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Immune Cell Signatures and Causal Association with Irritable Bowel Syndrome: A Mendelian Randomization Study

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Abstract

BACKGROUND

The intricate immune-brain interactions within the mucosal barrier, which influence neural development, survival, and function, could hold causal and therapeutic significance for irritable bowel syndrome. Previous research on the relationship between immune inflammation and irritable bowel syndrome (IBS) has yielded conflicting findings. Therefore, we analyzed 731 publicly available immune cells through Mendelian randomization studies and explored the impact of different immune phenotypes on IBS, to enhance our knowledge of the disrupted brain-gut interactions that define IBS. We expect that future studies will be based on the immunophenotypic results we have discovered, providing a mechanistic basis for drug development and systemic therapy efficacy.

AIM

we analyzed 731 publicly available immune cells through Mendelian randomization studies and explored the impact of different immune phenotypes on IBS, to enhance our knowledge of the disrupted brain-gut interactions that define IBS. We expect that future studies will be based on the immunophenotypic results we have discovered, providing a mechanistic basis for drug development and systemic therapy efficacy.

METHODS

An exhaustive two-sample Mendelian Randomization (MR) analysis was conducted to assess the causal link between immune cell markers and Irritable Bowel Syndrome (IBS) in this investigation. Utilizing publicly accessible genetic information, we investigated the causal relationships between 731 immune cell markers and IBS susceptibility. This included four distinct categories of immune cell signatures: median fluorescence intensity (MFI), relative cell abundance (RC), absolute cell count (AC), and morphological parameters (MP). To ensure the reliability, lack of heterogeneity, and absence of horizontal pleiotropy in our findings, we employed thorough sensitivity analyses.

RESULTS

We conducted bidirectional FDR correction, and IBS did not exhibit a statistically significant impact on immunophenotypes. However, in examining the causal influence of immune phenotypes on IBS, we identified that out of the four immune trait categories (MFI, RC, AC, and MP), IBS has a causal impact on 30 immunophenotypes ($p < 0.05$). Among them, 9 immune phenotypes have a protective effect on IBS (ivw<0.05, OR＜1), which are: IgD− CD27− %B cell, IgD− CD27− AC, HLA DR+ CD8br AC, CD28+ CD45RA− CD8dim %T cell, CD20 on IgD− CD38dim, CD24 on CD24+ CD27+, CD25 on IgD+ CD38−, CD127 on CD4+, PDL−1 on monocyte; Another 21 immune phenotypes can lead to the onset of IBS（ivw≥0.05, OR≥1）, namely IgD+ CD24− %lymphocyte, CM DN (CD4−CD8−) %DN, CD4+ AC, CD8dim NKT %T cell, CD28+ CD45RA− CD8dim AC, CD28− CD127− CD25++ CD8br %T cell, CD28− CD127− CD25++ CD8br AC, CD27 on IgD+ CD24+, CD27 on IgD− CD38−, CD27 on IgD− CD38dim, CD27 on unsw mem, CD27 on sw mem, IgD on transitional, CD3 on CD28+ CD45RA+ CD8br, CD28 on CD4 Treg, CD45 on lymphocyte, CD25 on resting Treg, CD45 on CD33− HLA DR+, CD8 on EM CD8br, CD4 on CD39+ resting Treg, CD4 on activated Treg.

CONCLUSION

Our study revealed a significant genetic correlation between immune cells and irritable bowel syndrome, which is of great significance for understanding the pathological mechanisms of IBS. It not only provides new ideas for future clinical diagnosis and treatment, but also helps to develop more accurate biomarkers and treatment methods for IBS. In addition, this discovery also helps to enhance our understanding of the role of immune cells in the pathogenesis of IBS, providing patients with more effective personalized treatment plans. On this basis, it is expected to improve the quality of life of IBS patients and reduce the burden of the disease on patients and their families.

INTRODUCTION

Irritable bowel syndrome (IBS) qualifies as a functional gastrointestinal (GI) condition, which is common with a global prevalence that ranges from 9% to 23%, depending on the criteria used for diagnosis and the methodology employed in the studies[1, 2]. Furthermore, IBS exhibits a varied clinical profile and is linked to considerable disease burden. The origins and development of IBS are complex and involve numerous factors, with many aspects yet to be fully understood[3]. Recent investigations have given rise to fresh theories and insights into the underlying pathophysiology of IBS. The range of hypotheses extends to include intestinal immunity, brain-gut-microbiota[4], visceral hypersensitivity and intestinal flora disorder[5], inflammation[6], postinfectious[7], food sensitivity[8], heredity[9], psychosocial dysfunction[10]and so on, and have contributed to the creation of numerous therapeutic options and effects. Among them, The brain-gut-microbiota axis[11]not only signifies a paradigm shift in neuroscience but also offers a new target for the treatment of irritable bowel syndrome (IBS). Within the gastrointestinal tract, the immune system preserves a physiological equilibrium in response to environmental triggers such as allergens, dietary antigens, or pathogens[12]. The variations in the luminal contents, anatomical structure, and physiological roles throughout the gut are correlated with the distinct

immune cell populations and immunological reactions observed in each portion of the intestinal tract[13].

Immune cells and cytokines are integral to the response against infections and the regulation of inflammation, and they are also essential in facilitating the dialogue between the brain and the immune system[10]. Alterations in lymphocyte populations, including changes in the counts and activation levels of B and T lymphocytes, have been observed in Irritable Bowel Syndrome (IBS). These changes are associated with increased eosinophil counts in the duodenum in patients with Functional Dyspepsia (FD) and an increase in colonic mast cells in those with IBS[14]. Meanwhile, the rising levels of α4+β7+ labeled gut-homing T cells in the blood may be linked to the pathophysiological mechanisms of functional dyspepsia (FD) and irritable bowel syndrome (IBS). The previously described physiological equilibrium within the GI tract can be disrupted in cases of gastrointestinal disorders. Enhanced infiltration of mast cells and T cells in the mucosal layer, along with their level of activation, cytokine production, and genetic variations, are suggested to influence bowel function and contribute to the manifestation of symptoms in Irritable Bowel Syndrome (IBS). Meta-analysis shows increased colonic infiltration with mast cells[15] and T cells (as CD3+, CD4+, or CD8+ T cells)in IBS patients. B cell-activating factor (BAFF) is considered to play a regulatory role in the immune responses of B cells and T cells, and it is linked to inflammatory activities that occur in conditions of autoimmunity and certain B cell cancers[16, 17].

In recent years, with the deepening of research, more and more evidence supports the role of immune cells in the pathogenesis of IBS. For example, changes in immune cells in the intestinal mucosa may lead to impaired intestinal barrier function, leading to intestinal infections and dysbiosis. In addition, immune cells can also affect the intestinal nervous system by regulating gut microbiota metabolites, leading to symptoms of IBS. Therefore, immune cell research is of great significance for IBS. On the one hand, it can help reveal the pathogenesis of the disease and provide a theoretical basis for finding new therapeutic targets; On the other hand, by monitoring

immune cells, the severity and progression of the disease can be evaluated, guiding clinical diagnosis and treatment.

Mendelian randomization (MR) is an analytical technique commonly employed in epidemiology to infer the causes of diseases, which is grounded in the principle of Mendelian randomization[18, 19]. The MR approach can be seen as a type of "natural" randomized controlled trial (RCT), which has the potential to mitigate the typical biases associated with observational studies and provide reliable causal evidence between exposures and outcomes through genetic variants[20, 21]. Previous observational research has uncovered numerous associations between immune cell characteristics and Irritable Bowel Syndrome (IBS), supporting the idea of a connection between these factors[16]. In the current study, we present a thorough analysis using a two-sample approach. We aimed to investigate whether there was a causal effect between immune cells and IBS using a Mendelian randomization approach. We are trying to determine whether immune cells might be a particularly potent predictor of this disease, which can contribute to the prediction and treatment of IBS.

MATERIALS AND METHODS

Study design

We evaluated the causal link between 731 immune cell signatures (spanning 7 groups) and Irritable Bowel Syndrome (IBS) through a two-sample Mendelian Randomization (MR) analysis. MR relies on genetic variants to act as proxy risk factors, and thus, credible instrumental variables (IVs) for causal estimation must meet three essential criteria: (1) genetic variants should be directly linked to the exposure; (2) these variants should not correlate with potential confounders between exposure and outcome; and (3) they should not influence the outcome *via* mechanisms other than the exposure itself. In the analysis we conducted, all the studies included have obtained ethical approval from the relevant institutional review boards, and all participants have provided their informed consent after being fully informed about the study's purpose,

methods, potential risks, and benefits. This ensures that the research adheres to stringent ethical standards and safeguards the rights of the participants.

Genome-wide association study (GWAS) data sources for IBS

For the IBS genome-wide association study (GWAS), we utilized GWAS summary statistics from the UK Biobank(UKB)[22, 23]. The research team performed a genome-wide association analysis involving 53,400 individuals with IBS and 433,201 controls, and they validated these findings in a 23andMe dataset that included 205,252 cases and 1,384,055 controls[22, 23]. Additionally, their work revealed and authenticated six genetic loci associated with an increased risk of IBS. The genes in question are NCAM1, CADM2, PHF2/FAM120A, DOCK9, CKAP2/TPTE2P3, and BAG6. Of these, the first four genes are linked to mood and anxiety disorders, with expression in the nervous system, or in some cases, both. Concurrently, the research uncovered a robust genome-wide association between IBS risk and indices of anxiety, neuroticism, and depression (with a correlation coefficient, rg, greater than 0.5). Further analysis indicates that this relationship likely stems from common underlying pathogenic mechanisms rather than, for instance, anxiety directly causing abdominal symptoms. These mechanisms deserve further investigation to elucidate the disrupted brain-gut interactions that characterize IBS.

Immunity-wide GWAS data sources

Publicly accessible GWAS summary statistics for various immune traits can be found in the GWAS Catalog, with accession numbers ranging from GCST0001391 to GCST0002121[25]. The dataset includes 731 immunological phenotypes, encompassing Absolute Cell (AC) counts (n = 118), median fluorescence intensities (MFI) representing surface antigen levels (n = 389), morphological parameters (MP) (n = 32), and Relative Cell (RC) counts (n = 192). The MFI, AC, and RC traits incorporate various immune cell types, including B cells, CDCs, mature T cells, monocytes, myeloid cells, TBNK (T cells, B cells, natural killer cells), and Treg panels. The MP trait focuses on CDCs and TBNK panels. The initial Genome-Wide Association Study (GWAS) focused on immune traits was carried out utilizing data from a total of 3,757 European individuals. It is important

to note that there was no overlap between the different cohorts included in this study. This approach allowed for a thorough examination of the genetic factors that may influence immune-related phenotypes. To refine the genetic analysis, a high-density array was utilized to genotype approximately 22 million single nucleotide polymorphisms (SNPs) in the study. These data were then employed to estimate the genetic variations using a reference panel based on the Sardinian sequence. After controlling for confounding factors such as gender, age, and two-year age group, the study conducted a thorough analysis of the associations between the genotyped SNPs and the immune traits of interest. This approach allowed for a more precise characterization of the genetic factors influencing immune-related phenotypes, taking into account the potential modifying effects of age and gender. By adjusting for these confounders, the study aimed to provide a clearer understanding of the genetic variants that may contribute to the immune traits, thereby enhancing the reliability and generalizability of the findings.

Selection of instrumental variables (IVs)

Based on recent research[26, 27] in the field of genetic epidemiology, the significance threshold for instrumental variables (IVs) associated with each immune trait has been set at 1×10-5, which also provides a standardized method for identifying relevant genetic factors that may influence immune-related phenotypes. In order to refine the selection of single nucleotide polymorphisms (SNPs) and identify and remove SNPs with high linkage disequilibrium with nearby SNPs, reduce the complexity of genetic data, and improve the accuracy of subsequent genetic analysis, we used PLINK software (v1.90 version) to apply a clustering program based on a linkage disequilibrium (LD) r2 threshold of less than 0.1 within a 500 kb window. For the Irritable Bowel Syndrome (IBS) trait, the significance level was adjusted to 5 × 10-8. To assess the strength of the IVs and to mitigate the impact of weak instrumental variables, we calculated the proportion of Phenotypic Variation Explained (PVE) and the F statistic for each IV.

Statistical analysis

All analyses were conducted using R 3.5.3 software (accessible at http://www.Rproject.org). To assess the causal relationship between 731 immunological phenotypes and Irritable Bowel Syndrome (IBS), we employed the inverse variance weighting (IVW)[28] method, along with weighted median[29] and mode-based approaches[30], utilizing the 'Mendelian Randomization' package (version 0.4.3)[31]. This approach can be understood as a way of treating data points "equally." It's like when you're cooking, and you might adjust the amount of ingredients based on their importance and impact. Inverse variance weighting does the same thing[28]; it adjusts the influence of each data point in the analysis based on its importance, ensuring that every data point is given fair consideration. In short, this method makes sure that every data point "counts," and if some data points have a "louder" voice, it uses other methods to balance that influence and ensure the accuracy of the results. Additionally, Cochran's Q statistic and the corresponding p-value help us determine whether there are real, non-random differences between the selected instrumental variables (IVs). If the null hypothesis of heterogeneity was rejected, we shifted from fixed-effects to random-effects IVW. To account for the possibility of horizontal pleiotropy, we used the MR-Egger method[32], which is like a detector that can identify whether any specific data points are unfairly affecting the results. Additionally, we employed the MR-PRESSO method[33], which is designed to identify and exclude outlier genetic variants that could significantly affect our results. These outliers are genetic features that might influence multiple outcomes; if not removed, they could interfere with the accuracy of our conclusions. We also used scatter plots and funnel plots to help us understand the data. Scatter plots help us identify if any outliers are affecting the results, while funnel plots show whether the correlation between data points is consistent. This way, we can ensure that our research findings are accurate and reliable.

RESULTS

Investigation of the causal influence of the onset of IBS on immunological phenotypes

To investigate the causal impact of IBS on immunological phenotypes, a two-sample Mendelian Randomization (MR) analysis was conducted, with the Inverse Variance Weighting (IVW) method serving as the primary analysis technique. Following adjustments for multiple testing using the False Discovery Rate (FDR) method, no significant associations were found at the 0.2 significance level for any of the immune traits. (Figure 1)

Investigation of the causal relationship between immunological phenotypes and IBS

Following False Discovery Rate (FDR) adjustment, with a P FDR threshold of less than 0.05, we identified that among the four categories of immune traits (MFI, RC, AC, and MP), IBS exhibits causal effects on 30 immunophenotypes at a significant level of $p < 0.05$(Figures 2).

Furthermore, the intercept of the MR-Egger regression and the global test from MR-PRESSO excluded the likelihood of horizontal pleiotropy for the four associations in question. The detailed results from the consistency analysis demonstrate the robustness of the observed causal relationship across different analyses(refer to Supplementary Figure 2 for detailed information). The scatter plot shows that the effects observed in different methods are relatively consistent, and the odds ratios (OR) calculated under different models are also relatively close. These results once again confirm the stability of the observed effects (see Supplementary Figure 2 for visual representation).

DISCUSSION

Utilizing extensive publicly accessible genetic datasets, we investigated the causal connections between 731 immune cell traits and irritable bowel syndrome (IBS). To the best of our understanding, this represents the inaugural Mendelian Randomization (MR) analysis to delve into the causal linkages between multiple immunophenotypes and IBS. Although the immune system is known to be involved in inflammatory bowel

disease (IBD), its contribution to the progression of irritable bowel syndrome (IBS) remains intricate and elusive. To our understanding, this marks the inaugural Mendelian Randomization (MR) analysis to investigate the potential causal links between various immune-related traits and irritable bowel syndrome (IBS). The research revealed that significant immune traits are present across different immune cell types, particularly within certain subsets of T cells and B cells. Collectively, these discoveries indicate a more extensive involvement of immune biomarkers in IBS than we had previously anticipated.

Human B cells can be categorized into four classical subsets, which are distinguished by their expression of CD27 and immunoglobulin (Ig)D[34]. Unlike the other three extensively investigated subsets, CD27−IgD− B cells are referred to as double-negative (DN) B cells[35]. DN B cells are increased in Autoimmune diseases, such as systemic lupus erythematosus （SLE）, myasthenia gravis(MG), multiple sclerosis (MS), and rheumatoid arthritis (RA). Chathyan Pararasa's research aimed to shed light on the different subsets of peripheral B cells in inflammatory bowel disease (IBD) and how these subsets might vary between active disease and a state of remission[36]. The study found a consistent decrease in the proportion of CD27−IgD− B cells that produce IgM, IgA, and IgG in the blood of IBD patients. Additionally, there was an increase in the CD27−IgD− subset within mucosal-associated lymphoid tissues (MALT) in those with IBD, suggesting that CD27−IgD− B cells are recruited from the blood into the gut during IBD[37].

Regulatory T cells (Tregs) are a pivotal component of the immune regulatory network[38], playing a critical role in maintaining intestinal homeostasis and moderating inappropriate immune responses. In the context of irritable bowel syndrome (IBS), our studies have highlighted the significance of specific Treg cell subsets, characterized by various surface markers. These include Tregs with a profile of CD28+, CD45RA-dim, CD8dim, and AC; CD28−, CD127−, CD25++, CD8br%; CD3+, CD28+, CD45RA+, CD8br; CD28+ on CD4 Tregs; CD25 on resting Tregs; CD4 on CD39+, resting Tregs; and CD4 on activated Treg molecules. These markers underscore the Tregs' involvement in

regulating intestinal immune responses and their potential influence on the pathogenesis of IBS. In addition, their activity is intricately linked to the human leukocyte antigen (HLA) gene[39], which can be an explanation for IBS. CD4+CD25highCD127 Low/−FOXP3+ regulatory T cells play a crucial role in maintaining immune tolerance and moderating excessive immune reactions[40]. Some researchers have delved deeper into the impact of retinoic acid on the human Treg cell subset (CD4+CD25+CD127 Low/−CD45RA+), demonstrating that the expansion of these cells results in a uniform and epigenetically stable population that lacks pro-inflammatory cytokine production. When injected into a SCID mouse with a xenograft of the human small intestine, these cells preferentially colonize the lamina propria[41, 42]. Exploring the use of Treg cells as a therapeutic intervention and developing strategies to enhance their therapeutic efficacy are areas of ongoing research[43].

TBNK cells represent a pivotal subset of the immune system, encompassing T lymphocytes, B lymphocytes, and natural killer (NK) cells[44]. They are integral to the human immune response, and alterations in their levels are indicative of shifts in immune status. Our research has uncovered that the TBNK cell population can markedly curtail the pathogenesis of irritable bowel syndrome (IBS), primarily through the modulation of CD4+AC, CD8dim NKT% T cells, and lymphocytes expressing CD45. Furthermore, TBNK cells serve as a critical intermediary, facilitating the interplay between various immune characteristics and IBS pathology. The migration of activated T lymphocytes to the gut, particularly to the small intestine, is mediated by two principal pathways[45, 46]:(1) the α4β7-mucosal vascular address in cell-adhesion molecule 1 (MAdCAM-1) pathway, and (2) the chemokine ligand (CCL)25 and CCR9 pathway. The migration of T and B lymphocytes to the intestine is primarily orchestrated by dendritic cells within Peyer's patches and mesenteric lymph nodes, which up-regulate specific integrins such as α4β7 or CCR9. α4β7 binds to its receptor, MAdCAM-1, and this protein is primarily found on the surface of post-capillary venules in lymphoid organs. Clinical and experimental evidence indicates that targeting B cells could be beneficial in some cases of human inflammatory bowel disease (IBD).

The targeting of CD20, a cell surface molecule primarily found on mature B lymphocytes, by rituximab has been shown to be highly beneficial in the treatment of numerous hematological and immune-mediated diseases[47]. While the relevance of this approach to irritable bowel syndrome (IBS) is not yet fully understood, it does offer a potential area for future investigation and insight into the treatment of IBS.

Some natural killer (NK) cells and monocyte cells also play a role in participating in consistent effects on IBS[48, 49]. Multiple investigations have revealed elevated levels of inflammatory cytokines in the bloodstream of individuals suffering from irritable bowel syndrome (IBS). Natural Killer (NK) cells can perform various immunoregulatory roles. For example, by expressing a high-affinity receptor for IL-2, NK cells may compete with other cells for access to IL-2. The Fc receptor CD16a (FcγRIIIa), which is present on CD56Dim NK cells, binds to the constant region of IgG, thereby activating antibody-dependent cell-mediated cytotoxicity (ADCC)[50]. Consequently, NK cells in the gut are likely to have a more significant impact on maintaining gut homeostasis than their circulating counterparts, due to their production of cytokines. In Dominik Aschenbrenner's study[51], Through transcriptomic analysis, he found that analyzing monocyte responses in inflammatory bowel disease reveals an IL-1 cytokine network that regulates IL-23 production in the presence of both genetic and acquired IL-10 resistance, which can provide a therapeutic way for the clinic. Some studies also showed that IL-6 receptor （IL6R） signaling is involved in the development of inflammatory bowel disease[52].

We performed a two-sample Mendelian Randomization (MR) analysis that utilized the published data from expansive Genome-Wide Association Study (GWAS) cohorts[24]. Our analysis included a substantial cohort of approximately 486,601 individuals, which enhanced our study's statistical efficiency. Our findings are rooted in genetic instrumental variables, and we employed a range of MR analysis approaches to draw causal conclusions. The results exhibit consistency and were not compromised by horizontal pleiotropy or additional confounding elements. Our investigation is not without its limitations. One major consideration is the incomplete assessment of

horizontal pleiotropy, despite the conduct of multiple sensitivity analyses. Additionally, the absence of individual-level data prevents us from performing more detailed stratified analyses of the population, such as considering average age, health status, social and environmental factors, and other relevant variables. This limitation can lead to bias in our Mendelian Randomization (MR) estimates. Thirdly, as our study was conducted using a European database, the findings are not suitable for other ethnic groups, therefore, this may restrict the applicability of our findings to a broader population. Lastly, our study only shows a clue between the immune cells and IBS, further basic experiments and clinical trials are supposed to be carried out to confirm our hypothesis.

CONCLUSION

In conclusion, We conducted a comprehensive analysis of four immune traits (MFI, RC, AC, and MP) through bidirectional MR and found a causal relationship between IBS and 30 immune phenotypes. Nine immune phenotypes were found to offer protection against IBS, while the remaining 21 were associated with an increased risk of developing the condition, revealing the multifaceted nature of the immune system's relationship with IBS. Moreover, our study has substantially diminished the effects of inevitable confounders, reverse causation, and other such variables. This finding could pave the way for a new understanding of IBS's biological basis and may ultimately contribute to the development of timely interventions and improved therapeutic approaches. The precise mechanisms behind the identified causal links require further investigation. The study has amplified the understanding of the immune system's genetic connection to irritable bowel syndrome, and the implications of these findings could potentially offer clear directions for the prevention and management of IBS in future clinical settings.

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