Original Article

Causal relationship between circulating immune cells and gastric cancer: a bidirectional Mendelian randomization analysis using UK Biobank and FinnGen datasets

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Background: The role of immune cells in cancer pathogenesis remains controversial due to conflicting reports, potentially arising from various confounding factors. Emerging evidence suggests that cancer can also influence immune cell populations and functions, making it challenging to investigate their causal relationship. Traditional observational studies often fail to eliminate all confounding factors and are prone to reverse causality. Therefore, we employ Mendelian randomization (MR) to determine the causal relationship between immune cells and cancer, as this method can identify causal relationships independent of confounding factors and avoid reverse causality.

Methods: Genome-wide association study (GWAS) summary statistics on immune traits, encompassing 310 immune cell phenotypes, were obtained from 3,757 European individuals, with peripheral blood immune cells tested using flow cytometry. GWAS summary statistics for gastric cancer were derived from 476,116 European individuals across two large-scale biobanks: the UK Biobank and FinnGen. Gastric cancer was identified by the International Classification of Diseases, 9th Revision (ICD-9), and 10th Revision (ICD-10) codes. Significant single nucleotide polymorphisms (SNPs) for immune traits were extracted at a threshold of $P<1\times10^{-5}$, while a threshold of $P<5\times10^{-8}$ was used for gastric cancer GWAS data. Linkage imbalance-based clumping was performed to obtain independent SNPs, and those with F<10 were excluded to mitigate weak instrument bias. Phenoscanner V2 was used to exclude SNPs directly associated with potential confounders or outcomes. Two-sample MR was conducted using five MR methods, with inverse-variance-weighted (IVW) as the primary analysis method. A false discovery rate (FDR) correction was used to reduce the likelihood of type 1 errors. In addition, we conducted MR-Egger intercept tests and Cochran's Q tests.

Results: The numbers of $CD4^-CD8^-$ T cells and IgD^-CD27^- B cells were positively correlated with the development of gastric cancer, with odds ratios (ORs) of 1.15 [95% confidence interval (CI), 1.07–1.24; P<0.001; P_{FDR}=0.041; IVW method] and 1.07 (95% CI, 1.03–1.11; P=0.001; P_{FDR}=0.187; IVW method), respectively. However, the percentage of IgD^+CD24^- B cells in lymphocytes were negatively associated with the development of gastric cancer (OR =0.90; 95% CI, 0.84–0.96; P=0.002; P_{FDR}=0.187; IVW method). MR analysis of the above three immune cell phenotypes showed no significant heterogeneity or horizontal pleiotropy. In the reverse MR analysis, gastric cancer was not causally associated with any of the immune cell phenotypes.

Conclusions: Circulating $CD4^-CD8^-$ T cells and IgD^-CD27^- B cells are positively correlated with the development of gastric cancer, while the percentage of IgD^+CD24^- B cells in lymphocytes are negatively

correlated. These findings provide insight into the relationship between immune cells and gastric cancer pathogenesis and may serve as a basis for the development of immunotherapies for gastric cancer.

Keywords: Immune cells; gastric cancer; Mendelian randomization (MR); genetic; causal association

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Introduction

Globally, gastric cancer is the fifth most common type of cancer, with over a million cases diagnosed each year (1). There is a poor prognosis for gastric cancer, and it is the third leading cause of cancer-related deaths (1,2). Currently, surgery and chemotherapy are the most commonly used treatments for gastric cancer (3-6). Immunotherapy is an emerging therapy that has been shown to have a number of advantages, including durable responses, long-term survival benefits, and a lower level of toxicity (7-10). Understanding the relationship between the immune system and gastric cancer may assist in the development of new immunotherapy targets.

Highlight box

Key findings

- The absolute numbers of $CD4^-CD8^-$ T cells and IgD^-CD27^- B cells were positively correlated with the development of gastric cancer.
- The percentage of IgD^+CD24^- B cells in lymphocytes was negatively associated with the development of gastric cancer.
- Gastric cancer was not causally associated with any of the immune cell phenotypes in the reverse Mendelian randomization (MR) analysis.

What is known and what is new?

- The immune system plays a crucial role in cancer immune surveillance, but the relationship between circulating immune cells and gastric cancer remains largely unknown.
- This study used bidirectional two-sample MR to demonstrate that certain immune cell traits, including $CD4^-CD8^-$ T cells, IgD^-CD27^- B cells, and IgD^+CD24^- B cells, are causally related to gastric cancer risk.

What is the implication, and what should change now?

- The findings provide insight into the relationship between immune cells and gastric cancer pathogenesis, which may aid in the development of new immunotherapy targets for gastric cancer.
- Further research is required to confirm these causal relationships and investigate the underlying mechanisms between the identified immune cell traits and gastric cancer.

The immune system of the host plays an important role in the immune surveillance of cancer and is capable of killing cancer cells through both innate and adapted immune responses (11). Despite this, there are contradictory reports regarding the role of immune cells in cancer pathogenesis (12-15). In gastric cancer, tumor-infiltrating dendritic cells and natural killer cells are generally associated with improved prognosis and enhanced anti-tumor immunity (16). In contrast, effector regulatory T cells and regulatory B cells can promote immune escape, leading to poorer outcomes (7). The prognostic implications of cytotoxic T cells ($CD8^+$) are particularly complex (17-19); while some studies link high $CD8^+$ T cell infiltration to better survival rates, others associate it with worse prognoses. These discrepancies likely arise from the intricate interplay between different immune cell subpopulations, the tumor microenvironment, and the stage of cancer progression. Thus, elucidating the causal relationships between immune cells and gastric cancer is crucial. However, traditional observational studies are often confounded by factors such as reverse causality, making it challenging to establish definitive causal links.

Mendelian randomization (MR) is an emerging genetic epidemiological method based on Mendel's genetic inheritance laws (20). MR uses genetic variations as instrumental variants (IVs), which are randomly assigned at conception prior to disease onset (21). Therefore, MR can be used to identify causal relationships independent of confounding factors and avoid reverse causality (22-24). In recent years, MR analysis has increasingly been utilized to identify possible risk factors for gastric cancer (25-27). Several MR studies have also examined the causal relationship between inflammatory biomarkers and gastric cancer, including interleukin-6 (28), interleukin-10 (29), and C-reactive protein (30).

While previous studies have primarily focused on the impact of immune cells on cancer development, emerging evidence suggests that cancer itself can influence immune cell populations and function. This bidirectional relationship

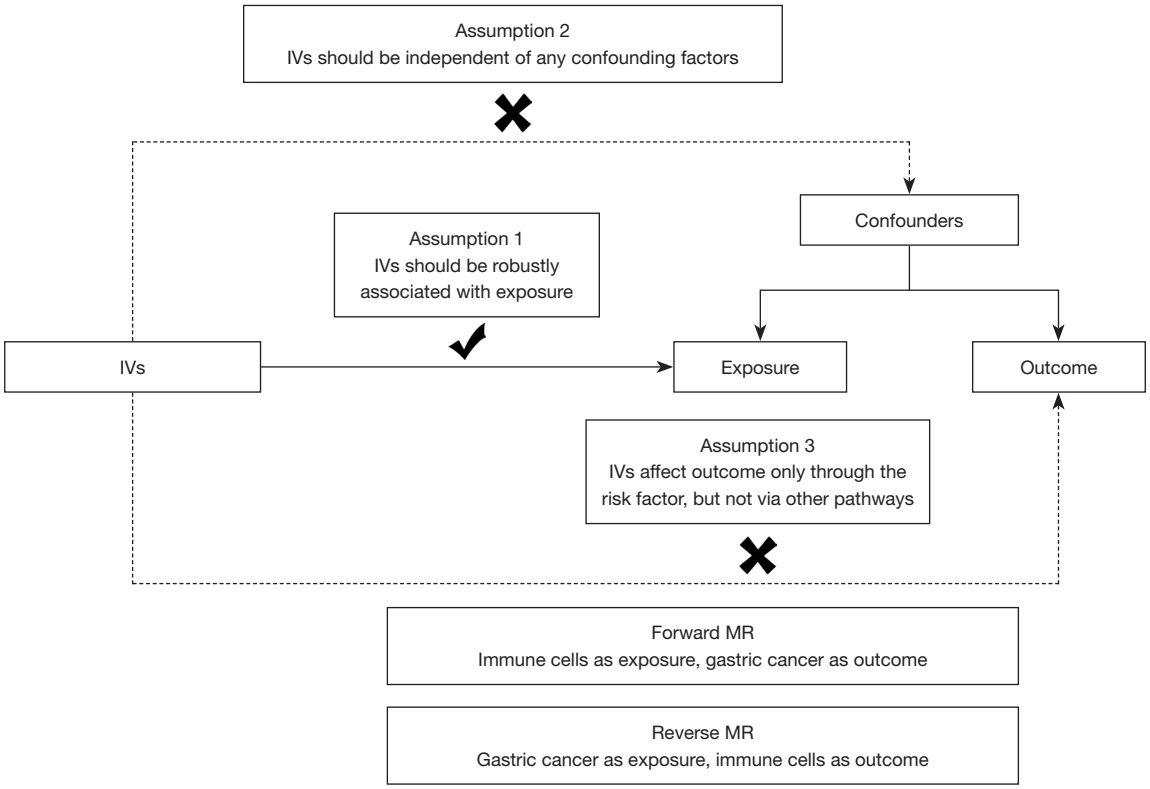

Figure 1 Three key assumptions of the mendelian analysis study and the design of our study design. MR, Mendelian randomization; IV, instrumental variable.

between cancer and the immune system is known as cancer immunoediting (31). During this process, the tumor can shape the immune response, potentially leading to changes in circulating immune cell populations (31,32). Therefore, investigating the bidirectional relationship between the immune cells and gastric cancer is crucial for understanding the complex interplay between them.

However, to our knowledge, no MR study has examined the possible causal link between circulating immune cells and gastric cancer risk. Therefore, in this study, we conducted a bidirectional two-sample MR analysis using large-scale genome-wide association study (GWAS) data to determine the causal relationship between immune cell traits and gastric cancer. We present this article in accordance with the STROBE-MR reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-480/rc).

Methods

Study design

This is a bidirectional two-sample MR study. In the forward MR analyses, immune cell traits were considered as exposures and gastric cancer was considered as outcome, whereas in the reverse MR analyses, gastric cancer was considered as exposure and immune cell traits as outcome. MR uses single nucleotide polymorphisms (SNPs) as IVs, and the valid IVs should fulfil the following three assumptions (33): first, IVs should be robustly associated with exposure (the relevance assumption); second, IVs should be independent of any confounding factors (the independence assumption); third, IVs affect outcome only through the risk factor, but not via other pathways (the exclusion limitation assumption). *Figure 1* illustrates our study design.

Data sources

GWAS-summary statistics for each immune trait are available through the GWAS Catalog (accession numbers GCST90001391 to GCST90001700) (34). A total of 310 immune cell phenotypes were analyzed, including absolute cell counts (n=118) and relative cell counts (n=192). The GWAS on immune traits was performed using data from

3,757 European individuals. Peripheral blood samples were collected from the donors in heparin tubes. Circulating immune cells were tested using flow cytometry, where the blood samples were stained with specific antibodies and analyzed with BD FACSCanto II flow cytometers (34). The data were processed using BD FACSDiva software, and cell populations were manually gated to ensure consistency (34). The GWAS included 20,143,392 SNPs, and the associations were examined after adjusting for covariates such as sex, age, and age^2 (35). GWAS-summary statistics regarding gastric cancer are available through the GWAS Catalog (accession number GCST90018849) (36). The statistics included 24,188,662 SNPs from 476,116 European individuals across two large-scale biobanks: the UK Biobank and FinnGen. In the UK Biobank cohort, gastric cancer cases were identified through linkage with national cancer registries (37). In the FinnGen cohort, gastric cancer cases were ascertained using a combination of nationwide health registries, including the Finnish Cancer Registry, the Care Register for Health Care, and the Cause of Death Register (38). Identification of gastric cancer was facilitated by corresponding International Classification of Diseases, 9th Revision (ICD-9), and 10th Revision (ICD-10) codes. 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. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Selection of IVs

We employed a systematic approach to select IVs for each immune trait and gastric cancer. For immune traits, we extracted significant SNPs at a threshold of $P<1\times10^{-5}$ (39,40), while for gastric cancer GWAS data, we used a more stringent threshold of $P<5\times10^{-8}$. To ensure independence among selected SNPs, we performed linkage disequilibrium (LD)-based clumping using PLINK. For immune traits, we applied an r^2 threshold of 0.1 within a 500 kb window (39), whereas for gastric cancer, we used a stricter r^2 threshold of 0.0001 within a 1,000 kb window. LD calculations were based on the 1,000 Genomes Project reference panel (41). To assess instrument strength, we calculated the F statistic for each SNP and included only those with F>10 to mitigate weak instrument bias (42). Furthermore, we utilized Phenoscanner V2 to exclude SNPs directly associated with potential confounders or outcomes (43,44), ensuring the validity of our selected IVs. This comprehensive selection process aimed to identify robust and independent IVs for our MR analysis.

Statistical analysis

To examine causal associations between different immune cell phenotypes and gastric cancer, we conducted five analyses: inverse variance weighting (IVW), MR Egger, weighted median, simple mode, and weighted mode, among which the IVW method (random effects) was the primary analysis (45). These methods make different assumptions about pleiotropy and instrument validity: IVW assumes all instruments are valid or that pleiotropic effects are balanced (46); MR Egger allows for directional pleiotropy and provides a test for its presence (47); weighted median is robust when up to 50% of the weight comes from invalid instruments (48); simple mode assumes that the most common estimate is the true causal effect (49); weighted mode provides a more robust estimate when there is heterogeneity among the SNPs (50). We used multiple methods to assess the robustness of our findings across different assumptions. Since there are multiple tests in this study, we used the false discovery rate (FDR) correction to avoid the likelihood of type 1 errors, and a $P_{FDR}<0.2$ was considered as significant (39,51). The Cochran's Q statistic was used to assess the heterogeneity among selected IVs, and a P value <0.05 indicates the existence of heterogeneity (45). The MR-Egger intercept test was employed to evaluate horizontal pleiotropy. If the MR-Egger intercept was significant (P<0.05), it suggested that association results may be influenced by horizontal pleiotropic effects of other traits (52). Forest plots and scatter plots were generated to visualize our results. Moreover, the leave-one-out sensitivity test was performed to test for the influence of a single SNP on the overall causal estimates. The statistical analyses were conducted with R version 4.3.1 (http://www.Rproject.org).

Results

Exploration of the causal effect of immune cell phenotypes on gastric cancer

We first used the IVW method to conduct MR analysis. The results showed that 27 immune cell phenotypes were associated with gastric cancer (P<0.05) (*Figure 2*). After FDR adjustment, three immune characteristics showed a causal association with gastric cancer ($P_{FDR}<0.2$), namely: the number of $CD4^-CD8^-$ T cells, the number of IgD^-

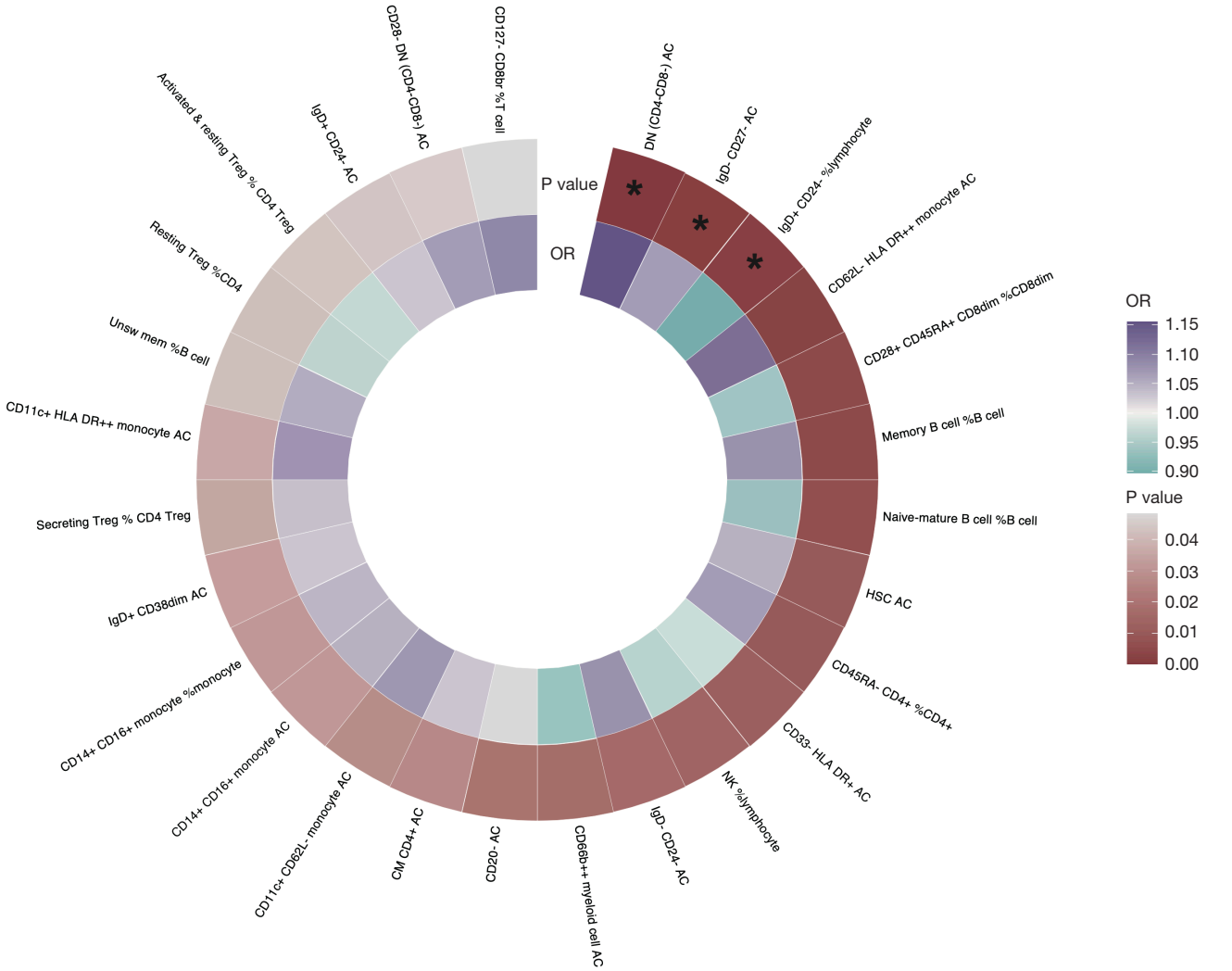

Figure 2 Heatmap for the causal effect of circulating immune cells on the risk of gastric cancer using the IVW method. The outer circle represents P values, whereas the inner circle represents ORs. *, the false discovery rate corrected P values <0.2. IVW, inverse variance weighting; OR, odds ratio.

CD27$^-$ B cells and the percentage of IgD$^+$CD24$^-$ B cells in lymphocytes.

The results showed that the absolute numbers of IgD$^-$CD27$^-$ B cells and CD4$^-$CD8$^-$ T cells were positively related to the development of gastric cancer, with odd ratios (ORs) of 1.15 [95% confidence interval (CI), 1.07–1.24; P<0.001; P_{FDR}=0.041] and 1.07 (95% CI, 1.03–1.11; P=0.001; P_{FDR}=0.187). However, IgD$^+$CD24$^-$ B cells in lymphocytes (OR =0.90; 95% CI: 0.84–0.96; P=0.002; P_{FDR}=0.187) were negatively associated with gastric cancer development (*Figure 3*).

In addition, we performed MR analysis using four other methods: the MR Egger, weighted median, simple mode, and weighted mode (*Figure 3*). Four methods yielded similar results regarding the causal relationship between CD4$^-$CD8$^-$ T cells and gastric cancer. In regard to the number of IgD$^-$CD24$^-$ B cells, only the weighted mode method provided comparable results to the IVW method. In terms of the percentage of IgD$^+$CD27$^+$ B cells in lymphocytes, only the weighted median method yielded comparable results to the IVW method. Regardless, the causal relationships suggested by the various MR methods are consistent with those suggested by the IVW method. *Figure 4A-4C* illustrate the scatter plots for the MR analysis of these immune cell phenotypes.

While conducting MR analysis on the above three

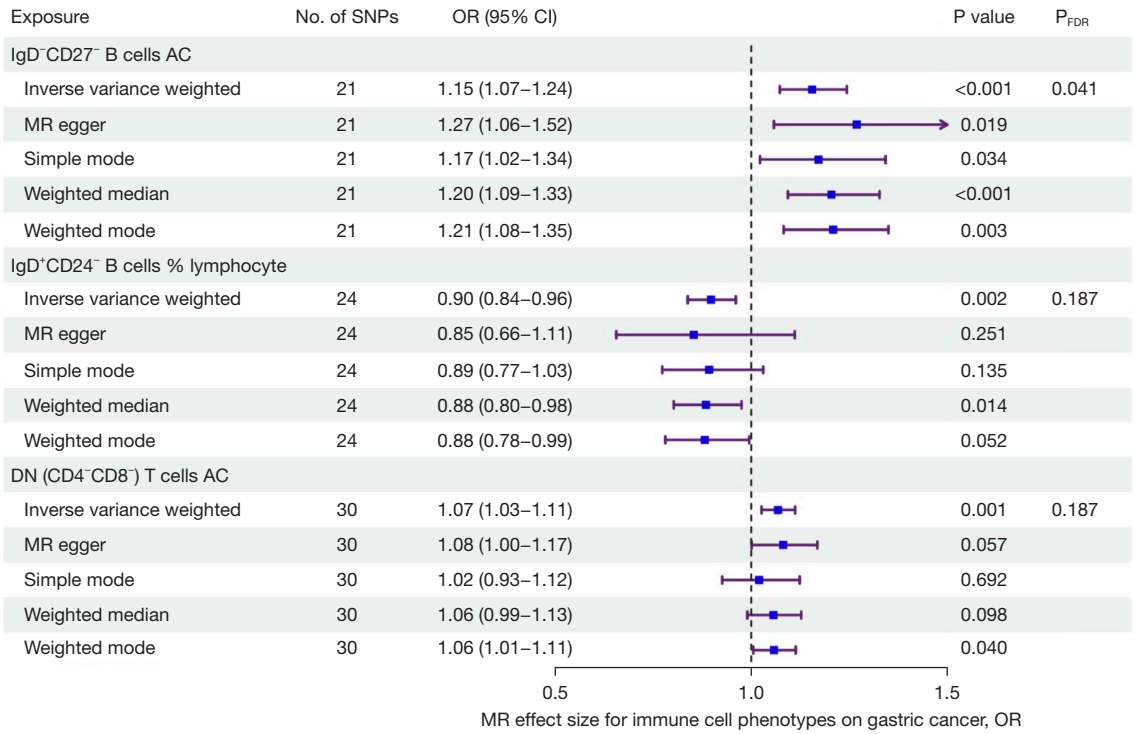

Figure 3 Forest plot for the causal effect of circulating immune cells on the risk of gastric cancer. AC, absolute count; MR, Mendelian randomization; DN, double negative; SNPs, single nucleotide polymorphisms; OR, odds ratio; CI, confidence interval; FDR, false discovery rate.

immune cell phenotypes, 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 (P>0.05) for each immune cell phenotype (*Table 1*), suggesting that the SNPs did not influence the outcome via factors unrelated to the exposure factors. Moreover, the leave-one-out sensitivity test showed that the overall causal estimates were not affected by individual SNPs (*Figure 4D-4F*).

For the other immune cell phenotypes, https://cdn.amegroups.cn/static/public/tcr-24-480-1.xlsx provides the results of MR analysis, and https://cdn.amegroups.cn/static/public/tcr-24-480-2.xlsx shows the results of heterogeneity and horizontal pleiotropy.

Exploration of the causal effect of gastric cancer on immune cell phenotypes

The IVW method was first used to conduct MR analysis. Following FDR adjustment, gastric cancer showed no causal association with any of the immune cell phenotypes (P_{FDR}>0.2). Gastric cancer did not have a causal effect on the number of $CD4^-CD8^-$ T cells, the number of IgD^-CD27^- B cells, or the percentage of IgD^+CD24^- B cells in lymphocytes, which was also demonstrated by the four other methods (MR Egger, weighted median, simple mode, and weighted mode) (*Figure 5*).

The https://cdn.amegroups.cn/static/public/tcr-24-480-3.xlsx presents MR results, and the https://cdn.amegroups.cn/static/public/tcr-24-480-4.xlsx presents heterogeneity and horizontal pleiotropy results for all immune cell phenotypes.

Discussion

In the present study, we used MR to investigate the causal relationship between 310 immune cell traits and gastric cancer. Our study demonstrated that the absolute number of $CD4^-CD8^-$ T cells and the absolute number of IgD^-CD27^- B cells were positively related to the development of gastric cancer, whereas the percentage of IgD^+CD24^- B cells in lymphocytes were negatively associated with gastric cancer development. Moreover, we found that gastric cancer did not have a causal effect on these immune cell phenotypes.

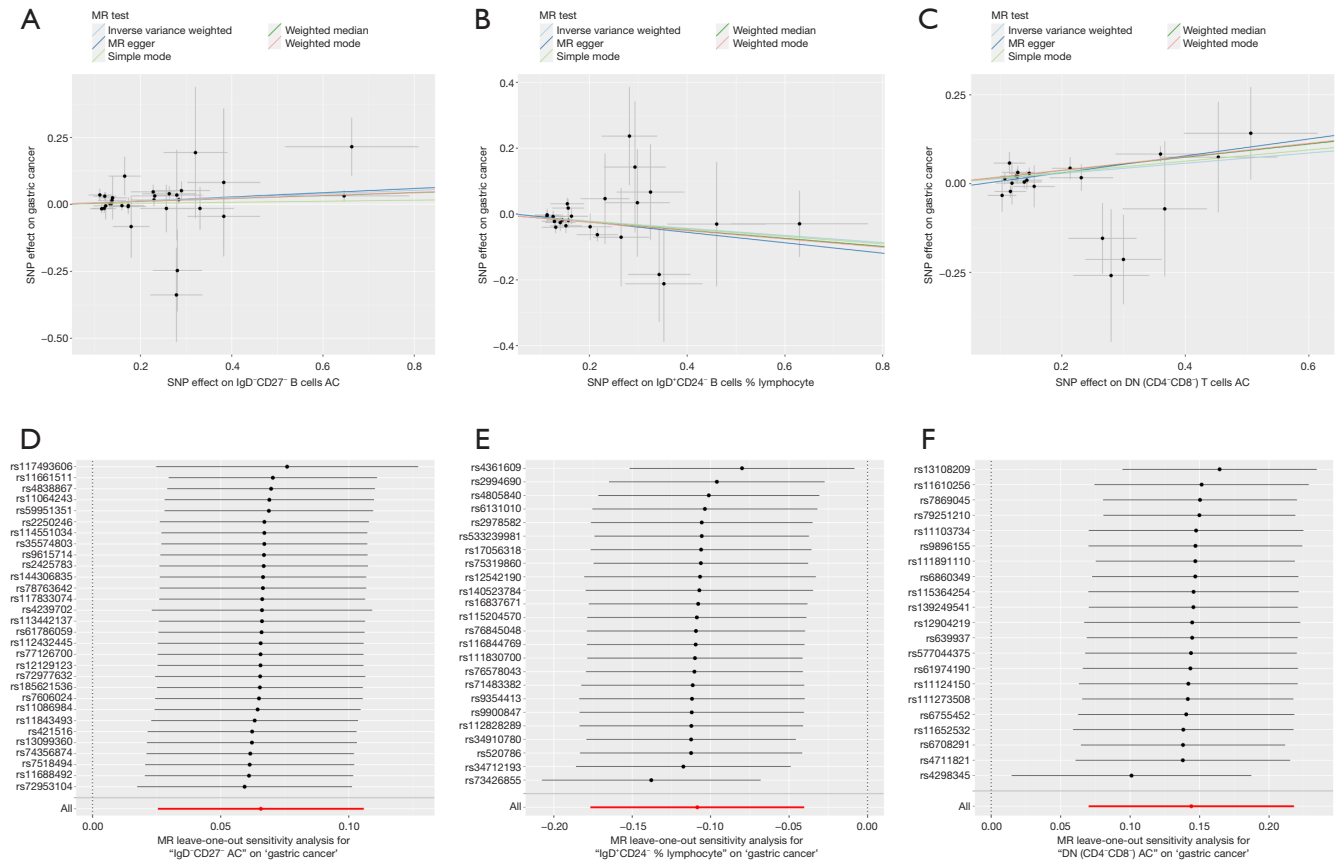

Figure 4 Scatter plots (A-C) and leave-one-out forest plots (D-F) for the causal effect of circulating immune cells on gastric cancer. MR, Mendelian randomization; SNP, single nucleotide polymorphism; AC, absolute count; DN, double negative.

Table 1 Heterogeneity and horizontal pleiotropy results for the Mendelian randomization analysis of immune cell phenotypes on gastric cancer

Exposure	P value (Q-Egger)	P value (Q-IVW)	Egger-intercept	P value (Egger-intercept)
IgD$^-$CD27$^-$ B cells AC	0.562	0.516	−0.003	0.712
IgD$^+$CD24$^-$ B cells %lymphocyte	0.401	0.353	0.008	0.704
DN (CD4$^-$CD8$^-$) T cells AC	0.255	0.271	−0.017	0.282

AC, absolute count; DN, double negative; IVW, inverse variance weighted.

According to our study, peripheral blood CD4$^-$CD8$^-$ T cell counts are positively associated with the development of gastric cancer. In the human body, the majority of adaptive immune cells contain either CD4 or CD8 as coreceptors. However, there is a small subpopulation of T lymphocytes that lack both CD4 and CD8 coreceptors which is called double-negative T cells (53). This subpopulation accounts for approximately 3–5% of peripheral blood T lymphocytes (54). It has been demonstrated that double-negative T cells are crucial to orchestrating immune responses. Numerous studies have examined the role of double-negative T cells in the development and progression of tumors. Lu *et al.* demonstrated that human double-negative T cells reduced pancreatic cancer cell proliferation and infiltration in co-culture (55). Double-negative T cells also showed enhanced cytotoxicity against lung cancer-derived cells *in vitro* (56). In some studies, activating double-negative T cells is suggested to be an alternative therapy for the treatment of leukemia (57,58). However, several studies reported inconsistent findings. Prins *et al.* found that decreasing the

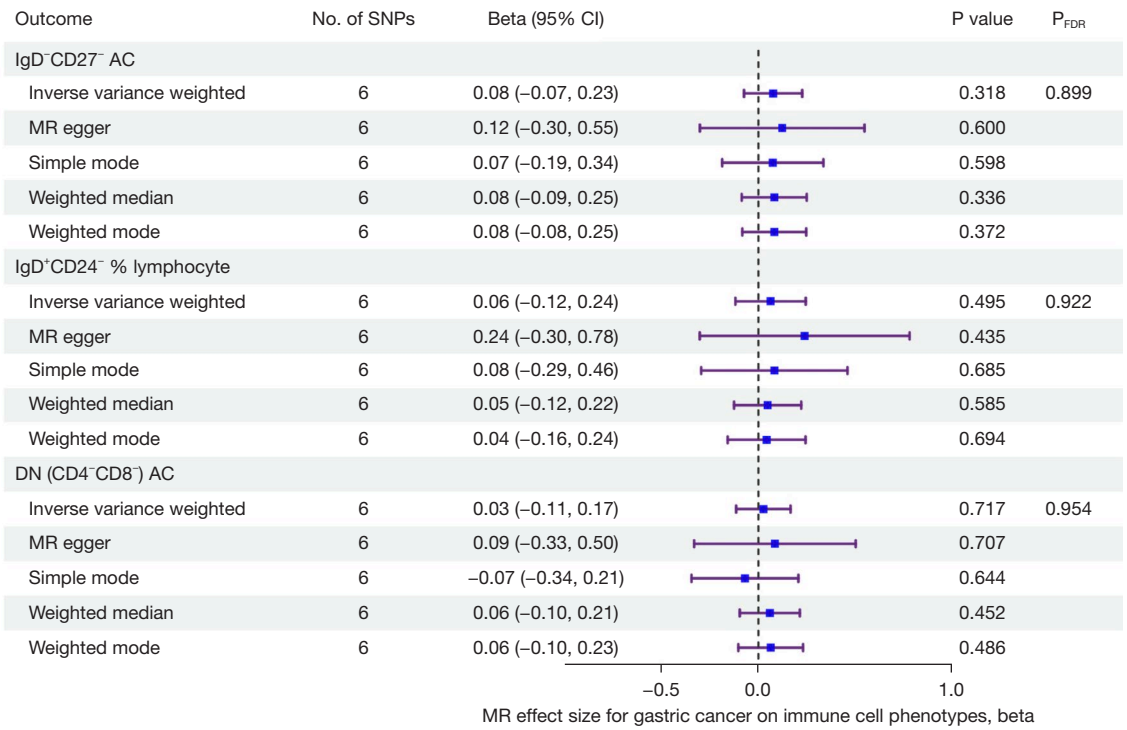

Figure 5 Forest plot for the causal effect of gastric cancer on circulating immune cells. AC, absolute count; MR, Mendelian randomization; DN, double negative; SNPs, single nucleotide polymorphisms; CI, confidence interval; FDR, false discovery rate.

number of double-negative T cells improved host survival in murine glioma and melanoma models, indicating the immunosuppressive properties of double-negative T cells (59). Furthermore, double-negative T cells were shown to contribute to tumor metastasis. An increase in double-negative T cells was observed in the lymph nodes of melanoma patients with disease progression as compared to those without progression (60,61). In view of these contradictory conclusions, it seems that double-negative T cells may be either pro-tumor or anti-tumor depending on the specific microenvironment and type of cancer (53). To date, little is known about the role of double-negative T cells in the development and progression of gastric cancer. Our results indicate a positive correlation between the number of double-negative T cells and the development of gastric cancer. However, further research will be required to confirm this finding.

Furthermore, we found that IgD$^-$CD27$^-$ B cell counts were positively associated with a higher risk of gastric cancer. IgD$^-$CD27$^-$ B cells are a rare subset of B cells known as double-negative B cells. They constitute approximately 5% of all peripheral B cells (62). Although IgD$^-$CD27$^-$ B cells are relatively rare in normal peripheral blood, they play an important role in a number of diseases, including systemic lupus erythematosus (63), coronavirus disease 2019 (COVID-19) (64), and malaria (65). Their functions include defending the host against pathogens as well as initiating autoimmune responses (66). IgD$^-$CD27$^-$ B cells have been reported to play an immunosuppressive role in the tumor microenvironment in some types of cancer. Centuori *et al.* demonstrate that infiltrating IgD$^-$CD27$^-$ B cells are enriched within the microenvironment of non-small cell lung cancer tumors, and that these cells are associated with tumor differentiation (67). Similarly, nasopharyngeal carcinoma is also enriched with IgD$^-$CD27$^-$ B cells, and the presence of IgD$^-$CD27$^-$ B cells is related to poorer clinical outcomes (68). These findings suggest that IgD$^-$CD27$^-$ B cells may be a potential immunotherapeutic target for these cancers. As of yet, no study has examined the role that double-negative B cells play in the development and progression of gastric cancer. According to our study, double-negative B-cell count was positively related to gastric cancer risk. These results are consistent with previous findings regarding other types of cancer, however more research is needed to illustrate the role and underlying mechanisms of IgD$^-$CD27$^-$ B cells in cancer development.

We also found that a higher percentage of IgD^+CD24^- B cells was associated with a lower risk of gastric cancer. The causality of this association is yet to be established by previous studies, but a number of studies have provided indirect evidence (69). Yang *et al.* examined the proportion of CD24-high-expressing B cells in the peripheral blood of patients with gastric cancer, and found that the proportion of these cells was lower in patients with non-progressive gastric cancer than in those with progression (69). Further research found that these cells are capable of suppressing the proliferation of autologous $CD4^+$ T cells and inhibiting the production of interferon-gamma by $CD4^+$ T cells (70). Thus, CD24 expression on B cells may contribute to immune suppression in gastric cancer. IgD is an antibody that is expressed on the surface of antigen-naive B cells (62). The main function of IgD is to control the survival, differentiation, and further utilization of the low-affinity autoreactive B cells that recognize structurally distinct antigens (71,72). The expression of IgD may counteract the restrictive pressure placed on the naive B cell receptor, thereby precipitating an adaptive immune response (73). Based on these findings, IgD^+CD24^- B cells may be capable of exerting anticancer immunity.

In the present study, we used large-scale GWAS data, examined the association between as many as 310 immune cell traits and gastric cancer and provided genetic evidence for the causal relationship between circulating immune cells and gastric cancer. However, there are several limitations in this study. First, the study was based on a European database, and the results may not be generalizable to other ethnic groups. Second, while we observed a causal relationship between certain immune cells and gastric cancer, the underlying mechanisms are still largely unknown, so our findings should be interpreted with caution.

Conclusions

Our study demonstrated that circulating $CD4^-CD8^-$ T cells and IgD^-CD27^- B cells were positively correlated with the development of gastric cancer, while the percentage of IgD^+CD24^- B cells in lymphocytes were negatively correlated. These findings provide insight into the relationship between immune cells and the pathogenesis of gastric cancer. Moreover, our findings may also serve as a basis for the development of immunotherapies for gastric cancer. Further research, however, is required to confirm these causal relationships, as well as investigating the underlying mechanisms.

Acknowledgments

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Footnote

Reporting Checklist: The authors have completed the STROBE-MR reporting checklist Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-480/rc

Peer Review File: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-480/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-480/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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