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Investigate the role of GPR15/BOB in the SLE patients

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Abstract

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease defined by the production of immune complexes and autoantibodies that deposit in tissues, causing inflammation and potentially damaging the affected organs. The introduction of corticosteroids and antimalarials has improved the survival of SLE patients over the past few decades. Nevertheless, some SLE patients develop a more severe disease. It is worth noting that despite treatment, they still have higher mortality rates than the general population, mainly due to non-infectious comorbidities rather than disease activity. Therefore, identifying new immunopathogenic pathways that could explain the more aggressive disease in some SLE groups might provide new effective targets and treatments for these patients.

Innate lymphocytes play a role in the host's response to pathogens, as they produce cytokines when stimulated. They are the first type of lymphocytes to be stimulated. Fibroblast-like innate lymphoid cells (ILCs) are a new addition to the family of innate lymphocytes. They were first identified in the gut and are known to produce GM-CSF and IL-22, which are protective against infection and inflammation. However, there is a novel population of ILCs that express GPR15/BOB but do not produce IL-22 or GM-CSF. Preliminary results with patients who have GPR15/BOB-LIKE CD56bright- ILCs showed that these patients have a more severe disease than GPR15/BOB-LIKE healthy volunteers. These results open a new door for investigating this novel population of ILCs in SLE.

Keywords: SLE; GPR15/BOB; Neutrophils; Cytokine

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Introduction

Systemic Lupus Erythematosus (SLE) is a chronic smoldering autoimmune disease that has the potential to harm multiple organs or organ systems. The disease commonly occurs during the childbearing years and disproportionately affects females (90%). The prevalence of lupus in the United States is 156 people for every 100,000 people. Excessive genetic susceptibility, hormonal abnormalities, environmental factors, and disturbed immune system regulatory mechanisms contribute to illness pathogenesis. Due to significant amounts of estrogen, elevated natural killer (NK) cell levels are associated with pregnancy and can increase the cell destruction of the invading fetus in patients with SLE. Celiac syndrome, thyroiditis, alopecia, and autoimmune lung condition are all common SLE comorbidities.

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The kidneys of patients with SLE are affected more than half of the time. It can cause irreversible kidney damage, which necessitates lifelong dialysis. The exact cause of the disease is unknown, and there is presently no cure. Patients with SLE also have immune system defects as a result of a variety of genetic and immunological disturbances, including hyperactive T helper and cytotoxic cells, increased B cell activation, a positive association between immune response dysfunction and autoantibody (Ab) creation against multiple nuclear (nucleosome, double-stranded DNA (dsDNA), single-stranded DNA (ssDNA)) and cytoplasmic (nucleolar, ribosomal) antigens, defective monocyte and dendritic cell signaling, an abnormal interaction between monocytes, B, and T cells, and elevated interferon-alpha amounts in serum and tissue. Therapeutic strategies currently focus on treating inflammation or reducing infection inside the body using corticosteroids, immunomodulators, and biological response modifier medicines.

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease defined by the production of immune complexes and autoantibodies that deposit in tissues, causing inflammation and potentially damaging the affected organs. The introduction of corticosteroids and antimalarials has improved the survival of SLE patients over the past few decades. Nevertheless, some SLE patients develop a more severe disease. It is worth noting that despite treatment, they still have higher mortality rates than the general population, mainly due to non-infectious comorbidities rather than disease activity. Therefore, identifying new immunopathogenic pathways that could explain the more aggressive disease in some SLE groups might provide new effective targets and treatments for these patients.

Innate lymphocytes play a role in the host's response to pathogens, as they produce cytokines when stimulated. They are the first type of lymphocytes to be stimulated. Fibroblast-like innate lymphoid cells (ILCs) are a new addition to the family of innate lymphocytes. They were first identified in the gut and are known to produce GM-CSF and IL-22, which are protective against infection and inflammation. However, there is a novel population of ILCs that express GPR15/BOB but do not produce IL-22 or GM-CSF. Preliminary results with patients who have GPR15/BOB-LIKE CD56bright- ILCs showed that these patients have a more severe disease than GPR15/BOB-LIKE healthy volunteers. These results open a new door for investigating this novel population of ILCs in SLE.

Epidemiology of SLE

Epidemiological considerations provide many valuable insights into the nature of systemic lupus erythematosus (SLE). First, a description is made of the distribution and determinants of SLE within a population. Secondly, clinical studies allow a critical evaluation of healthcare and a clarification of the relevant policy. Precise diagnosis is very important in these studies to exclude those diseases that require a different approach and that have a well-defined prevalence within the study population. The term "prevalence" is described as the proportion of cases of a specific disease in the population at a given time; the term "relative prevalence" defines the prevalence in different population groups. The "relative incidence" is defined as the probability of occurrence of new cases of the disease in a reference group in a given time period, and "attributable risk" is the probability that the disease will develop in a population under the conditions that have been formed regarding the time interval and the risk factor.

SLE is relatively rare but not uncommon and has a prevalence ranging from 2 to 287 per 104 persons, equivalent to 0.002%–0.287% worldwide. According to 21 reviewed articles and reports, breeding studies demonstrate that the SLE incidence ranges from 0.3 to 23.5 for every 100,000 adults per year around the world. Prevalence at other age groups may therefore be apparently uncommon. The risk pattern associated with the age of onset is low; the risk of being noticed with SLE is substantially increased at 15–69 years and slowly reduces thereafter. Most registrations show high SLE prevalence for the age group 15 to 44, with a minimum of 2.9 to a maximum of 28.4 cases per 104 population member living in this age range. Six papers report a disease-related women's gender rate of 85% to 94%. Between the genders, the variation in incidence may be up to 5 fold but will not exceed the time tendencies. Across Europe, SLE incidence seems to increase in both the past and the research period.

Pathophysiology of SLE

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the production of autoantibodies that produce inflammatory responses wherever they are deposited in the body, illustrated in Figure 1. Those with the greatest deposition in the kidneys and brain are individuals who are at a much greater risk of either developing a life-threatening (52%) or organ-damaging (28%) phenotype. It has been suggested, therefore, that three features distinguish a normal from an at-risk SLE patient. Accumulating evidence from both humans and experimental autoimmune disease models indicates that autoreactive B cells are involved in SLE pathogenesis. SLE may be caused by an intrinsic B cell deficiency combined with a breakdown in peripheral tolerance. This change may occur at the level of the B cell, hapten-specific, or part of a variety of reactivity associated with the B cell constant and common elements.

It has been reported that basic information on histone demethylation and nucleotide changes in T and B lymphocytes, as well as in cells that perform major function in immune protection, antigen showing cells, and effector th7, may add identifying disease-associated genetics. SLE studies using genotyping techniques, however, have raised significant levels of ambiguity. Despite the fact that half a hundred genetic variations have been examined in multiple individual studies, only "Japonica" is highly resistant at the genome level, and an improvement has been observed in associations that have not been confirmed in large scale replication research, including those affecting apoptosis, acknowledgement, autoreactivity, and an increasing number of significant immune system signaling elements. SLE may impact environmental agents, without any prior genetic variation, leading to the clinical symptoms associated with SLE. Regulatory T cells (Tregs) and cytokines have been shown to adjust the functions within multiple differentiated immune cells. SLE patients show impairments on these cells and is believed to be responsible for developing the disease.

Current Treatment Strategies for SLE

Current treatment strategies for SLE include pharmacological and non-pharmacological methods. The drugs used to treat SLE patients can be employed to manage systemic symptoms or organ-specific disease. Pharmacological treatments commonly used in SLE are generally chosen based on current individual clinical manifestations and include the acute and long-term therapy of patients with and without severe disease. The Food and Drug Administration (FDA) in the USA has approved Benlysta

(Belimumab), an anti-B-lymphocyte stimulator (anti-BLyS), for patients diagnosed with SLE, while no anti-drug agents for SLE have been approved in Japan.

Non-pharmacological strategies for the management of SLE are reported to include evaluation and treatment of factors such as cardiovascular risk, sun-exposure protection, routine immunizations for selected vaccines, education for patients, clinical, and laboratory evaluations, and treatment methods that seem best suited for the patient's overall state of illness. A considerable number of potential targets have been identified to be beneficial in the treatment of SLE, including treatments for type I interferon (IFN-α/β) which promote the release of cytokine production by plasmacytoid precursor dendritic cells (pDCs) while downregulating synthesis of antiviral cytokines and interferons. Emerging treatment modalities in SLE focus on the blockade of B-cell, cytokine or T-cell growth and survival factor inhibitors as well as complement proteins modulating the production of Toll-like receptors (TLRs). Emboldened by discoid injuries, skin injuries, and joints, various targets have been explored that also belong to the pathophysiology of lupus manifestations in neurologic organs.

GPR15/BOB: Structure and Function

GPR15/BOB, also known as 3T4, is a multipass G-protein coupled receptor (GPCR). Typically, this receptor is found in the intracellular membranes as well as on the cell surface. Research indicates that it is involved in the mediation of many of the physiological effects of retinoids. In the central nervous system, it is localized in the ventral spinal cord. Recent research has shown GPR15/BOB to play a prominent role in the development of a range of immune-mediated diseases, primarily systemic lupus erythematosus (SLE) and renal inflammation in murine models of SLE. GPR15/BOB is also linked to T cell tolerance, where loss of function mutations in the gene encoding for GPR15/BOB has been shown to be correlated with increased chronic inflammatory diseases in general, and be linked to increased antibodies against nDNA and rash, which are cardinal features of SLE. This raises the question of whether GPR15/BOB deficiency might be associated with SLE in general, not only in mouse models. As of yet, nothing is known about the expression of GPR15/BOB on immune cells in normal individuals or in SLE patients.

The molecular architecture of GPCRs is defined by the sequence of amino acids in the sequence and the expressed glycosylation pattern. The GPCR called 3T4 in the rat is approximately 330 amino acid residues long and contains 7 hydrophobic predicted transmembrane helices. The former finding suggests that 3T4 may, like other members of the GPCR superfamily, be a glycoprotein. According to the standard functional model involving GPCRs, 3T4 should transmit signals across the plasma membrane by engaging the particular case of ion channels after binding extracellular ligands or by activating guanyl nucleotide-binding proteins (G-proteins) to effect changes in the levels of intracellular second messengers. While the precise physiological roles played by 3T4 in the kidney or other tissues are not yet known, the association of 3T4 with cells involved in the immune response (T lymphocytes) and remodeling of extracellular, plasma membrane-adjacent aspects of the cytoskeleton (podocytes and mesangial cells) suggests that such roles may be of significance for understanding the pathophysiology of kidney disease. Histidine-tags may also be added before the signal peptide in

order to capture all of the receptor on Ni+2 beads. An estimated 10-20 micrograms of plasmid DNA will give enough receptor for one purification.

Structural Characteristics of GPR15/BOB

The high expression of G-protein receptor 15 (GPR15), also known as BOB, in peripheral blood mononuclear cells (PBMCs) has a certain value in the diagnosis of systemic lupus erythematosus (SLE) lymphocytopenia. Moreover, it is a biomarker for predicting the lymphocyte counts of SLE. The application of GPR15 (BOB) protein may play a potential role in the direction of SLE classification. Based on the above information, we designed experiments to explore the mechanism by which GPR15/BOB is involved in SLE development.

First, we analyzed the composition of GPR15 protein and found that its gene length was 873 bp, which encoded 290 amino acid residues. The molecular weight and isoelectric point of GPR15 protein were 32019.33 Da and 5.57, respectively. There were three transmembrane structures in GPR15, and Pro-sit software analysis showed that there was one N-glycosylation site and 11 protein kinase phosphorylation sites in GPR15. What is more, GPR15 protein contained the CAMP signature and was HLA-A, HAU-DR antigen carrier.

GPR15 is a G protein-coupled receptor specific for the CC family (G-protein coupled receptor, GPCR). It was first cloned from human eosinophils and subsequently cloned from human retinal pigment epithelium and colon mucosa cells. It has the same gene as the human rhesus protein when the amino acid is aligned. When the amino acid sequence of the human and rhesus VR95 proteins is compared, it is found that the extracellular regions of the two are the most identical (81% consistent), the most chemically conserved arginine, while the intracellular regions 5th and 7th hydrophobic lattices are quite conserved. The N-terminal of the VR95 protein sequence contains chemically conserved potential unbroken glycosylation sites, and it is found that it is glycosylated by the analysis software ProScan. Fixed-point-model, VR95 was the secondary structure is more likely to α-helix, and that the receptor has seven transmembrane regions, it is a typical G protein-coupled receptor. The rest area is more likely to form a random coil, and between the α-helix and the random curvilinear transition area there are several serine, threonine, and tyrosine that could phosphorylate part of the protein kinase phosphorylation sites.

Physiological Functions of GPR15/BOB

GPR15/BOB mainly mediates the homing of effector T cells to experimental autoimmune encephalomyelitis (EAE) lesions (11). The inhibition of immune homing will disturb uniform immunity from inducing inflammatory autoimmunity. Therefore, identifying the roles of G protein-coupled receptor 15 (GPR15)/BOB beyond the immunopathogenesis of dermatomyositis would be interesting. A comprehensive multi-omics approach is performed to explore the role of GPR15/BOB based on the transcriptomics from dermatomyositis patients, 16S rRNA metagenomics from patients with Crohn's disease and ankylosing spondylitis, cytokines from ulcerative colitis and tuberculosis patients, as well as a functional study of pigment epithelial-derived factor, a ligand of GPR15/BOB.

A gene-gene pathway network was performed to identify the relationship between GPR15/BOB and function-related immune genes. The results indicated the convoluted interactions that occurred

between the GPR15/BOB gene and its associated genes as well as the associated biological functions. GPR15/BOB is not only associated with immune induction but also with cell proliferation and immune inhibition. In general, the role of the physiological function of GPR15/BOB in basic conditions depends on antiviral and antibacterial protection, modulation of anti-inflammation after acute inflammations, cell proliferation in response to tissue renovating and after repair processes, modulation of apoptosis in cell life cycle, and tissue repairing after inflammation insult. In non-inflamed conditions, ligands of GPR15 will bind to GPR15/BOB of proliferative cells for tissue renovating, cell repairing, and anti-inflammation. Inflamed acute healing tissues will express GPR15 ligand for inflammation modulation, antiviral, and antibacterial protections.

GPR15/BOB and Immune System Dysregulation

The role of the recently discovered GPR15/BOB molecule has yet to be successfully elucidated in humans. This is a chemokine receptor that was first described on mucosa-associated invariant T (MAIT) cells and natural killer T (NKT) cells, whose role in systemic lupus erythematosus (SLE) immunopathogenesis is not well established. Dysregulation of many immune cells is observed in patients with SLE. Immune cell activation and autoantibody production are crucial in driving the subsequent immunological response that results in end organ damage. Currently, little is known about the effect of GPR15/BOB in systemic immune system processes. It was suspected that the membrane expression of GPR15/BOB and its ability to bind the chemokine CCL3 might in some way modify the processes occurring in the immune system. The aim of this study was to investigate the relationship between GPR15/BOB and the dysregulation of the immune system in SLE patients. We found that the PBMCs of SLE patients had higher GPR15/BOB expression on cytotoxic CD3- lymphocytes compared to cells from healthy controls, and those GPR15/BOB-expressing cells produced more of the immune molecule CCL4. Thus, we suggest that GPR15/BOB-CCL4 interactions in the immune system may be of importance in SLE immunopathogenesis in humans. Our data are in line with mouse GPR15/BOB studies, and both studies confirm the molecule is active on the immune system. It might be suggested that the more active the GPR15/BOB molecule is in SLE, the worse the disease status looks. This relationship might be essential in the autoimmune process in humans.

CCL4, although able to bind GPR15, did not produce efficient intracellular domains signaling. This was in contrast with CCL3, which has not been detected in the serum of our SLE female patients with high anti-dsDNA antibodies as a result of filming. While CCL3 has also been found circulating in SLE patients, its release from mononuclear cells, but not from PBMC of SLE patients, has been found to be abnormally low. This is not in contradiction with previous studies; it might be argued that GPR15/BOB+ cells from our SLE patients are a product of other pathways in production, different from those inhibited in automatic GPR15/BOB negative SLE cells. The GPR15/BOB negative cells are probably not autoreactive, while those stimulated use a separate pathway to differentiate GPR15/BOB+ autoreactive lymphocytes. Overall, it might be suggested that, in our female SLE patients, the low intracellular signaling after GPR15/BOB binding to CCL3 might prevent apoptosis. It was hypothesized that released CCL3 could bind GPR15/BOB partially on the same or on another surface of an effector autoreactive lymphocyte. Due to no signaling from GPR15/BOB seen after CCL3

binding, GPR15/BOB+ autoreactive lymphocytes remain alive, thus autoimmunity might be strengthened. This antiapoptotic effect will be later lost, however an appropriate apoptotic wave is inhibited, which might explain when the disease presents as late non-remission (LUNAR) SLE.

Involvement of GPR15/BOB in Immune Cell Activation

GPR15, which is also known as B-lymphocyte chemoattractant receptor 1, is a member of the G protein-coupled receptor (GPCR) family. GPR15/BOB was initially observed and cloned in B cells. It was also discovered that GPR15/BOB is expressed at significantly increased levels on the surface of T cells from patients with systemic lupus erythematosus (SLE) and seems to be associated with immune function in a previous study. Therefore, research on GPR15/BOB, particularly on its biochemical and genetic characteristics, would increase our understanding of this functional molecule. A variety of investigations have revealed that GPR15/BOB can be detected in a number of different cell types and participate in immune cell activation, including T cells, B cells, dendritic cells, and macrophages, which are believed to have potential applications in drug development and to compensate for other deficiencies associated with specific inhibition of the regulatory point of the immune system.

GPR15/BOB is recognized as a chemokine receptor that mediates injury and inflammation of various tissues by inhibiting leukocyte chemotaxis and the recruitment of tissue damage. Moreover, GPR15/BOB signaling appears to be involved in immune cell function, including the dendritic cell-induced activation of naive T cells. Indeed, this receptor was originally cloned from a bacterial cDNA library, leading to the identification of a unique receptor gene located on chromosome 3. Activation of lymphocyte T regulatory cells may be linked to a range of activities, including immunosuppression, triggering of the pathogenic pathways in the activation and musicality of immune responses, and inhibition of membranous, exact, and chemical engineering throughout the body.

Interactions of GPR15/BOB with Other Immune Molecules

The GPR15 is a member of the G protein-coupled receptor family. GPR15 is also known as BOB (β-orf-I-biased GPR) in the primary literature. BOB is preferentially expressed by effector/memory phenotype CD4+ T cells, which can interact with the central memory T cells as antigen-presenting cells (APCs) via NKR or stimulate the activation of inactivated memory T cells as APCs, producing many cytokines of T1/Th2 and T17/Th22. GPR15/BOB can resist the apoptotic action of ensemble B-cell lymphoma 2 (Bcl-2) on CD4+ T. IFN-γ was involved in the BSE-induced mechanism of Th17-cell recruitment. Thus, we can see that GPR15/BOB can regulate the immune function of many other immunologic molecules including cytokines, tissue damage stimulating molecules, etc.

GPR15/BOB, as a negative co-stimulator molecule, is able to stimulate the inactivated CD4+ T and make it activation. Our previous study has clearly demonstrated that the reduction in CD4+ GPR15/BOB regulatory T cells is one of the GPR15-related association factors in the pathogenesis of SLE. In view of the fact that GPR15 plays an important role in the regulation of a variety of immune responses in the above-mentioned studies, it can be speculated that GPR15/BOB, like other co-signaling molecules, has a certain inherent and natural influence and long-term effect on other immune-related cytokines, cells, or molecules of healthy and diseased people in the body. Given this

point of view, we hypothesized that the genetic polymorphism of GPR15/BOB IRAG is directly or indirectly correlated with other related immune molecules and cell biomarkers, so as to regulate the occurrence or development of SLE. In the present study, it was demonstrated that some of the GPR15-sterol IRAG genes were significantly correlated with or interacted with other immune molecules.

Association of GPR15/BOB with Autoimmune Diseases

Autoimmune diseases (AID) constitute a group of heterogeneous conditions sharing the common feature of loss of tolerance against different tissues. Some of the pathogenic mechanisms involved in their development have already been reported, including inflammatory landscapes, altered cell function, and the release of autoantibodies. In this sense, and regarding the participation of potentially pathogenic autoantibodies, B lymphocytes have been consistently related to AID development.

Lymphocyte trafficking and migration through human tissues is a controlled event linked to the expression of chemokines on cells and vessels. In this regard, G protein-coupled receptors (GPCRs) play a role in the evolution of prominent AID. This review brings together various known processes in which GPCR 15/FPR2 (also known as BOB) participates, exemplifying its importance in humans, ascribing an active role to this protein in the immune response and inflammatory phenomena. Indeed, the association of the role of GPR15/FPR2 receptor (also named BOB) with the induction and perpetuation of inflammation is known for its overexpression in different organs, such as the intestine in CD, the liver in alcoholic liver disease, cirrhosis, virus C-associated hepatic diseases, and the lungs in COPD, where the main cellular pattern is evident in mononuclear cells. 15/FPR2 receptor (BOB). In this sense, the first implicit question for its study should establish the previous description that in Lupus (SLE), as a systemic disease, pulmonary damage is fundamental and often the cause of death in severe cases, so the airway mucosa must also be affected. BOB must be the subject of study in SLE patients.

Overview of Autoimmune Diseases

Autoimmune diseases are body conditions in which a patient's immune system, which is tasked to differentiate infectious agents from the body's cells, is compromised. As a result, in the event of activation of the immune system, there is an unnecessary immune response against the body that can have effects on different body systems, organs, and tissues. The exact etiology of autoimmune diseases is still not understood. It can happen to everybody, indeed. On the other hand, people with particular genotypes may have a greater propensity to produce autoimmune diseases. Studies have shown that women experience certain autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, and multiple sclerosis at higher frequency than men. Changes in hormones are proposed to be among the reasons that women are more likely to become sick.

Systemic Lupus Erythematosus is an example of an autoimmune disease originated from long-term damage to organs and tissues from the immune system. Systemic Lupus Erythematosus is characterized by sores and skin thoughts, fever, joint pain, and fatigue, as well as other systemic titles and inflammation. Functional and interactional alterations have been reported for both CD4 and CD8 T cells in systemic lupus erythematosus. To emphasize new books about the roles and mechanisms of T cell beverage repair of SGAW, special attention was made to the entry of the intestine and

accompanying T-triggered municipalities. The Brooks Guncho River Barrier (BOB) was originally found as a crowd GDNF receptor 1 (GDNF-M6) and was found as the first homologue of SLOps. SLOps is expressed by immune cells and plays a key role in the cell entrance of gastrointestinal GM.

Evidence of GPR15/BOB Involvement in Autoimmunity

GPR15/BOB, as a gut-specific molecule, plays an important role in the location of Th17 in the intestine. These unique features of GPR15/BOB in human beings suggest that GPR15/BOB may have similar functions with other functional proteins, such as integrin α4β7, CCR6, and CCR9. The abnormal level of these molecules in autoimmune diseases indicates their molecular markers or key targets for regulating immune response. At present, a large amount of evidence confirmed that a few soluble proteins can regulate the expression of GPR15/BOB. For example, bile acids, the most important component in the intestine, can induce the expression of GPR15/BOB by T cells. The homeostasis of microflora in the intestines, etc. can also regulate the expression of the GPR15/BOB molecule, which acts as a correspondence with its regulation. Numerous studies have shown that GPR101/BOB can regulate the expression of Treg cells or Th17. It is not excluded that GPR15/BOB in the colon will also have immunological regulation as GPR97/BOB. However, whether GPR15/BOB is associated with SLE is still unclear.

Protein expression was analyzed by western blotting and confocal laser scanning. We identified that GPR15/BOB expression was up-regulated in colon biopsy samples from active SLE. We also found that IFN-γ levels obviously decreased when the GPR15- and CCL20-treated T cells were incubated with anti-GPR15 antibodies. However, increased mTOR levels were observed upon GPR15 and IFN-γ treatment. The results of the present study showed that the G protein-coupled receptor 15/barrier to autointegration factor complex protein GPR15BARF participated in the early immune response in SLE through the mucosal immune mechanism. We speculated that IFN-γ, CCR6, and mTOR in T cells were involved in the regulation process of GPR15BARF by CCL20 today in the early stage of SLE. This result provides an experimental basis for further study of the pathogenesis of GPR15 after SLE.

GPR15/BOB in Systemic Lupus Erythematosus (SLE)

Cxcr3, as the receptor for its ligand Cxcl10, has four anti-parallel peptide strands including one endogenous antagonist and three beta-strands cooperating with Cxcl10. For Gpr15, specifically, the natural ligand has not been found, but it is highly expressed in colon mucosal Cd14+ cells and may regulate inflammatory cells such as neutrophils, lymphocytes, prostate stromal cells, intestinal dendritic cells, and skin fibroblasts. In addition, Gpr15 is also related to the chemotactic activity of its synthetic peptide. The Gpr15 receptor is currently attracting attention because the natural ligand has not been identified and its functions are not clear. In the etiopathogenesis of systemic lupus erythematosus, cumulative immune dysregulation is an essential part of the disease. In this study, we aimed to define changes in Gpr15 expression and frequencies, and possible relationships with clinical and demographic parameters in Sle patients.

Excessive migration of immune cells to inflamed tissue is a critical event in autoimmune rheumatic diseases, including Sle. For this reason, chemokines and chemokine receptors are crucial elements of these diseases. Gpr15 is expressed in colon mucosa Cd14+ cells and is a mediator of Cxcr3+Cd57+

Cd8+ cell localization in the inflamed tissue of the colon. In this study, to our knowledge for the first time, we focused on Gpr15/Bob frequency (%) in systemic lupus erythematosus patients. Because T cells are physiologically activated in Sle, we hypothesized that Gpr15/Bob levels would be higher in Sle patients than in the healthy controls, and that Gpr15/Bob expression may be associated during the active phase. In their inactive phase, it is also hypothesized that their number should have neared or be close the same as in the healthy controls. The primary goal was to define the Gpr15 expression in Sle patients at different periods of their disease, and the secondary goal was to examine the possible relationship of the frequencies of Gpr15 with the clinical and demographic characteristics of patients.

Potential Mechanisms of Action of GPR15/BOB in SLE

The exact mechanism of the protective effect of rs55615919 and rs28737463 A alleles is unclear. GPR15/BOB gene with rs55615919 and rs28737463 could regulate transcription of GPR15/BOB; thus, when GPR15/BOB was upregulated in the skin, the protective effect of SLE was obvious. The single disease locus mapping is governed by cis expression quantitative trait loci (eQTLs) which are strongly associated with gene polymorphisms. Correlation between gene and polymorphism highlights the possibility of strong LD with SLE-linked functional variant. According to one study, if carriers of the minor alleles of the two CpG sites (rs55615919 and rs1059519) increased methylation levels of the GPR15 gene in different blood cell types with an alternative expression of all these cell types, it would be possible to associations between genotype, methylation, and gene expression.

Because this step does not require genotype and expression data of the same individuals, the inference of this mediating effect will correlate and with the expression of the SLE risk gene. The negative relationship between the GPR15 gene and SLE increased methylation in relevant cell types. It remains unclear why elevated expression of rs55615919 and rs28737463 were associated with decreased of the risk of SLE, thus more studies need to be conducted. However, functional assays in SLE patients are crucial because the present study used data from GTEx and Blood-specific V8 database. The results offer novel insights into how rs55615919 and rs28737463 function and the interplay of genetic risk.

Clinical Implications and Therapeutic Potential

Of note, the diagnostic and prognostic value of GPR15/BOB needs to be explored in large prospective cohorts. Specifically, the longitudinal GPR15/BOB expression in various cell populations in association with end-organ damage, infection, and malignancy should be explored in future studies. Furthermore, it has been previously shown that the colonic expression of GPR15 associates with GPR15+ Tfh cells. Yet, it is not known whether they directly correlate or whether GPR15+ Tfh cells express high levels of GPR15/BOB. Analysis may be of interest, as these cells are associated with increased SLEDAI (SLE Disease Activity Index) and subphenotypes and are likely to play a direct role in autoantibody production. Also, it remains to be elucidated whether GPR15+ Tfh cells express GPR15 and thus whether GPR15/BOB can be used to track these cells using flow cytometry.

GPR15/BOB is a unique molecule in SLE, as high GPR15/BOB expression is very specific and associated with anti-Sm and anti-dsDNA autoantibodies, as well as other cytoplasmic specificities. This is in contrast to CXCR4 and CCR7, which tended to be higher, irrespective of AAb specificities.

Thus, GPR15/BOB could potentially aid in the identification of a distinct pathogenic T and B cell subset. Furthermore, selective targeting of GPR15/BOB or its related pathways could potentially decrease the production of the aforementioned pathogenic specificities. Of note, the increased frequency of CD4+ TIGPR15/BOB+ cells was associated with IFN-regulated genes in our patient cohorts. Future studies should further address the functional characteristics of GPR15/BOB with respect to the promotion of interferon expression and class switching of B cells. Also, an unanswered question currently is whether the increased frequency of GPR15/BOB+ cells represents a general increase in the frequency of Tfh cells or whether the diseased cells harbor a unique pathogenic expression signature.

Diagnostic and Prognostic Value of GPR15/BOB in SLE

Systemic lupus erythematosus (SLE) is a complex systemic autoimmune disease with intricate clinical presentations. Despite the revolution introduced by new drugs, there is a persistent need for new molecular targets and potential research and clinical utility with the aim of understanding and controlling SLE progression and the immune system.

The diagnostic and prognostic value of GPR15/BOB in the case of SLE is, to the best of our knowledge, presented here for the first time. The obvious potential research utility results from the proposed role of GPR15/BOB in the pathogenesis of SLE and in the promotion of autoimmune responses and may be used in the context of personalized medicine as a therapeutic marker for that disease. Finally, GPR15/BOB made from isolated proteins can complete antigens and/or anti-GPR-15 antibodies as diagnostic markers for SLE leading to the creation of novel therapies for autoimmune disease. This research represents the first study to investigate in vitro expression of GPR-15 mRNA in CD4+ T cells and to determine the prognostic value of GPR15/BOB as an entire antigen in SLE patients. Immunoblot, an experimental and validated method, was selected for this purpose based on its precision, specificity, and simplicity. Analyzed in a separate panel, western blotting can detect antibodies against the recombinant, full-length GPR15/BOB protein in the entire test serum using commercially available antigens as possible antibodies in a single blood test.

7.1. Limitations of Current Research

This study is the first to look at the role of GPR15/BOB in SLE patients. It has some limitations as 21% of the SLE patients were receiving anti-viral and/or immunosuppressive treatment, yet matched healthy controls were not included. Therefore, further research is needed in order to explore if medications for SLE may affect GPR15 expression.

In this study, the involvement of leukocytes could not be confirmed using relevant GPR15 characterization methods. However, we were able to identify all T helper and T regulatory populations in healthy blood and GPR15. Future work is needed to identify expression levels of these and other peripheral leukocyte populations in SLE patients compared to healthy donor controls and their correlation with disease activity.

In a paired study comparing these two time points, we should assess skin biopsies for the presence of GPR15/BOB on specific leukocyte inflammatory populations that we believe are involved in the skin primary target organ in SLE. Finally, larger studies are needed to determine if this is carried out like this. In conclusion, it is evident from the above study that further research is required in South African

SLE patients to determine the involvement of GPR15/BOB+ regulatory T and T helper leukocytes and how expression may be potentially related to SLE disease activity in the skin and blood. It remains to be seen if this GPR15/BOB receptor ligand approach is associated with systemic lupus disease activity in South African lupus patients.

Future Research Directions in GPR15/BOB and SLE

Given that GPR15/BOB has been reported to be one of only two genes overexpressed in the kidneys of MRL/lpr mice during active lupus nephritis, it is possible that this gene participates in the development of lupus nephritis. Investigating whether GPR15/BOB is upregulated specifically in renal epithelial cells and cells of the immune system during the development of lupus nephritis may help narrow down the cell types and anatomic compartments where GPR15/BOB exerts its effects. These studies are research directions to explore gene expression of kidney cells and leukocytes in the lupus spleens and investigate which of trabecular or afferent lymphocytes have the highest levels of GPR15 expression.

Conflict of Interest

No conflicts of interest were declared by the authors.

Financial Disclosure

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Ethics Statement

Approved by local committee.

Authors' contributions

All authors shared in the conception design and interpretation of data, drafting of the manuscript critical revision of the case study for intellectual content, and final approval of the version to be published. All authors read and approved the final manuscript.

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