How Insomnia Stimulates Inflammation in Rheumatoid Arthritis, and how the treatment by neutralization can affect the bones by the utilization of ACPA as a biomarker: A proposal

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September 20, 2023

Contents

1 Abstract

The research paper explores the impact of Rheumatoid arthritis (RA) and its effects on the joints and bones of humans. RA has shown potential for an issue that people suffer from all of their lives. There are many major reasons for injury with RA such as insomnia and stress, which are discussed in this paper. In addition, the treatment by neutralization has a gigantic potential for developing a way to decrease the effects of RA. Using ACPA as a biomarker can detect abnormal B cells causing damage in rheumatic arthritis. Neutralizing them with an artificial antibody will be made by Dar 12 by mutating its genetic code by genetic engineering to turn its function into an antibody that matches Siglec 15's epitope to stop it from working. It has the potential to increase treatment. This antibody will close siglec 15's trigger sites, deactivating hyperactive B cells. This paper proposes valuable insights into this field of immunology, discussing the implications and how we can utilize neutralization as a powerful way to face the consequences of RA.

2 Introduction

2.1 What is Rheumatoid Arthritis?

Rheumatoid arthritis (RA) is an autoimmune disease that mistakenly attacks the cells in the body confusing between its tissue and the foreign cells. It causes many problems like inflammation and damage to the joints and bones,so the person will be in pain due to bone damage; also, it has many symptoms, for instance, swelling in joints with pain and fatigue; moreover, it is characterized by symmetric, polyarticular disease involving the

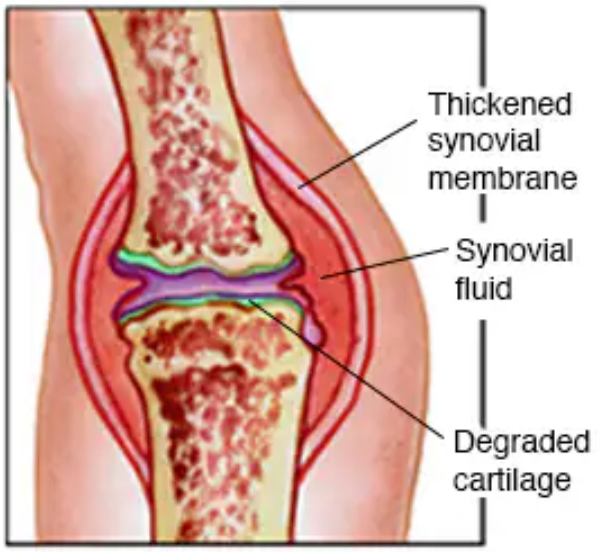

Figure 1: The implications of Rheumatic arthritis

small joints of the hands, feet, and joints; furthermore, it also attacks the whole body by affecting organs like the heart, lungs, and other organs [1]. Figure 1 demonstrates the implications of Rheumatic arthritis.

As a genetic and environmental disease, it is a systemic inflammatory disorder that is chronic and progressive. Several inflammatory pathways can lead to joint inflammation and destruction, resulting in the formation of pannus, which is an abnormal tissue growth in the joints. This tissue can spread to your bones and cartilage, the smooth, shiny, and white connective tissue covering the ends of the bone. The degeneration of this cartilage may produce arthritis and result in chronic inflammation of the joints. [2]

The synovial tissue, which is a connective tissue that locates the inner surface of the capsules of synovial joints, secretes synovial fluid that serves as a lubricating function, allowing joint surfaces to smoothly move across each other; consequently, rheumatoid arthritis aims to such a complex crosstalk between T cells,

B cells, fibroblasts. Locally produced cytokines and chemokines regulate it. Interleukin beta (IL-6), tumor necrosis factor (TNF), and other hormones like inflammatory factors stimulate systemic disease implications outside the joints, resulting in pain and alterations of sleep such as insomnia [3].

Additionally, the inflammatory synovial tissue (pannus) is directly responsible for the development of marginal bone erosions that are evident in radiographic evaluation; however, the pannus can access the bone marrow, causing inflammatory changes that enhance osteoclastic resorption at the subchondral bone surface. Articular cartilage can be subjected to inflammatory attack from below through subchondral bone and from above by the pannus moving across the surface. [4].

In addition, Chronic inflammation in RA leads to focal articular bone erosions within inflamed joints, as well as generalized osteoporosis in the axial and appendicular skeleton. This bone loss progresses throughout the disease, correlates with disease severity, and if left untreated, can lead to joint deformity and fractures. Indeed, recent studies have shown that proinflammatory cytokines not only induce bone resorption but also contribute to bone loss by direct inhibition of osteoblast differentiation [5]. In the next section, we will discuss the relationship between Insomnia and Rheumatoid Arthritis and how socioeconomics contributes to increasing Insomnia

3 Literature Review

3.1 Insomnia and Rheumatoid Arthritis

Insomnia and pain are noticeable behavioral symptoms in individuals with RA. Inflammation results from the dysregulation of

sleep and wake activity causing pain. Insomnia has a role in the activation of inflammation. The relation between Insomnia and rheumatic arthritis is produced in part by social adversities such as life stress or personal difficulties that contribute to a dysregulation of sleep and wake activity [6]; moreover, the relationship between socioeconomic status and rheumatoid arthritis slightly increase among the people with a low socioeconomic status(SES), which is more prevalent and has many disadvantages such as the risk of death is higher, because they are considered less healthy due to the ability of finance and healthcare services.

Furthermore, it increases the level of stress, so it has a role in increasing the percentage of insomnia. In experiment illustrates the difference between high and low socioeconomics. They found that in the group with a disease duration of up to 5 years. Patients with low SES produced more obvious health impact than patients with high SES and also suffered more often from high disease activity than high SES; however, within the group with a 5 to 15 disease duration and with a disease duration of 15 years or more, there is no statistically obvious impact of SES on health outcomes was found. The causes for that were demonstrated for a reason, they observed that patients with a low SES in 0 to 5 disease duration received less health care than patients with high SES with the same disease duration. [7]

The study [8] showed that socioeconomic deprivation was associated with reduced response to TNFi and increased risk of treatment discontinuation. Median time to discontinuation differed by 1 year between the 20% most deprived and the 40% least deprived groups. The most deprived subgroup had lower odds of achieving remission, LDA, or EULAR response compared with the least de-

prived. Reduced response to treatment, as measured using DAS28 and its components, in more deprived groups appears to be driving the greater risk of treatment discontinuation, whereas discontinuation due to adverse events was similar.

Stress contributes badly to responding to medicine and Insomnia, which leads to excessive increases in inflammation, as coordinated by the central nervous system, and multiple neural and endocrine effector mechanisms. Inflammation can worsen sleep disturbances, which accelerate RA symptoms. Sleep disruption leads to increased pain sensitivity and RA disease activity. We can consider that Insomnia and Inflammation dysregulate each other which is mediated by the activation of inflammatory cytokines that attack joints of the body. Next section, we will elaborate on how can we face these implications of RA [9].

3.2 Pharmacological treatment by the neutralization

The research paper suggests that pharmacological treatment can be achieved by the neutralization of inflammatory cytokines, which means surrounding them; in other words, the ability of antibodies to block the sites on the pathogen that they use to enter their target cell. (A target cell is a cell that responds to a hormone because it bears receptors for the hormone, and the cell is influenced due to this bearing.

The treatment by neutralization has the advantage that we can use it for defense and deactivate the inflammatory cytokinesis (a signaling molecule) that is secreted from immune cells and certain other cell types that promote inflammation. Therefore, the deactivation by neutralization for these signaling molecules will decrease the inflammation that happens in joints. Blocking the sites of inflammation cytokines that are used for entering the im-

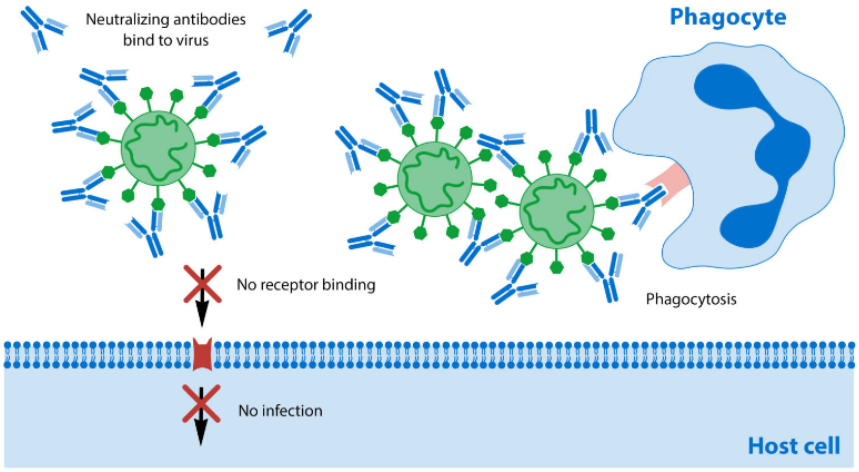

Figure 2: The neutralization's mechanism

mune cell will end the inflammation

It exhibits a better fit than the receptor itself, resulting in the virus surface becoming covered by antibodies. This in turn prevents the virus from entering the immune cell and the beginning of inflammation. [10]. Figure 2 illustrates how the neutralization occurs. Major cells participate in defending against inflammation such as the B cells and T cells. B cells have a variety of immune functions including antigen presentation, antibody production, and direct antigen pickup through their B cell receptor. However, we will focus on B cells.

Adaptive immunity relies on T and B cells, which are white blood cells called lymphocytes. lymphocytes originate from stem cells in the bone marrow. Some migrate from the bone marrow to the thymus, an organ in the thoracic cavity above the heart. These lymphocytes mature into T cells. Lymphocytes that remain and mature in the bone marrow develop as B cells. B cell function is essential for bone remodeling and osteoclast function. Studies are showing that B cell depletion therapy seems to be very effective in inhibiting bone erosion and synovitis in RA. Therefore,

B-cell activation must be tightly regulated to prevent autoimmune diseases, and any deficiency or inhibition of the B-cell antigen receptor (BCR) signaling pathway can lead to B-cell hyperreactivity and subsequent autoimmune progression. These studies will be elaborated in the following paragraphs.

Activated B cells secrete RANKL, thereby increasing osteoclast activity [11]. In inflammatory bone diseases such as RA, B cells have a major impact on focal bone erosion [12].

The most specific autoimmunity known for rheumatoid arthritis (RA) is reflected by a generation of anti-citrullinated protein antibodies (ACPA), which are a collection of autoantibodies with different isotypes usage (i.e.: IgG, IgA, IgM) that recognize the nonessential amino acid and works as a hallmark of RA, rheumatoid factor autoantibodies, and anti-citrullinated protein memory B cells, all implicated in RA pathogenesis [13].

High levels of ACPA in the serum of patients with seropositive RA have positively correlated with the frequency of ACPA-producing class-switched memory B cells [14]. ACPA-expressing B cells from seropositive patients exhibit a memory cell phenotype and express IgG or IgA [15]. A variety of molecules can negatively regulate B cell responses to rheumatoids, including FcRIIb and CD22. FcRIIb is an inhibitory Fc receptor that binds to IgG ICs and regulates B cell responses.[16]

CD22 (Siglec-2) and Siglec-G are the only inhibitory Siglec-G expressed on the surface of B cells that suppress BCR signaling and play an important role in preventing excessive immune responses and inducing tolerance to autoantigens. CD22 belongs to the lectin family and down-regulates B cell receptor (BCR)/Tolllike receptor (TLR) signaling in B cells [17].

Studies have shown that neither CD22-deficient nor Siglec-G-

deficient mice on a pure C57BL/6 or BALB/c background develop autoimmunity; In contrast, senescent Siglec-G × CD22 double-deficient mice develop a progressive immune response with spontaneous production of large amounts of IgG autoantibodies (specifically including ssDNA, dsDNA, and RNA). as well as autoimmune antibodies against proteins IgG-Fc/rheumatoid factor [18]. It is important to consider that Siglec-G is a CD33 group of siglec-expressed in B cells and plays an important role in B cell tolerance.[19]

Therefore, Considering the importance of FcRIIb, FcμR, and CD22 in B cell responses and their association with autoimmunity, alterations in these molecules in patients with seropositive RA may contribute to the pathogenesis of this disease.[20] In the next section, we will discuss the Methods of collecting data and the hypothetical experiment for treatment of Rheumatoid arthritis. Also, we will demonstrate the purpose of the study, and how we will use neutralization against B cells that secreted RANKL that promote RA and use ACPA as biomarkers to specify B cells in patients with RA

4 Methodology

4.1 Sample collection and analysis

Discovery cohort:

The same samples that were used in the experiment [21] will be used in our proposed experiment. We used data from six case-control collections (UK, US, Dutch, Spanish, Swedish Umea, and Swedish Epidemiological Investigation of rheumatoid arthritis. All individuals provided informed consent and were recruited through protocols approved by institutional review boards. Each collec-

tion consisted of individuals who were self-described as white and of European descent, and all cases either met the 1987 American College of Rheumatology diagnostic criteria or were diagnosed by board-certified rheumatologistsfrom these samples, we defined a total of 2,406 ACPA-RA cases and 13,930 control subjects for discovery from five collections (excluding the Swedish EIRA). The ACPA will work as a biomarker for the abnormal B cells that stimulate Rheumatic arthritis by producing a high amount of RANKl protein blocking Siglec-15.

Validation cohort:

We followed standard clinical practice to identify ACPARA subjects as those who were not reactive to anti-CCP antibodies by using reference cutoff levels defined at local clinical labs. In the UK cohort, we used the commercially available DiastatTM ACPA Kit (Axis-Shield Diagnostics Limited). In the US samples, we used a second-generation commercial anti-CCP enzyme immunoassay (Inova Diagnostics).16 We used the Immunoscan-RA Mark2 ELISA test (Euro Diagnostica) for Spanish samples. For the Swedish Umea and Dutch collections, we used the Immunoscan-RA Mark2 ELISA test (Euro Diagnostica).17 These assays are the standard commercially available assays that are currently being widely used in clinical practice. In this cohort we followed these criteria we considered samples ACPAonly if they were negative for all four of these tests. After applying this assay, we removed 106 case individuals who were reactive to the sensitive assay and 381 case individuals to whom we did not apply the assay. We also excluded 73 cases and 249 control subjects who were positive for HLA-B*27. Because HLA-B*27 is highly sensitive for AS (¿90effectively removed the effect of possible confounding

from AS or related spondyloarthropathies. The resulting replication collection consisted of 427 cases and 1,691 control subjects.

4.2 Experiment's procedures:

In this experiment, we will use anti-citrullinated protein antibodies as a biomarker for guiding the abnormal B cells that produce RANKL protein. Siglec-15 is a highly conserved member of the Siglec family and is expressed primarily on macrophages and dendritic cells, including OCs, which are macrophages specific to the skeletal system [22].

Structurally, Siglec-15 comprises two Ig-like structural domains, a transmembrane structural domain containing lysine residues, and a short cytoplasmic tail. Siglec-15 is involved in OC differentiation and bone remodeling as an activating signaling molecule in the immune system and is highly upregulated during OC differentiation and maturation.

OC differentiation is a highly regulated process that requires the synergistic stimulation of multiple signaling pathways, such as macrophage colony-stimulating factor (M-CSF), RANKL, and Ig-like receptor-induced costimulatory signals for initiation and regulation.[23]

RANKL (NFkB ligand or receptor activator of TNFSF25) is a member of the tumor necrosis factor (TNF) superfamily and is an essential signaling molecule for OC differentiation and a key molecule linking the skeletal and immune systems [24]

Binding between RANKL and its receptor RANK induces OC formation and activation and recruits TRAF family proteins such as TRAF6, which in turn activates six key signaling pathways in OCs: nuclear factor of activated T cells (NFAT) c1, nuclear factor B (NF-B), Akt/protein kinase B, Jun N-terminal kinase, extracel-

lular signal-regulated kinase (ERK), and p38, all of which contribute to OC differentiation and survival. However, the binding between Siglec-15 and DAP-12, tyrosine residues in ITAM are phosphorylated by Src family kinases, which in turn recruit Syk [25].

Activated Syk and SRC promote activation of the phospholipase Cg (PLCg)-calcium pathway, which ultimately leads to activation of NFATc1 [25] [26]. A master transcriptional regulator of OC differentiation and transducer of the terminal OC differentiation transcriptional program [26].

RANKL-induced TRAF6 recruitment can also activate intracellular calcium-related signaling pathways, suggesting that RANKL and ITAM synergistically activate the PLCg-calcium-NFATc1 signaling pathway [27]. The RANKL-RANK pathway only partially activates NFATc1. The full activation of NFATc1 is dependent on the ITAM signaling pathway.

This emphasises on the ability of Siglec-15 to promote RANKL-mediated OC differentiation and maturation through the ITAM structure of DAP12. All this process is illustrated in Figure 3. As mentioned above Siglec-15 is involved in RANKL-mediated OC differentiation. The neutralization of siglec-15, which blocks their sites, is potentially a suitable target for treating bone loss in inflammatory arthritis and can reduce bone loss. Artificial antibody for siglec 15 will be used in the abnormal B cell to inhibit the excess production of RANKL protein. the utilization of this artificial antibody, which will have the same epitope as siglec-15, for blocking it in the abnormal B cells.

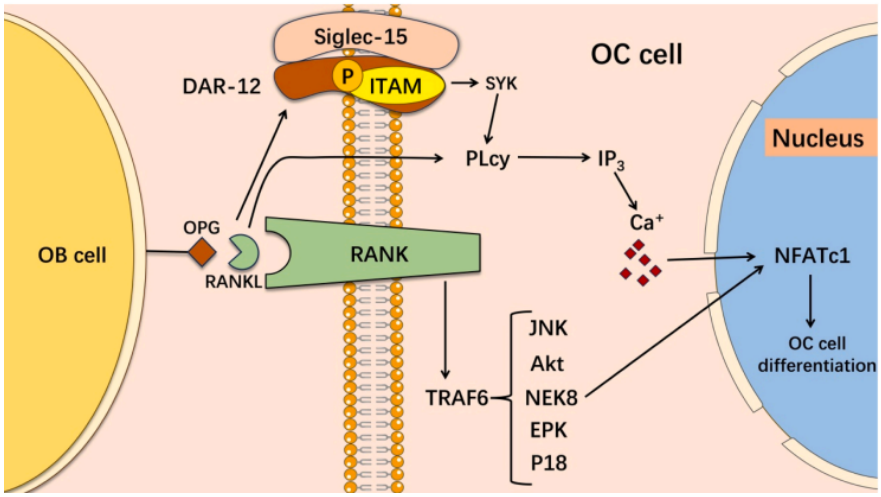

Figure 3: Relevant signaling network is for osteoclast differentiation centered on NFATc1. RANKL stimulates signaling pathways activated through TRAF6

5 Results

5.1 Objectives

The efficient usage of anti-citrullinated protein (ACPA) as a biomarker for detecting the abnormal B cells that cause damage; moreover, the utilization of these biomarkers might help us for guiding to the genetic coding in the human genome, which is expressed proteins that participate in the rheumatic arthritis.

Therefore, this idea can facilitate the way for using the neutralization mechanism to deactivate these abnormal B cells by finding a way to create an artificial antibody that matches the same epitope to siglec 15. This has a gigantic potential for increasing the percentage of treatment for Rheumatic arthritis. The usage of the antibody will close the sites that trigger siglec 15 and will stop the hyperactivation of these B cells, in which this mechanism makes such a quarantine for these abnormal cells. Finally, all of this will assist us in a better way for helping us in understanding and helping the patients who suffer from the effects of the disease.

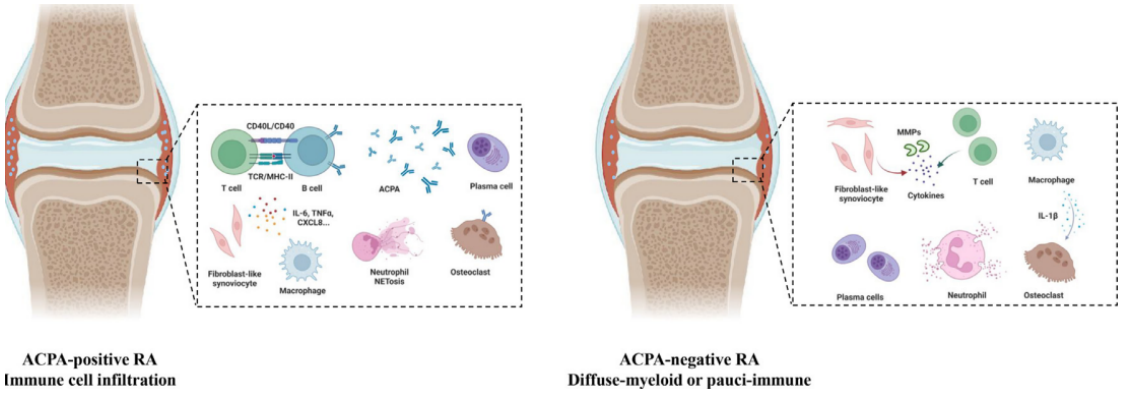

Figure 4: illustrate the difference between ACPA+RA and ACPA-RA

5.2 Expected results

The usage of ACPA as a biomarker will guide the abnormal B cells that stimulate rheumatic arthritis. The development of RA starts from a preclinical phase, followed by a transition phase, and then to clinical RA.1 The transition is considered a result of a complex interplay between predisposing genes and environmental triggers. Interestingly, ACPA+ RA and ACPA- RA differ in genetic and environmental risk factors. Different responses to targeted therapies between the ACPA+ RA patients and ACPA-RA patients implied differential underlying pathogenesis [27].

Therefore, determining the kind of ACPA is important in the treatment. Figure 4 demonstrates the Patterns of immune cell involvement in the synovial tissue of ACPA-positive and ACPA-negative RA. Both innate and adaptive immune cells are involved in the pathogenesis of RA in synovial tissues, but different patterns may exist in ACPA-positive RA and ACPA-negative RA.

Compared with ACPA-positive RA with rich immune cell infiltration and ACPA-mediated cytotoxic effects, ACPA-negative RA is characterized by less lymphocyte infiltration but increased proinflammatory cytokine profile in CD4+ T cells. By producing robust MMPs and pro-inflammatory cytokines and chemokines

(IL-6, CXCL12, CCL2), synovial stromal cells may play a more important role in ACPA-negative RA, and the outcomes of using neutralization, if we can mutate the Dap 12 protein from promote sigce 15, so it will stop the hyperactivity. It is anticipated that the procedures have the potential to be successful in treatment the of Rheumatic arthritis patients.

5.3 Potential concerns

The utilization of ACPA as a biomarker for Rheumatic arthritis has some problems. There are genetic and environmental differences between ACPA+ and ACPA-RA, resulting in varied responses to targeted therapies and implying different underlying pathogenesis. It should be pointed out that multiple key challenges remain to be addressed.

There are two main issues to consider regarding immune triggers in ACPARA. First, the immune triggers in ACPA- RA are not well understood, compared to ACPA+ RA. Second, most studies on this subject have been conducted on the general RA population, and it is unclear if these mechanisms apply to ACPA-RA patients. While some studies have claimed to include both ACPA+ and ACPA- RA patients, it is not clear whether the positive findings were from ACPA+ or ACPARA patients. Therefore, further research is needed to make substantial comparisons between ACPA+ and ACPA- RA patients.

Although some genetic risk variants for ACPA- RA have been identified by genome-wide association studies (GWAS) on genetic risk variants in ACPA- RA, the precise mechanisms associated with these causal variants remain unclear. Some mechanistic explanations for ACPA- RA have been developed based on animal models, without epidemiological evidence. It is important

to verify these findings in real-world studies with ACPA- RA patients. A better understanding of immune mechanisms in ACPA-RA is essential for designing more precise targeting therapies against putative pathogenic subsets, and for developing personalized treatment strategies. Additionally, identifying new biomarkers for early recognition of ACPA-RA will significantly improve outcomes by promoting early treatment. [27]

Additionally, it is important to consider the role of genetic engineering. From genetic engineering, we could turn the Dap 12 to work as an antibody, which can stop the promotion of the siglce 15, if the genetic engineering success in finding a way to mutate Dap 12 to work as an antibody or discover a protein from the human genome, it will stop the abnormal antibody. Therefore, additional studies should be done in order to determine the difference between ACPA+ RA and ACPA-RA, and some working in the genetic engineering field to mutate the Dap 12 protein to the antibody that fits with the epitope of siglce 15.

6 Conclusion

As an autoimmune disease category, rheumatic arthritis is a disease that millions of people suffer from, making life difficult and painful for them [28]. The usage of APCA as a biomarker for detecting the abnormal B cells that cause the disease needs more investigation to know the difference between the effects of positive and negative ACPA RA, it is important to know because it will reveal information about the Rheumatic arthritis and how we can overcome it. additionally, more research and experiments in genetic engineering have the potential to find a way for the antibody that closes the epitope of sigce-15. The proposal goes deep into

finding the way to stop this disease, which is neutralization, and it proves its efficiency in beating the antibodies that are not part of the human body; in addition, it provides a better understanding of the importance of the neutralization mechanism as a better solution for rheumatic arthritis and has the potential for developing effective therapeutic strategy and gigantic diagnosis.

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