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Original Research

Bayesian-based analysis of the causality between 731 immune cells and erectile dysfunction: a two-sample, bidirectional, and multivariable Mendelian randomization study

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Abstract

Background: The causal relationship between certain immune cells and erectile dysfunction (ED) is still uncertain.
Aim: The study sought to investigate the causal effect of 731 types of immune cells on ED through Mendelian randomization (MR) using genome-wide association studies (GWAS).
Methods: Genetic instruments for 731 immune cells were identified through GWAS, and ED data were obtained from the FinnGen database. Univariable and multivariable bidirectional MR studies were conducted to explore potential causal relationships between these immune cells and ED. The inverse-variance weighted method was primarily used, with Cochran's Q test and MR-Egger intercept test assessing pleiotropy and heterogeneity. Bayesian weighted Mendelian randomization (BWMR) was also employed.
Outcomes: Six immune cells were identified as related to ED. CD45 on Natural Killer (NK) cells, CD33dim HLA DR+ CD11b + Absolute Count, CD19 on IgD- CD38dim B cells, and CD3 on CD39+ resting CD4 regulatory T cells were identified as risk factors, whereas CD20 on IgD+ CD38dim B cells and Activated & resting CD4 regulatory T cell %CD4+ T cells were protective factors. Further multivariable MR analysis confirmed that 5 of these immune cells independently impacted ED, except for CD45 on NK cells. Reverse MR analysis indicated that ED occurrence decreases certain immune cell counts, but BWMR found no causal relationship for CD20 on IgD+ CD38dim B cells.
Results: Our MR analysis confirmed a potential bidirectional causal relationship between immune cells and ED, providing new insights into potential mechanisms and therapeutic strategies.
Clinical Translation: This study provides evidence for the impact of certain immune cells on the development of ED and suggests potential therapeutic targets.
Strengths and Limitations: We performed both univariable and multivariable MR to strengthen the causal relationship between exposures and outcomes. However, the population in this study was limited to European ancestry.
Conclusion: Our MR analysis confirmed a potential bidirectional causal relationship between immune cells and ED. This provides new insights into potential mechanisms of pathogenesis and subsequent therapeutic strategies.

Keywords: Mendelian randomization; erectile dysfunction; immune cells.

Introduction

Erectile dysfunction (ED) is defined as the inability of the penis to achieve or maintain an erection sufficient to satisfy sexual intercourse, representing a common disease that significantly affects male sexual function, particularly in men over 40.[1] The prevalence of ED varies due to regional and cultural differences but remains generally high. In Brazil, the prevalence is reported at 37.2%, in Italy at 48.6%, and in some Asian countries, it astonishingly reaches 63% among men aged 50 to 80.[2,3] With an aging population and other contributing factors, the prevalence and burden of ED are anticipated to increase, underscoring the necessity for updated and comprehensive epidemiological data to better understand and address this growing health issue.

Although ED is not life-threatening, studies show it severely impacts the psychological health of patients and significantly affects their sexual partners, leading to substantial disruptions in life, family, and work, thus posing a considerable societal challenge. ED is increasingly recognized as a chronic vascular inflammatory disease. As research deepens on the relationship

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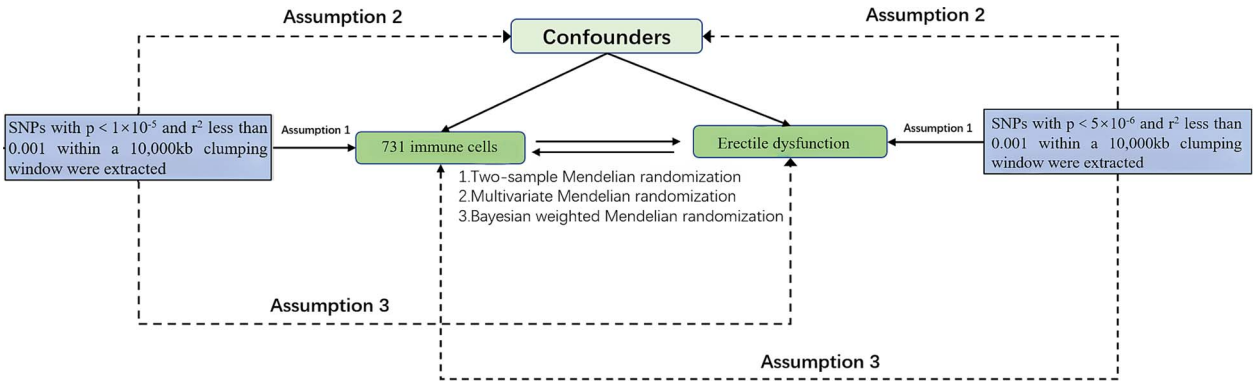

Figure 1. Causal relationship model between immune cells and erectile dysfunction (ED).This figure illustrates the framework for investigating the causal relationship between 731 immune cell types and ED using Mendelian randomization. SNPs were extracted with $P < 1 \times 10^{-5}$ and $r^2 < 0.001$ (left side) and $P < 5 \times 10^{-6}$ and $r^2 < 0.001$ (right side) within a 10 000 kb clumping window. The model includes assumptions that SNPs directly influence immune cells (assumption 1), confounders may affect both immune cells and ED (assumption 2), and SNPs indirectly influence ED through immune cells (assumption 3). Three Mendelian randomization methods are applied: 2-sample, multivariate, and Bayesian weighted.

between ED and inflammatory and immune status, the links between ED and various chronic diseases such as cardiovascular diseases, diabetes, and metabolic syndrome become more pronounced. These conditions indirectly affect erectile function by impacting vascular and neural functions, thereby increasing the risk of ED. Studies indicate elevated levels of inflammatory markers in patients with ED, such as C-reactive protein (CRP), interleukin-6 (IL-6), and von Willebrand factor, which correlate positively with the severity of ED.[4,5] Immune cells play roles through the regulation of inflammatory responses, as seen in alternative therapies like immune cell therapy replacing stem cell approaches, using autologous in vitro peripheral blood mononuclear cell (PBMNC) concentrate for treating ED.[6] This also suggests new mechanisms and targets for innovative therapeutic approaches to ED. In the absence of randomized controlled trials, Mendelian randomization (MR) serves as a feasible alternative. Typically, single nucleotide polymorphisms (SNPs) are used as instrumental variables (IVs) to infer the causal effects of exposure variables on outcome variables.[7] This study aims to answer the following questions: Which specific immune cell types causally influence the risk of ED? Additionally, does the occurrence of ED, in turn, lead to an increase or decrease in the numbers of these immune cells?

Materials and methods

Study design

We conducted a 2-sample MR study to explore the causal relationships between 731 immune cell types and ED. This study employed 2-sample MR, reverse MR, multivariable MR, and BWMR analysis methods (Figure 1). The analysis was based on 3 core assumptions: the relevance assumption, which posited a connection with the risk factor; the independence assumption, which ensured no common causes with the outcome; and the exclusion restriction assumption, which held that the influence on the outcome did not operate through pathways other than the risk factor.[8,9]

Data source

New Data on ED were obtained from the FinnGen database (https://www.finngen.fi/en/access_results), which includes 1154 cases and 94 024 controls. In the FinnGen study, ED cases were classified based on clinical diagnosis using specific International Classification of Diseases, 10th Revision (ICD-10) codes, ensuring standardized and consistent categorization. Specifically, the ICD-10 code N52.9 was utilized to identify ED cases. This code is part of the larger ICD-10 classification system used globally to ensure uniformity in disease classification. Additionally, data on 731 immune cell types were sourced from public GWAS databases, covering IDs GCST90001391 to GCST90002121 based on a sample of 3757 Europeans.[10] The immune cell phenotypes were classified into 4 main categories: absolute cell counts with 118 types, median fluorescence intensity representing surface antigen levels with 389 types, relative cell counts with 192 types, and morphological parameters with 32 types. These immune cells were further divided into 7 groups, including B cells, conventional dendritic cells, mature T cells, monocytes, myeloid cells, TBNK cells, and regulatory T cells (Treg cells). All participants were of European descent to minimize potential biases due to population heterogeneity. To eliminate the potential issue of sample overlap, which could result in false positives in 2-sample MR analyses, we rigorously ensured that the exposure data, comprising 731 immune cell types, were exclusively derived from an Italian population. Furthermore, the outcome data for ED were sourced from the FinnGen database, representing a distinct Finnish cohort. By selecting data from entirely different national populations, we completely eliminated any possibility of sample overlap in our analysis, thereby ensuring the validity of our results and mitigating the risk of bias. No additional ethical declarations or consents were required for this study.

Selection of genetic instrumental variables

Under the assumptions of hypotheses 1, 2, and 3, for a forward MR analysis, we used MR to screen for *P*-values $<1 \times 10^{-5}$ across the whole genome, SNPs were selected to be independently distributed with an r^2 threshold of <0.001, ensuring a distance of up to 10 000 kb between each pair in immune cells. For reverse MR, setting ED as the exposure and finding insufficient SNPs at $P < 5 \times 10^{-8}$ necessitated setting a more lenient *P*-value threshold of $<5 \times 10^{-6}$ to extract more SNPs. The process further involved combining databases of exposures and outcomes, removing incompatible alleles and palindromic sequences, and calculating the final set of IVs. Subsequently, we computed the F-statistic for each SNP using the formula $F = \beta^2/se^2$, where β represented the effect

of the SNP on the exposure and se was the standard error. To ensure accuracy, SNPs with an F-statistic greater than 10 were typically selected as IVs to minimize bias due to weak instruments.[11]

Statistical methods

To establish causality, we employed 5 methods: MR Egger, weighted median, IVW, simple mode, and weighted mode to examine the causal relationship between immune cells and ED. Among these, the IVW method is the primary approach for MR analysis. It combines effect estimates from different genetic variants to provide an overall estimate of the causal effect, assigning more weight to estimates with smaller variances. The other methods serve as auxiliary validation. MR Egger Regression: Evaluates and adjusts for horizontal pleiotropy by estimating the intercept term, ensuring the accuracy of causal estimates. Weighted Median Method: Provides robust causal estimates even if some SNPs are biased, ensuring the reliability of the results. Simple Mode Method: Estimates causal effects using a simple linear regression model under the assumption of no pleiotropy. Weighted Mode Method: Takes pleiotropy into account by using a more complex weighting method for causal effect estimation. All MR analyses were conducted using R version 4.3.1, with the TwoSampleMR package version 0.6.1 and MR-PRESSO version 1.0.[12]

Heterogeneity and pleiotropy analysis

This study utilized Cochran's Q statistic, MR-Egger, and a method used to detect pleiotropy between samples and remove outlier SNPs (MR-PRESSO) methods. Cochran's Q Statistic: Used to assess heterogeneity among different SNPs. By calculating the deviation of each SNP from the overall effect, Cochran's Q test can determine if there is significant heterogeneity. A significant Q statistic indicates substantial differences among SNPs, suggesting that some SNPs may have effects that differ from others, necessitating further inspection and adjustment. MR-Egger Regression: Besides estimating causal effects, MR-Egger regression uses its intercept term to evaluate horizontal pleiotropy. Horizontal pleiotropy occurs when an SNP influences the outcome variable through multiple pathways, not just through the exposure variable. A significant intercept term in MR-Egger indicates the presence of pleiotropy, which can bias causal effect estimates. By adjusting for pleiotropy, MR-Egger provides more accurate causal relationship assessments. MR-PRESSO Method: Specifically designed to detect and correct for outlier SNPs. This method improves the accuracy and robustness of causal estimates by identifying and removing outlier SNPs, thus reducing their influence on the causal estimate. MR-PRESSO also adjusts for pleiotropy, further ensuring the reliability of causal relationship assessments.

Bayesian weighted Mendelian randomization

Bayesian weighted Mendelian randomization (BWMR) significantly enhanced the accuracy of causal inference by integrating Bayesian statistics with MR. It effectively used genetic variation as an IV to accurately assess the causal effects of high-volume exposure on outcomes. BWMR not only addressed the weak instrument variable problem inherent in traditional methods but also incorporated prior knowledge and updated posterior distributions to provide more reliable effect estimates. This ensured the robustness and reliability of causal relationships in complex settings.[13]

Results

Forward MR: Screening results for immune phenotypes associated with ED risk

In our MR analysis, we primarily utilized the IVW method. To identify immune cells with a stronger association with ED, we set a *P*-value threshold of <.01. We identified causal relationships between 7 immune cells and ED. MR-Egger regression was conducted to assess horizontal pleiotropy, and all immune cells had *P*-values >0.05, suggesting no pleiotropy. Cochran's Q test was used to evaluate the heterogeneity of SNPs, with *P*-values for both IVW and MR-Egger >.05, indicating no heterogeneity. Except for HLA-DR on B cells, when MR-PRESSO was used to remove all outlying SNPs, the result remained <0.05 (Table 1, Figure 2). This indicates the presence of aberrant SNPs in this specific immune cell. Due to this finding, HLA-DR on B cells was excluded from further analysis, as MR-PRESSO indicated potential pleiotropy. As a result, only 6 immune cells were found to have causal relationships with ED. The immune cells acting as protective factors (odds ratio [OR] < 1) were Activated & Resting CD4 Regulatory T cells %CD4+ T cells (IVW *P*-value = .0088, OR: 0.812–0.970) and CD20 on IgD+ CD38dim B cells (IVW *P*-value = .002, OR: 0.783–0.946). The immune cells acting as risk factors (OR > 1) were CD33dim HLA DR+ CD11b + Absolute Count (IVW *P*-value = .0085, OR: 1.031–1.232), CD19 on IgD- CD38dim B cells (IVW *P*-value = .0033, OR: 1.047–1.263), CD3 on CD39+ Resting CD4 Regulatory T cells (IVW *P*-value = .0078, OR: 1.034–1.247), and CD45 on Natural Killer (NK) cells (IVW *P*-value = .004, OR: 1.045–1.263). Further multivariable analysis identified which immune cells independently influence the risk of ED. Including all 6 immune cells as exposures, with ED as the outcome, it was found that apart from CD45 on NK cells, the other 5 immune cells still had an independent impact on the risk of ED (Table 1, Figure 2). To ensure the accuracy of the results, we also carried out Bayes weighted Mendelian verification. The findings were consistent with previous results, except for CD20 on IgD+ CD38dim B cells, which did not show statistical significance (*P*-value >.05) in the BWMR analysis (Supplementary Material), while the other immune cells met the criteria for Bayesian-weighted verification. The results of the other Bayes-weighted methods are included in the Supplementary Material. Except for CD20 on IgD+ CD38dim B cells, the Bayes-weighted *P*-values for each immune cell were also <.05, indicating they passed BWMR verification.

Reverse MR: Causal effects of ED on immune phenotypes

To explore the causal impact of ED on immune phenotypes, we conducted a 2-sample MR analysis using the IVW method as the primary analytical approach. We set a *P*-value threshold of <.01. The results indicate that the onset of ED reduces the number of CCR2 on CD14+ CD16+ monocytes(IVW *P*-value = .003, OR: 0.851–0.967), SSC-A on monocytes(IVW *P*-value =.007 OR: 0.842–0.973), SSC-A on CD14+ monocytes(IVW *P*-value =.002, OR: 0.837–0.962), SSC-A on HLA

Table 1. Summary of genetic associations with erectile dysfunction (ED).

Main exposure	Method	nSNP	*P*- value	or	or_lci95	or_uci95	Heterogeneity test *P*	MR-Egger intercept *P*	MR-PRESSO
ebi-a-GCST90001500 Activated & resting CD4 regulatory T cell %CD4+ T cell	MR Egger	25	.0749	0.8884	0.7845	1.006	.772		
	Weighted median	25	.0634	0.8914	0.7895	1.0064			
	Inverse-variance weighted	25	.0088	0.888	0.8125	0.9706	.816	.99	0.84
	Simple mode	25	.8172	0.9756	0.7927	1.2006			
	Weighted mode	25	.094	0.8957	0.7914	1.0137			
	MVMR	23	<.01	0.8527	0.7883	0.9224			
ebi-a-GCST90001525 CD33dim HLA DR+ CD11b + Absolute Count	MR Egger	27	.0128	1.2148	1.0538	1.4003	.163		
	Weighted median	27	.0047	1.1834	1.0528	1.33			
	Inverse-variance weighted	27	.0085	1.1275	1.0311	1.2328	.134	.2	0.148
	Simple mode	27	.382	1.0978	0.8938	1.3483			
	Weighted mode	27	.0151	1.1808	1.0419	1.3383			
	MVMR	23	.0005	1.1339	1.056	1.2176			
ebi-a-GCST90001735 CD19 on IgD- CD38dim B cell	MR Egger	28	.0142	1.2113	1.0499	1.3975	.788		
	Weighted median	28	.0077	1.2056	1.0506	1.3835			
	Inverse-variance weighted	28	.0033	1.1506	1.0477	1.2636	.789	.36	0.806
	Simple mode	28	.5664	1.0774	0.8377	1.3857			
	Weighted mode	28	.0232	1.1876	1.0324	1.366			
	MVMR	18	.0032	1.1422	1.0457	1.2477			
ebi-a-GCST90001752 CD20 on IgD+ CD38dim B cell	MR Egger	29	.0016	0.7658	0.6596	0.8892	.948		
	Weighted median	29	.0122	0.8334	0.7227	0.961			
	Inverse-variance weighted	29	.002	0.8616	0.7839	0.9469	.856	.06	0.812
	Simple mode	29	.3323	0.8821	0.6876	1.1317			
	Weighted mode	29	.0092	0.8263	0.7229	0.9445			
	MVMR	24	.0375	0.9128	0.8376	0.9948			
ebi-a-GCST90001852 CD3 on CD39+ resting CD4 regulatory T cell	MR Egger	19	.0225	1.195	1.0398	1.3734	.942		
	Weighted median	19	.0357	1.1463	1.0092	1.302			
	Inverse-variance weighted	19	.0078	1.1359	1.0342	1.2476	.936	.35	0.932
	Simple mode	19	.334	1.1087	0.9044	1.3591			
	Weighted mode	19	.0574	1.1529	1.0049	1.3227			
	MVMR	18	.0089	1.1105	1.0266	1.2013			
ebi-a-GCST90001911 CD45 on Natural Killer	MR Egger	27	.1054	1.1286	0.98	1.2997	.438		
	Weighted median	27	.0276	1.1762	1.018	1.359			
	Inverse-variance weighted	27	.004	1.1494	1.0455	1.2637	.488	.73	0.535
	Simple mode	27	.3918	1.1188	0.869	1.4405			
	Weighted mode	27	.0848	1.1406	0.9877	1.3171			
	MVMR	20	.2618	1.0485	0.9652	1.1391			
ebi-a-GCST90002116 HLA DR on B cell	MR Egger	21	.3173879	0.9169	0.777048	1.081998	.033		
	Weighted median	21	.0041353	0.8394	0.74472	0.946092			
	Inverse-variance weighted	21	.0080939	0.8695	0.783952	0.964305	.035	.43	0.038
	Simple mode	21	.001629	0.6956	0.572119	0.845749			
	Weighted mode	21	.003373	0.8289	0.742052	0.925821			

This table presents the results of genetic association studies investigating the relationship between various genetic markers and ED. Each row corresponds to a specific genetic marker and includes the method used for analysis, the number of SNPs (nSNP) involved, the *P*-value indicating the significance of the association, the odds ratio (OR) with its 95% confidence interval (CI), and additional statistical tests for heterogeneity and pleiotropy. Significant associations ($P < .05$) are highlighted, suggesting a potential causal link between the genetic markers and ED.

DR+ T cells(IVW *P*-value =0.002, OR: 0.837–0.963), and SSC-A on NK T cells(IVW *P*-value =.008, OR: 0.849–0.976). Table 2 contains all the results from the reverse MR analysis, and the tests for pleiotropy and heterogeneity were both >0.05, indicating no pleiotropy or heterogeneity.

Discussion

Utilizing extensive publicly available GWAS genetic data, we examined the causal relationships between 731 immune cells and ED. To our knowledge, this is the first analysis and discussion to explore such relationships with a comprehensive set of 731 immune cells. Applying a stringent *P*-value threshold, we identified 7 immune cells associated with both risk and protective factors for ED. After excluding one cell due to pleiotropy, 6 cells remained. Further multivariable analysis showed that 5 of these cells still exhibited strong correlations. Additionally, treating ED as the exposure and immune cells as the outcome revealed no evidence of bidirectional causal relationships among these 6 immune cells. But the CD20 + CD38dim on IgD B cell did not pass the BWMR test and verification. A stricter *P*-value (<.01) used in reverse analyses also demonstrated causal relationships between the onset of ED and changes in 5 immune cells.

ED is considered a vascular disease resulting from endothelial dysfunction and atherosclerosis [14]. Although not life-threatening, the occurrence of ED significantly impacts sexual and family life, work efficiency, and consequently

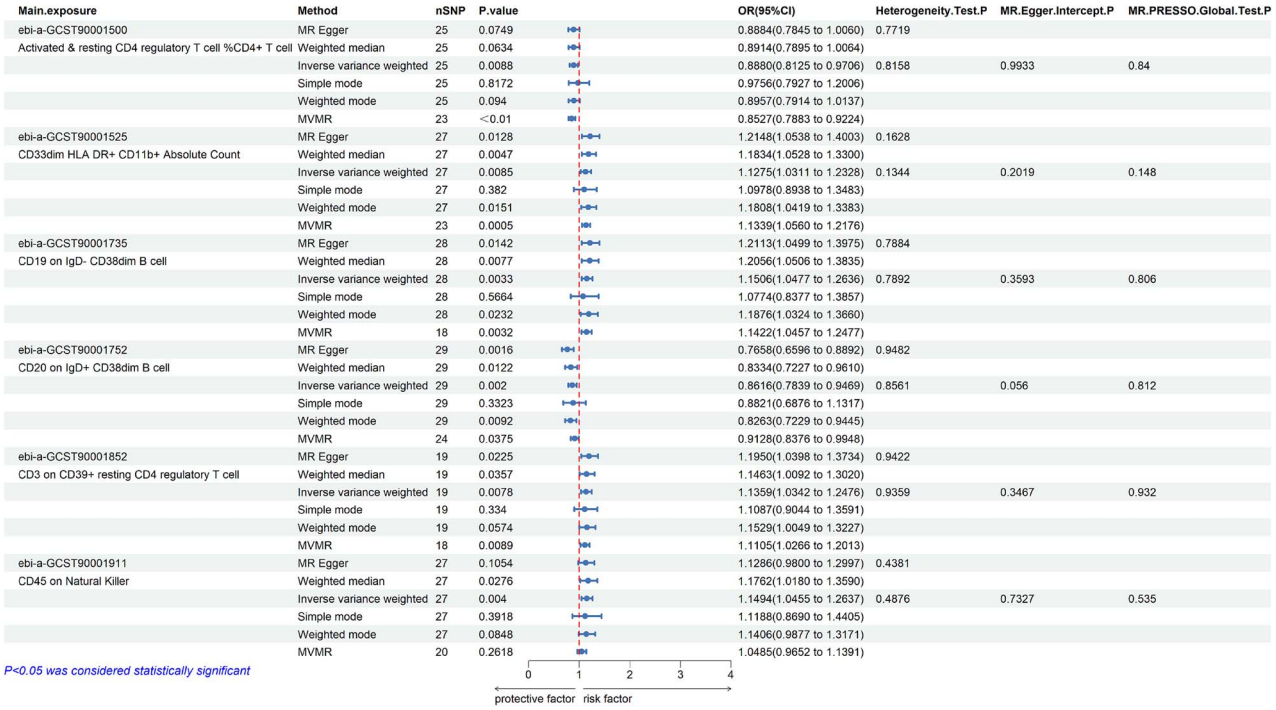

Figure 2. Mendelian randomization analysis of immune cell types and erectile dysfunction (ED). This figure shows the results of Mendelian randomization (MR) analyses investigating the association between various immune cell types and ED. Each row represents a specific immune cell type, analyzed using different MR methods (MR Egger, weighted median, inverse-variance weighted, simple mode, weighted mode, MVMR). The columns include the number of SNPs used (nSNP), *P*-values, odds ratios (OR) with 95% confidence intervals (CIs), and heterogeneity and pleiotropy test *P*-values. The forest plots illustrate the effect sizes, with statistically significant results ($P < .05$) indicating potential causal relationships.

Table 2. Mendelian randomization analysis results for immune cell types and erectile dysfunction (ED).

Main outcome	Method	nSNP	*P*-value	or	or_lci95	or_uci95	Heterogeneity test *P*	MR-Egger intercept *P*	MR-PRESSO
ebi-a-GCST90001992 CCR2 on CD14+ CD16+ monocyte	MR Egger	11	0.1451	0.8794	0.7510	1.0298	.6013		
	Weighted median	11	0.0468	0.9113	0.8315	0.9987			
	Inverse-variance weighted	11	0.0030	0.9074	0.8511	0.9676	.6750	.6799	0.7240
	Simple mode	11	0.2614	0.9178	0.7968	1.0571			
	Weighted mode	11	0.2027	0.9050	0.7840	1.0447			
ebi-a-GCST90002073 SSC-A on monocyte	MR Egger	11	0.0734	0.8342	0.7000	0.9941	.4511		
	Weighted median	11	0.0923	0.9179	0.8308	1.0142			
	Inverse-variance weighted	11	0.0070	0.9055	0.8424	0.9732	.4528	.3412	0.5120
	Simple mode	11	0.3674	0.9278	0.7942	1.0840			
	Weighted mode	11	0.3934	0.9347	0.8057	1.0843			
ebi-a-GCST90002074 SSC-A on CD14+ monocyte	MR Egger	11	0.1110	0.8595	0.7267	1.0167	.7866		
	Weighted median	11	0.0041	0.8725	0.7949	0.9577			
	Inverse-variance weighted	11	0.0023	0.8978	0.8377	0.9622	.8290	.5906	0.8410
	Simple mode	11	0.0970	0.8627	0.7366	1.0105			
	Weighted mode	11	0.0860	0.8627	0.7411	1.0043			
ebi-a-GCST90002083 SSC-A on HLA DR+ T cell	MR Egger	11	0.2091	0.8897	0.7511	1.0539	.7527		
	Weighted median	11	0.0360	0.9080	0.8296	0.9937			
	Inverse-variance weighted	11	0.0025	0.8981	0.8376	0.9630	.8247	.9072	0.8430
	Simple mode	11	0.2151	0.9059	0.7826	1.0487			
	Weighted mode	11	0.2135	0.9080	0.7876	1.0469			
ebi-a-GCST90002084 SSC-A on Natural Killer T	MR Egger	11	0.6347	0.9584	0.8091	1.1353	.9631		
	Weighted median	11	0.0978	0.9266	0.8467	1.0141			
	Inverse-variance weighted	11	0.0084	0.9104	0.8490	0.9762	.9686	.5302	0.9800
	Simple mode	11	0.5663	0.9586	0.8336	1.1024			
	Weighted mode	11	0.4889	0.9518	0.8317	1.0892			

This table shows the results of Mendelian randomization (MR) analyses exploring the association between various immune cell types and ED. It includes the immune cell types analyzed (Main outcome), the MR methods used (Method), the number of SNPs (nSNP), *P*-values, odds ratios (OR) with 95% confidence intervals (CI), and *P*-values for heterogeneity, MR-Egger intercept, and MR-PRESSO global tests. Statistically significant results ($P < .05$) are highlighted, indicating potential causal relationships.

our overall well-being.[14] Current treatments, including sildenafil, tadalafil, and vardenafil, among other PDE5 inhibitors, are not curative and provide only symptomatic relief.[15,16] Moreover, these drugs may have potential side effects that make them unsuitable for long-term use, such as headaches, visual impairments, flushing, and persistent ED following discontinuation.[17-19] As research into ED deepens, a significant link has been identified between ED and the human immune system. For those unresponsive to traditional oral PDE5 inhibitors and suffering from intractable vasculogenic ED related to diabetes, new treatment strategies like stem cell therapy are increasingly being utilized.[20,21] However, the effectiveness of stem cell therapy may be affected by the immune response. Increasingly, experimental evidence suggests that immunotherapy-based approaches might be safer and more effective. Recent studies are transitioning from traditional stem cell treatments to therapies using ex vivo isolated PBMNCs for treating ED, with significant results.[6,22]

B cells are a crucial component of the human immune system and part of the adaptive immune response. They primarily function in antibody production, antigen presentation, and modulation of the immune response through the secretion of various cytokines, playing a central role in immune regulation.[23,24] However, the role of immune cells in ED, a disease often overlooked until recently, has begun to be recognized as fundamentally an inflammatory vascular condition. The importance of B cells has become increasingly apparent in this context. Studies have shown that B cells significantly influence the disease course through their antibody production, antigen presentation, and cytokine secretion functions.[25] Additionally, B cells play a role in vasculitis by regulating inflammatory responses and promoting tissue repair and fibrosis.[26] In the context of ED, a vascular inflammatory disease, B cells play a unique role. Our results suggest that CD19 on IgD- CD38dim B cells is a risk factor for ED. CD19, a primary B cell surface molecule, plays a critical role in B cell activation and signal transduction. Research by Blair et al. indicated that CD19 enhances immune responses by modulating B cell receptor signaling.[27] Lin et al.'s research on IgG4-related disease found that a significant increase in CD19+ CD24- CD38hi B cells correlates with disease severity, highlighting the critical role of specific CD19+ B cell subsets in inflammatory diseases.[28] In the context of vasculitis, these CD19+ B cell subsets may influence vascular wall stability and hemodynamics by promoting inflammation or modulating immune responses, key factors in the development of ED. Moreover, CD19's signaling pathway, particularly its role in regulating key pathways like PI3-K and mitogen-activated protein kinase (MAPK) in B cells, is especially significant.[29,30] Upon activation, CD19 can affect B cell function by promoting the activation of PI3-K (phosphatidylinositol 3-kinase), whose activation produces phosphatidylinositol (3,4,5)-trisphosphate (PIP3), a critical secondary messenger that further activates Akt.[31] Akt, a multifunctional kinase, participates in transmitting cell survival signals, regulating the cell cycle and metabolic regulation. By activating Akt, B cells receive anti-apoptotic signals, enhancing their survival and promoting the release of inflammatory cytokines, which may have a significant impact on the inflammatory state within blood vessels.[32] CD19 can also activate the MAPK pathway, a group of signal transduction proteins essential for cell proliferation, differentiation, and response to external stress. In B cells, the activation of the MAPK pathway can promote the production of inflammatory cytokines such as IL-6 and TNF-α, key mediators in regulating local and systemic inflammatory responses. Thus, CD19 signaling might exacerbate or sustain the chronic inflammatory environment, particularly in pathological states related to vascular health such as atherosclerosis, potentially affecting the development of ED. Conversely, CD20 on IgD+ CD38dim B cells acts as a protective factor, possibly because CD19 is a co-stimulatory molecule in B cells that primarily promotes B cell activation and proliferation by enhancing B cell receptor signaling. This enhanced signaling may lead to an overactive antigen response, especially in IgD- CD38dim B cells, which are more active in inflammation and autoimmune responses.[33] In the context of ED, this might exacerbate the inflammation in blood vessels and local tissues, thereby increasing the risk of ED. In contrast, the expression of CD20 in IgD+ CD38dim B cells is associated with a protective role. CD20's primary function is as a regulator of B cell activation and survival, especially in IgD+ CD38dim B cells, which typically have a higher regulatory potential, possibly involving immune suppression and anti-inflammatory actions. CD20 helps maintain B cells in a resting state by regulating their calcium signaling, potentially preventing these cells from overresponding to inflammatory stimuli.[34] Therefore, in ED, CD20-expressing regulatory B cells might help suppress excessive immune activation and inflammation, thereby protecting vascular function from damage. However, it did not pass validation with Bayesian weighting. Possible reasons for this include insufficient sample size, inadequate effect strength of genetic variants, the influence of confounding factors, and limitations of the method itself. Therefore, additional clinical studies are needed to further verify whether there is a causal relationship with ED. HLA DR on B cells also acts as a protective factor because B cells with HLA-DR molecules are involved in presenting antigenic peptides to T cells, thereby activating them.[35] Tabata et al. found that this activation could induce an enhanced expression of the IgM heavy chain gene associated with Syk activation without leading to proliferation or apoptosis.[36] This activation is crucial for adaptive immune responses, especially in coordinating effective targeted responses to specific pathogens or infected cells. However, our findings indicate significant pleiotropy in MR-PRESSO tests for HLA-DR on B cells, suggesting that its role in disease may be more complex than initially thought. According to a genome-wide association study, it is associated with the risk of multiple diseases, including chronic lymphocytic leukemia (CLL), Hodgkin lymphoma, and multiple myeloma. These findings reveal a possible common genetic susceptibility role in multiple diseases, reflecting its pleiotropic characteristics.[37] This pleiotropy suggests that genetic variations may affect various types of diseases through one or more biological pathways.

T cells also play a crucial role in vasculitis, commonly divided into CD4+ T cells and CD8+ T cells. CD4+ T cells, known as helper T cells, primarily assist and regulate the activity of other immune cells through cytokine secretion.[38] CD8+ T cells, also known as cytotoxic T cells, mainly function to directly kill virus-infected cells.[39] Both CD4+ and CD8+ T cells have been proven to play significant roles in various vasculitis diseases. Deliyanti and colleagues discovered that tumor necrosis factor (TNF), interferon-gamma (IFNγ), perforin, and granzyme A/B can be mediated by CD8+ T cells in retinal vascular diseases. By inhibiting the chemokine

receptor CXCR3, the number of CD8+ T cells in the retina can be reduced, thereby mitigating pathological vasculitis.[40] In another study, Winchester et al. found that cytomegalovirus infection enhances the activation of inflammatory CD8+ T cells through the interaction of CD2 and its ligand LFA-3. This activation leads CD8+ T cells to release more inflammatory cytokines, such as TNF and IFNγ, in vasculitis, especially in atherosclerosis, exacerbating vascular inflammation, and structural damage.[41] Our results indicate that CD3 on CD39+ resting CD4 regulatory T cells (Tregs) is a risk factor, potentially because high expression of most Treg cells is generally associated with worse clinical outcomes.[42,43] CD39 is a crucial immunoregulatory molecule that plays a key role in maintaining immune balance by hydrolyzing extracellular ATP to produce adenosine, which has an immunosuppressive effect.[44] Perry and colleagues found that CD39+ CD4+ T cells might regulate inflammatory responses and suppress excessive immune activity, and are associated with late-stage disease and therapeutic intervention needs in CLL.[45-47] This might be due to CD39's ability to induce CD4+ cells indirectly after exposure to ATP or upon B cell receptor binding. Conversely, the percentage of activated and resting CD4 regulatory T cells (%CD4+ T cells) acts as a protective factor. We believe this could be due to the bidirectional role of Tregs in ED. Tregs help maintain immune homeostasis and suppress excessive inflammatory responses, which is beneficial for protecting vascular health, particularly in conditions like ED that are closely linked to vascular function. However, in some types of cancer and other chronic diseases, high expression of Tregs is associated with poorer clinical prognosis, potentially because high concentrations of Tregs in cancer may promote tumor escape from immune surveillance, thus correlating with worse survival rates. In this scenario, high expression of Tregs is not a protective factor but rather a promoter of disease progression. Another study found that CD4+ T cells can exacerbate tissue damage and disease progression through their pro-inflammatory functions in transmission electron microscopy cells, but the presence of regulatory T cells (Tregs) is crucial for countering these effects and providing protection in vasculitis diseases.[48]

NK cells, integral to the innate immune system, play a crucial role in the first line of defense, particularly in immune surveillance against malignant transformations. CD45, a transmembrane protein tyrosine phosphatase, is critical for the regulation of NK cells. It modulates cytokines and through multiple signaling pathways (such as activation of the Ly49D promoter) balances NK cell production and cytotoxicity.[49] CD45 also enhances NK cell activation by antagonizing inhibitory signals and may play a vital role in NK cell maturation and cellular homeostasis through key signal phosphorylation.[50,51] CD45 on NK cells might indeed be a protective factor against cancer or inflammation. However, specific studies have shown that T cells targeting CD45, when transplanted into mice with the same CD45 genotype, can cause early graft-vs-host disease characterized mainly by pulmonary vasculitis.[52] This suggests that CD45 regulation in NK cells may influence vascular inflammatory responses, potentially linking it to certain types of vasculitis diseases. We hypothesize that modulating immune cell functions could consequently affect the inflammatory state of vessels. Given that ED is a vasculitis disease, it would inevitably be impacted. This aligns with the results from multivariate analysis in MR, indicating that CD45 cannot independently exert its protective effect on NK cells; its function is likely the result of interaction with other factors. Therefore, further research on CD45 in NK cells is warranted.

CD33dim HLA DR+ CD11b + Absolute Count is a composite marker identified as a risk factor, with potential reasons being multifaceted. Although HLA DR alone, a cell surface receptor part of the Class II Major Histocompatibility Complex, is responsible for presenting exogenous antigens to CD4+ helper T cells and correlates positively with lymphocyte infiltration.[53-55] it serves as a favorable prognostic indicator in colorectal cancer research and is significantly beneficial for immunotherapy interventions.[56] However, as part of a complex, the mechanisms may shift. Research has shown that in individuals with elevated serum CRP, a marker of inflammation, there is an increase in CD11b+/CD33+/HLA-DR- myeloid cells.[57] This might imply a connection to inflammatory diseases. Another study on melanoma found that CD33 + CD11b + HLA-DR- is highly expressed in myeloid-derived suppressor cells (MDSCs), which aid in immune suppression.[58] Thus, MDSCs expressing CD33 + CD11b + HLA-DR- might suppress local or systemic immune responses by releasing anti-inflammatory and immunosuppressive molecules such as nitric oxide and arginase, influencing the local inflammatory state.[59] Additionally, MDSCs may affect vascular function through immunosuppressive mechanisms, similarly impacting penile blood flow and erectile function, which could influence the development of ED.[60]

In the reverse MR results, we primarily observed changes in the SSC-A parameter, showing declines in monocytes, Treg cells, T cells, and NK cells. SSC-A reflects the internal complexity and granularity of cells, generally associated with the abundance and distribution of intracellular structures such as granules and organelles. However, we found no direct evidence linking ED to a decrease in SSC-A. We speculate that this might be due to endothelial dysfunction related to systemic vascular dysfunction associated with ED, which could lead to increased inflammatory factors and oxidative stress, altering the state of surrounding immune cells.[61,62] Long-term inflammation might induce changes in the internal structure of immune cells, such as a reduction in internal granules and organelles. Additionally, ED affects immune regulation, including changes in hormone levels.[63,64] Thus, we hypothesize that reduced testosterone levels could impact the activity and maturation of monocytes and other immune cells, subsequently affecting their internal structure and SSC-A expression. Immune regulation might also lead to further programmed cell death. During apoptosis, internal cellular structures change, such as the breakdown of organelles and reduction of granules, which could appear as a decrease in SSC-A in flow cytometry analyses. The decrease in CCR2 on CD14+ CD16+ monocytes might be due to ED's association with cardiovascular diseases, diabetes, and metabolic syndrome, all involving inflammatory pathways.[65] The expression of the chemokine receptor CCR2 on these cells is crucial for their transport and functional responses in various physiological and pathological contexts. Typically, CD14+ CD16+ monocytes exhibit lower CCR2 expression compared to other subsets, reflecting their unique roles in immune response and tissue homeostasis.[66]

Our study also has limitations that might introduce bias into our results. First, the number of SNPs is limited, insufficient for a comprehensive overview. Second, many results

were positive only with the IVW method, while other tests were not. Third, the selected databases comprised entirely of European populations, meaning the results might not be directly applicable to other ethnic groups.

Conclusion

Our use of MR to investigate the causal relationships between various immune cells and ED could offer new directions for research into using immune cells as biomarkers for ED treatment. However, to fully understand the relationship between immune cell behavior and ED, it is essential to conduct further high-quality clinical studies and explore the underlying mechanisms. This comprehensive approach will help refine the potential therapeutic role of immune cells in managing and treating ED.

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Author contributions

J. Chen, Y. Liu, P. Zhan and T. Gao contributed equally to this work.

Supplementary material

Supplementary material is available at *Sexual Medicine* online.

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Conflicts of interest

None declared.

References

1. Shamloul R, Ghanem H. Erectile dysfunction. *Lancet*. 2013;**381**(9861):153–165. https://doi.org/10.1016/S0140-6736(12)60520-0.
2. Goldstein I, Goren A, Li VW, Tang WY, Hassan TA. Epidemiology update of erectile dysfunction in eight countries with high burden. *Sex Med Rev*. 2020;**8**(1):48–58. https://doi.org/10.1016/j.sxmr.2019.06.008.
3. Li MK, Garcia LA, Rosen R. Lower urinary tract symptoms and male sexual dysfunction in Asia: a survey of ageing men from five Asian countries. *BJU Int*. 2005;**96**(9):1339–1354. https://doi.org/10.1111/j.1464-410X.2005.05831.x.
4. Das UN. Is erectile dysfunction a low-grade systemic inflammatory condition? *Eur Heart J*. 2007;**28**(5):642–643author reply 643-644. https://doi.org/10.1093/eurheartj/ehl531.
5. Liu G, Zhang Y, Zhang W, *et al*. Novel predictive risk factor for erectile dysfunction: serum high-sensitivity C-reactive protein. *Andrology*. 2022;**10**(6):1096–1106. https://doi.org/10.1111/andr.13206.
6. Bonanni M, Rehak L, Massaro G, *et al*. Autologous immune cell-based regenerative therapies to treat vasculogenic erectile dysfunction: is the Immuno-centric revolution ready for the prime time? *Biomedicines*. 2022;**10**(5):1091. https://doi.org/10.3390/biomedicines10051091.
7. Bae SC, Lee YH. Causal association between body mass index and risk of rheumatoid arthritis: a Mendelian randomization study. *Eur J Clin Investig*. 2019;**49**(4):e13076. https://doi.org/10.1111/eci.13076.
8. Staley JR, Burgess S. Semiparametric methods for estimation of a nonlinear exposure-outcome relationship using instrumental variables with application to Mendelian randomization. *Genet Epidemiol*. 2017;**41**(4):341–352. https://doi.org/10.1002/gepi.22041.
9. de Leeuw C, Savage J, Bucur IG, Heskes T, Posthuma D. Understanding the assumptions underlying Mendelian randomization. *Eur J Hum Genet*. 2022;**30**(6):653–660. https://doi.org/10.1038/s41431-022-01038-5.
10. Orru V, Steri M, Sidore C, *et al*. Complex genetic signatures in immune cells underlie autoimmunity and inform therapy. *Nat Genet*. 2020;**52**(10):1036–1045. https://doi.org/10.1038/s41588-020-0684-4.
11. Burgess S, Thompson SG, Collaboration C C G. Avoiding bias from weak instruments in Mendelian randomization studies. *Int J Epidemiol*. 2011;**40**(3):755–764. https://doi.org/10.1093/ije/dyr036.
12. Hemani G, Zheng J, Elsworth B, *et al*. The MR-base platform supports systematic causal inference across the human phenome. *Elife*. 2018;**7**:e34408. https://doi.org/10.7554/eLife.34408.
13. Zhao J, Ming J, Hu X, Chen G, Liu J, Yang C. Bayesian weighted Mendelian randomization for causal inference based on summary statistics. *Bioinformatics*. 2020;**36**(5):1501–1508. https://doi.org/10.1093/bioinformatics/btz749.
14. Woolf B, Rajasundaram S, Cronje HT, *et al*. A drug target for erectile dysfunction to help improve fertility, sexual activity, and well-being: Mendelian randomisation study. *BMJ*. 2023;**383**:e076197. https://doi.org/10.1136/bmj-2023-076197.
15. Kuthe A. Phosphodiesterase 5 inhibitors in male sexual dysfunction. *Curr Opin Urol*. 2003;**13**(5):405–410. https://doi.org/10.1097/00042307-200309000-00008.
16. Hatzimouratidis K, Hatzichristou DG. A comparative review of the options for treatment of erectile dysfunction: which treatment for which patient? *Drugs*. 2005;**65**(12):1621–1650. https://doi.org/10.2165/00003495-200565120-00003.
17. Raina R, Lakin MM, Agarwal A, *et al*. Long-term effect of sildenafil citrate on erectile dysfunction after radical prostatectomy: 3-year follow-up. *Urology*. 2003;**62**(1):110–115. https://doi.org/10.1016/s0090-4295(03)00157-2.
18. Razdan S, Greer AB, Patel A, Alameddine M, Jue JS, Ramasamy R. Effect of prescription medications on erectile dysfunction. *Postgrad Med J*. 2018;**94**(1109):171–178. https://doi.org/10.1136/postgradmedj-2017-135233.
19. Bernard BA, Metman LV, Levine L, Ouyang B, Leurgans S, Goetz CG. Sildenafil in the treatment of erectile dysfunction in Parkinson's disease. *Mov Disord Clin Pract*. 2017;**4**(3):412–415. https://doi.org/10.1002/mdc3.12456.
20. Furtado TP, Saffati G, Furtado MH, Khera M. Stem cell therapy for erectile dysfunction: a systematic review. *Sex Med Rev*. 2023;**12**(1):87–93. https://doi.org/10.1093/sxmrev/qead040.
21. Al Demour S, Adwan S, Jafar H, *et al*. Safety and efficacy of 2 intracavernous injections of allogeneic Wharton's jelly-derived mesenchymal stem cells in diabetic patients with erectile dysfunction: phase 1/2 clinical trial. *Urol Int*. 2021;**105**(11-12):935–943. https://doi.org/10.1159/000517364.
22. Cengiz T, Kaya E, Oral DY, *et al*. Intracavernous injection of human umbilical cord blood mononuclear cells improves erectile dysfunction in streptozotocin-induced diabetic rats. *J Sex Med*. 2017;**14**(1):50–58. https://doi.org/10.1016/j.jsxm.2016.11.314.
23. Rastogi I, Jeon D, Moseman JE, Muralidhar A, Potluri HK, McNeel DG. Role of B cells as antigen presenting cells. *Front Immunol*. 2022;**13**:954936. https://doi.org/10.3389/fimmu.2022.954936.
24. Upasani V, Rodenhuis-Zybert I, Cantaert T. Antibody-independent functions of B cells during viral infections. *PLoS Pathog*. 2021;**17**(7):e1009708. https://doi.org/10.1371/journal.ppat.1009708.

25. Sage AP, Tsiantoulas D, Binder CJ, Mallat Z. The role of B cells in atherosclerosis. *Nat Rev Cardiol*. 2019;**16**(3):180–196. https://doi.org/10.1038/s41569-018-0106-9.
26. Adamo L, Rocha-Resende C, Mann DL. The emerging role of B lymphocytes in cardiovascular disease. *Annu Rev Immunol*. 2020;**38**(1):99–121. https://doi.org/10.1146/annurev-immunol-042617-053104.
27. Blair PA, Norena LY, Flores-Borja F, *et al*. CD19(+) CD24(hi)CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic lupus erythematosus patients. *Immunity*. 2010;**32**(1):129–140. https://doi.org/10.1016/j.immuni.2009.11.009.
28. Lin W, Jin L, Chen H, *et al*. B cell subsets and dysfunction of regulatory B cells in IgG4-related diseases and primary Sjogren's syndrome: the similarities and differences. *Arthritis Res Ther*. 2014;**16**(3):R118. https://doi.org/10.1186/ar4571.
29. McCaleb MR, Miranda AM, Ratliff KC, Torres RM, Pelanda R. CD19 is internalized together with IgM in proportion to B cell receptor stimulation and is modulated by phosphatidylinositol 3-kinase in bone marrow immature B cells. *Immunohorizons*. 2023;**7**(1):49–63. https://doi.org/10.4049/immunohorizons.2200092.
30. Banihashemi SR, Rahbarizadeh F, Zavaran Hosseini A, Ahmadvand D, Khoshtinat Nikkhoi S. Liposome-based nanocarriers loaded with anthrax lethal factor and armed with anti-CD19 VHH for effectively inhibiting MAPK pathway in B cells. *Int Immunopharmacol*. 2021;**100**:107927. https://doi.org/10.1016/j.intimp.2021.107927.
31. Buhl AM, Cambier JC. Phosphorylation of CD19 Y484 and Y515, and linked activation of phosphatidylinositol 3-kinase, are required for B cell antigen receptor-mediated activation of Bruton's tyrosine kinase. *J Immunol*. 1999;**162**(8):4438–4446. https://doi.org/10.4049/jimmunol.162.8.4438.
32. Otero DC, Omori SA, Rickert RC. Cd19-dependent activation of Akt kinase in B-lymphocytes. *J Biol Chem*. 2001;**276**(2):1474–1478. https://doi.org/10.1074/jbc.M003918200.
33. Klasener K, Jellusova J, Andrieux G, *et al*. CD20 as a gatekeeper of the resting state of human B cells. *Proc Natl Acad Sci U S A*. 2021;**118**(7):e2021342118. https://doi.org/10.1073/pnas.2021342118.
34. Shan D, Ledbetter JA, Press OW. Apoptosis of malignant human B cells by ligation of CD20 with monoclonal antibodies. *Blood*. 1998;**91**(5):1644–1652. https://doi.org/10.1182/blood.V91.5.1644.
35. Yasukawa M, Shiroguchi T, Inatsuki A, Kobayashi Y. Antigen presentation in an HLA-DR-restricted fashion by B-cell chronic lymphocytic leukemia cells. *Blood*. 1988;**72**(1):102–108. https://doi.org/10.1182/blood.V72.1.102.bloodjournal721102.
36. Tabata H, Matsuoka T, Endo F, Nishimura Y, Matsushita S. Ligation of HLA-DR molecules on B cells induces enhanced expression of IgM heavy chain genes in association with Syk activation. *J Biol Chem*. 2000;**275**(45):34998–35005. https://doi.org/10.1074/jbc.M002089200.
37. Law PJ, Sud A, Mitchell JS, *et al*. Genome-wide association analysis of chronic lymphocytic leukaemia, Hodgkin lymphoma and multiple myeloma identifies pleiotropic risk loci. *Sci Rep*. 2017;**7**(1):41071. https://doi.org/10.1038/srep41071.
38. Swain SL, McKinstry KK, Strutt TM. Expanding roles for CD4(+) T cells in immunity to viruses. *Nat Rev Immunol*. 2012;**12**(2):136–148. https://doi.org/10.1038/nri3152.
39. Kitchen SG, Jones NR, LaForge S, *et al*. CD4 on CD8(+) T cells directly enhances effector function and is a target for HIV infection. *Proc Natl Acad Sci U S A*. 2004;**101**(23):8727–8732. https://doi.org/10.1073/pnas.0401500101.
40. Deliyanti D, Figgett WA, Gebhardt T, Trapani JA, Mackay F, Wilkinson-Berka JL. CD8(+) T cells promote pathological angiogenesis in ocular neovascular disease. *Arterioscler Thromb Vasc Biol*. 2023;**43**(4):522–536. https://doi.org/10.1161/ATVBAHA.122.318079.
41. Winchester NE, Panigrahi S, Haria A, *et al*. Cytomegalovirus infection facilitates the costimulation of CD57+CD28- CD8 T cells in HIV infection and atherosclerosis via the CD2-LFA-3 Axis. *J Immunol*. 2024;**212**(2):245–257. https://doi.org/10.4049/jimmunol.2300267.
42. Raffin C, Vo LT, Bluestone JA. T(reg) cell-based therapies: challenges and perspectives. *Nat Rev Immunol*. 2020;**20**(3):158–172. https://doi.org/10.1038/s41577-019-0232-6.
43. Togashi Y, Shitara K, Nishikawa H. Regulatory T cells in cancer immunosuppression - implications for anticancer therapy. *Nat Rev Clin Oncol*. 2019;**16**(6):356–371. https://doi.org/10.1038/s41571-019-0175-7.
44. Xia C, Yin S, To K K W, *et al*. CD39/CD73/A2AR pathway and cancer immunotherapy. *Mol Cancer*. 2023;**22**(1):44. https://doi.org/10.1186/s12943-023-01733-x.
45. Perry C, Hazan-Halevy I, Kay S, *et al*. Increased CD39 expression on CD4(+) T lymphocytes has clinical and prognostic significance in chronic lymphocytic leukemia. *Ann Hematol*. 2012;**91**(8):1271–1279. https://doi.org/10.1007/s00277-012-1425-2.
46. Vignali PDA, DePeaux K, Watson MJ, *et al*. Hypoxia drives CD39-dependent suppressor function in exhausted T cells to limit antitumor immunity. *Nat Immunol*. 2023;**24**(2):267–279. https://doi.org/10.1038/s41590-022-01379-9.
47. Kortekaas KE, Santegoets SJ, Sturm G, *et al*. CD39 identifies the CD4(+) tumor-specific T-cell population in human cancer. *Cancer Immunol Res*. 2020;**8**(10):1311–1321. https://doi.org/10.1158/2326-6066.CIR-20-0270.
48. Lintermans LL, Stegeman CA, Heeringa P, Abdulahad WH. T cells in vascular inflammatory diseases. *Front Immunol*. 2014;**5**:504. https://doi.org/10.3389/fimmu.2014.00504.
49. Huntington ND, Xu Y, Nutt SL, Tarlinton DM. A requirement for CD45 distinguishes Ly49D-mediated cytokine and chemokine production from killing in primary natural killer cells. *J Exp Med*. 2005;**201**(9):1421–1433. https://doi.org/10.1084/jem.20042294.
50. Meza Guzman LG, Hyland CD, Bidgood GM, *et al*. CD45 limits early natural killer cell development. *Immunol Cell Biol*. 2024;**102**(1):58–70. https://doi.org/10.1111/imcb.12701.
51. Ren J, Jo Y, Picton LK, Su LL, Raulet DH, Garcia KC. Induced CD45 proximity potentiates natural killer cell receptor antagonism. *ACS Synth Biol*. 2022;**11**(10):3426–3439. https://doi.org/10.1021/acssynbio.2c00337.
52. Chen W, Chatta GS, Rubin WD, *et al*. T cells specific for a polymorphic segment of CD45 induce graft-versus-host disease with predominant pulmonary vasculitis. *J Immunol*. 1998;**161**(2):909–918. https://doi.org/10.4049/jimmunol.161.2.909.
53. van Vreeswijk H, Ruiter DJ, Brocker EB, *et al*. Differential expression of HLA-DR, DQ, and DP antigens in primary and metastatic melanoma. *J Invest Dermatol*. 1988;**90**(5):755–760. https://doi.org/10.1111/1523-1747.ep12560951.
54. Walsh MD, Dent OF, Young JP, *et al*. HLA-DR expression is associated with better prognosis in sporadic Australian clinicopathological stage C colorectal cancers. *Int J Cancer*. 2009;**125**(5):1231–1237. https://doi.org/10.1002/ijc.24484.
55. Warabi M, Kitagawa M, Hirokawa K. Loss of MHC class II expression is associated with a decrease of tumor-infiltrating T cells and an increase of metastatic potential of colorectal cancer: immunohistological and histopathological analyses as compared with normal colonic mucosa and adenomas. *Pathol Res Pract*. 2000;**196**(12):807–815. https://doi.org/10.1016/S0344-0338(00)80080-1.
56. Dunne MR, Phelan JJ, Michielsen AJ, *et al*. Characterising the prognostic potential of HLA-DR during colorectal cancer development. *Cancer Immunol Immunother*. 2020;**69**(8):1577–1588. https://doi.org/10.1007/s00262-020-02571-2.
57. Latifi A, Ghanizadeh-Vesali S, Hosseini S, Mohsenzadegan M. Clinical significance of peripheral blood CD11b(+)/CD33(+)/HLA-DR(-) myeloid cells in infants and children with infectious diseases and increased CRP. *Med J*

Islam Repub Iran. 2020;**34**:92. https://doi.org/10.34171/mjiri.34.92.
58. Sade-Feldman M, Kanterman J, Klieger Y, *et al*. Clinical significance of circulating CD33+CD11b+HLA-DR- myeloid cells in patients with stage IV melanoma treated with Ipilimumab. *Clin Cancer Res*. 2016;**22**(23):5661–5672. https://doi.org/10.1158/1078-0432.CCR-15-3104.
59. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol*. 2009;**9**(3):162–174. https://doi.org/10.1038/nri2506.
60. Ostrand-Rosenberg S. Myeloid derived-suppressor cells: their role in cancer and obesity. *Curr Opin Immunol*. 2018;**51**:68–75. https://doi.org/10.1016/j.coi.2018.03.007.
61. D'Andrea S, Micillo A, Francavilla F, *et al*. Serum from patients with erectile dysfunction and vascular risk factors triggered an oxidative stress-dependent mitochondrial apoptotic pathway in ex vivo expanded circulating Angiogenic cells of healthy men. *J Sex Med*. 2016;**13**(7):1063–1070. https://doi.org/10.1016/j.jsxm.2016.04.067.
62. Zhu B, Niu Y, Guo H, Jin X, Liu F. Pyroptosis and inflammation-mediated endothelial dysfunction may act as key factors in the development of erectile dysfunction (review). *Mol Med Rep*. 2023;**28**(3):165. https://doi.org/10.3892/mmr.2023.13052.
63. Trebaticky B, Zitnanova I, Dvorakova M, *et al*. Role of oxidative stress, adiponectin and endoglin in the pathophysiology of erectile dysfunction in diabetic and non-diabetic men. *Physiol Res*. 2019;**68**(4):623–631. https://doi.org/10.33549/physiolres.934129.
64. Yao G, Liang J, Han X, Hou Y. In vivo modulation of the circulating lymphocyte subsets and monocytes by androgen. *Int Immunopharmacol*. 2003;**3**(13-14):1853–1860. https://doi.org/10.1016/j.intimp.2003.09.002.
65. Williams DW, Byrd D, Rubin LH, Anastos K, Morgello S, Berman JW. CCR2 on CD14(+)CD16(+) monocytes is a biomarker of HIV-associated neurocognitive disorders. *Neurol Neuroimmunol Neuroinflamm*. 2014;**1**(3):e36. https://doi.org/10.1212/NXI.0000000000000036.
66. Weber C, Belge KU, von Hundelshausen P, *et al*. Differential chemokine receptor expression and function in human monocyte subpopulations. *J Leukoc Biol*. 2000;**67**(5):699–704. https://doi.org/10.1002/jlb.67.5.699.