduct MR analysis. Following FDR adjustment, OA showed no causal association with any of the immune cell phenotypes (P_{FDR} > 0.1).

We also found that OA did not have a causal effect on the expression of HLA-DR on CD14+ monocytes, which was also demonstrated by the four other MR methods (Fig. 4).

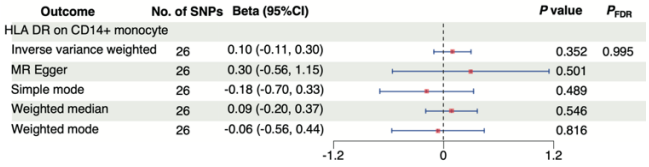

Fig. 4: Forest plot for the causal effect of osteoarthritis on HLA-DR expression on circulating CD14+ monocyte. SNPs, single nucleotide polymorphisms; CI, confidence interval; FDR, false discovery rate; HLA, human leukocyte antigen

Discussion

In the present study, we used MR method to investigate the causal relationship between immune cell traits and RA as well as OA. According to our study, the expression of CD27 on memory B cells was negatively related to the risk of RA, and the expression of HLA-DR on CD14+ monocyte was negatively related to the risk of OA. We also found that RA was negatively associated with the expression of HLA-DR on mDCs, but significant horizontal pleiotropy was observed.

We found that the expression of CD27 on memory B cells was negatively related to the risk of RA. This finding is consistent with previous studies that have reported altered frequency and function of CD27+ memory B cells in RA patients. The frequencies and IgM-producing capacities of CD27+IgD+ B cells were significantly decreased in RA patients, and these cells exhibit-

ed altered B cell receptor repertoire diversity and proinflammatory biased features (36). After effective therapy and disease remission, the frequency of these cells recovered (36), suggesting that CD27+ memory B cells may play a role in the pathogenesis and treatment response in RA. Furthermore, the basal level of CD38+ and/or CD27+ memory B cell count was important for an abatacept response to abatacept (37), a biologic drug used in the treatment of RA, indicating that the presence of memory B cells may be crucial for the efficacy of certain RA treatments. The decreased frequency and altered function of CD27+ memory B cells in RA patients may reflect a dysregulated B cell response that contributes to the production of pathogenic autoantibodies and the maintenance of chronic inflammation. This dysregulation could be due to various factors, such as genetic susceptibility, environmental triggers, and immune system imbalances (8). Moreover, the interaction between memory B cells and other immune cells, such as T cells and macrophages, may also contribute to the pathogenesis of RA (38). Our findings, along with previous studies, suggest that CD27+ memory B cells play a complex role in the pathogenesis and treatment response of RA. While their frequency and function are altered in RA patients, the exact mechanisms by which they contribute to the disease process remain to be fully elucidated. Further research is needed to better understand the interplay between memory B cells, inflammation, and treatment response in RA.

Our MR analysis revealed that RA causally reduces the expression of HLA-DR on circulating mDCs. This finding is interesting, as mDCs play a crucial role in the pathogenesis of RA by presenting autoantigens and activating autoreactive T cells (39, 40). Previous studies have shown that mDCs from RA patients exhibit an activated phenotype with increased expression of costimulatory molecules and pro-inflammatory cytokines (41, 42). However, the specific role of HLA-DR expression on mDCs in RA has not been extensively explored. The causal relationship between RA and reduced HLA-DR expression on mDCs may suggest a novel mechanism by which RA influences the immune system. In RA, the synovial microenvironment is enriched with pro-inflammatory mediators, such as TNF-α, IL-6, and IL-1β, which can modulate the function and phenotype of mDCs (43). It is possible that the chronic inflammatory state in RA may contribute to the downregulation of HLA-DR on mDCs, thereby affecting their ability to present antigens and regulate T cell responses. Furthermore, the reduced HLA-DR expression on mDCs in RA may have implications for disease progression and treatment response. HLA-DR is a key molecule involved in the presentation of antigenic peptides to CD4+ T cells, and its expression levels have been associated with the efficiency of antigen presentation (38, 44). In the context of RA, lower HLA-DR expression on mDCs could potentially lead to inadequate presentation of autoantigens and impaired regulation of autoreactive T cells, thus perpetuating the autoimmune response. This finding may also have implications for the development of new therapeutic strategies targeting mDCs in RA. Modulating the expression of HLA-DR on mDCs could potentially restore their antigen-presenting function and help regulate the autoimmune response. However, we observed significant horizontal pleiotropy when investigating their causal relationships. Horizontal pleiotropy occurs when genetic variants affect the outcome through pathways independent of the exposure, potentially biasing the causal estimate. In this case, the presence of horizontal pleiotropy suggests that the genetic variants associated with RA may influence HLA-DR expression on mDCs through multiple pathways, not solely through the direct effects of RA. This complexity underscores the need for cautious interpretation of this particular finding and highlights the intricate nature of the relationship between RA and mDC function. Further investigations are necessary to validate this finding and unravel the complex interplay between RA, mDCs, and the immune system.

We also found that the expression of HLA-DR on circulating CD14+ monocytes was negatively related to the risk of OA. Synovial fluid mono-

cytes/macrophages in knee OA patients exhibit an activated phenotype characterized by high levels of HLA-DR expression (45). This activated phenotype of synovial fluid monocytes has been associated with patient-reported outcome measures, suggesting their potential role as biomarkers and therapeutic targets for OA (45). Interestingly, our findings suggest that the HLA-DR expression on circulating CD14+ monocytes was be associated with a reduced risk of OA. One possible explanation for this discrepancy is that the activated monocytes expressing high levels of HLA-DR may migrate from the circulation into the joints, contributing to the local inflammatory milieu and the progression of OA (46). This migration could lead to a reduction in the number of HLA-DR expressing monocytes in the peripheral blood. Another potential explanation is that the functional properties of monocytes may differ between the systemic circulation and the local joint environment. It has been demonstrated that the synovial fluid in OA joints contains a complex mixture of pro-inflammatory mediators, such as cytokines, chemokines, and damage-associated molecular patterns (47). These factors may contribute to the activation and polarization of monocytes/macrophages towards a pro-inflammatory phenotype within the joint, which may not be reflected in the circulating monocyte population. However, it is important to note that while the IVW method showed a negative association between HLA-DR expression on CD14+ monocytes and OA risk, the other four MR methods did not support this finding. This discrepancy highlights the need for further research to clarify the relationship between monocyte HLA-DR expression and OA pathogenesis.

In this study, we used extensive GWAS data to investigate the relationship between 731 immune cell characteristics and traits associated with RA and OA. Our findings provide genetic evidence supporting a causal link between circulating immune cells and the development of both RA and OA. Several immune cell traits were identified as having a causal relationship with the risk of developing these conditions. These results offer potential new insights into the immunological mechanisms underlying RA and OA, which may guide future research into novel immunotherapies. Nevertheless, future research may need to incorporate longitudinal data to further validate these findings. Prospective cohort studies that measure immune cell traits over time and track the development of RA and OA could provide additional evidence for the temporal nature of these relationships. Such studies could also help elucidate the dynamic changes in immune cell populations throughout disease progression and in response to treatment. Moreover, integrating longitudinal data with genetic information in approaches such as longitudinal MR could offer a more comprehensive understanding of how genetic predisposition interacts with changes in immune cell traits over time to influence disease risk and progression.

However, it is important to acknowledge the limitations of our study. Firstly, the data used was derived from a European database, and therefore, the findings may not be applicable to other ethnic populations. Moreover, while we observed causal relationships between specific immune cells and RA and OA, the underlying mechanisms remain unclear, and thus, our results should be interpreted with caution. Additionally, significant heterogeneity was detected in some of the MR analyses. Although the application of a random effects model ensures the reliability of the conclusions, caution should still be exercised when interpreting these results. Lastly, when examining the relationship between RA and HLA-DR expression on circulating mDCs, evidence of horizontal pleiotropy was observed, indicating that this causal relationship may not be robust.

Conclusion

Overall, our study shows a causal relationship between specific immune cell traits and RA as well as OA. The results of this study provide further insight into the role of immune cells in the pathogenesis of RA and OA, potentially contributing to the development of targeted immuno-

therapies to treat these conditions. Nevertheless, further studies are necessary to explore the underlying mechanisms driving these associations and validate the causal relationships.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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