International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2024.1271.1280

Research Article
Impact of *Saccharomyces cerevisiae* Probiotic on Influenza Vaccine Efficacy in Diabetic Rats: An Immunological Perspective

[1,2]Sahar El Hadad, [1,3]Alia Aldahlawi, [1]Abeer Al Hashmi, [1]Shahira Hassoubah and [1,3]Jehan Alrahimi

[1]Department of Biological Science, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia
[2]Research Center of Genetic Engineering and Bioinformatics, VACSERA, Dokki, Cairo, Egypt
[3]Immunolgy Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia

Abstract

Background and Objective: In previous fascinating studies, the potential role of *Saccharomyces cerevisiae*, a yeast essential in bread and beer production, as a probiotic in regulating immune responses has been illuminated. This study investigated the effects of administering *S. cerevisiae* alongside influenza vaccinations in diabetic rats. **Materials and Methods:** The rats were divided into four groups: A non-diabetic control group (UN), an induced diabetic group (UD), diabetes with flu vaccine group (DF) and diabetes with a combination of flu vaccine and the *S. cerevisiae* group (DFS). Immune responses were measured by analyzing serum cytokine levels (IL-15, IL-18, IL-23 and IL-25) and antibody levels (total IgG, IgM, IgG and IgG subclasses). The statistical analysis was performed using ANOVA to determine significant differences among the groups. **Results:** Analysis of immune responses revealed significant differences among the groups' serum. The DF rats showed elevated levels of IL-15, IL-18, IL-23 and IL-25 cytokines compared to the UN group. However, the DFS group exhibited decreased cytokine levels compared to the UD and DF groups. Antibody analysis showed lower IgG and IgM antibody levels in UD rats compared to UN. In contrast, DF rats had increased antibody levels, indicating an enhanced immune response. Interestingly, DFS rats had reduced total IgG and IgM levels compared to DF. Examination of IgG subclasses revealed elevated levels in DF and DFS groups compared to UD. However, the DFS group showed a decrease in one subclass but an increase in others compared to DF. **Conclusion:** These findings suggested that while *S. cerevisiae* has immune-modulating potential, its use as a probiotic may compromise the response to influenza vaccination in diabetic rats, necessitating further research to understand quality, infection monitoring and medication interactions on immune-compromised individuals.

Key words: Probiotics, IL-15, IL-18, IL-23, IL-25, diabetic diseases, IgG, IgM, IgA, IgG1, IgG2, IgG3, IgG4, autoimmune proinflammatory cytokines, influenza vaccine

Citation: El Hadad, S., A. Aldahlawi, A. Al Hashmi, S. Hassoubah and J. Alrahimi, 2024. Impact of *Saccharomyces cerevisiae* probiotic on influenza vaccine efficacy in diabetic rats: An immunological perspective. Int. J. Pharmacol., 20: 1271-1280.

Corresponding Author: Sahar El Hadad, Department of Biological Science, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

High blood sugar levels, indicative of hyperglycemia, stem largely from diabetes mellitus, which manifests predominantly as Type 1 Diabetes (T1D) and the more common Type 2 Diabetes (T2D). The T2D occurs when insulin resistance develops in the body's cells, despite normal insulin production by the pancreas[1]. On the other hand, T1D results from an autoimmune attack on pancreatic beta cells, leading to reduced insulin production, with harmful cytokines and cytotoxic lymphocytes are implicated in this process[2]. Diabetes not only affects glucose metabolism but also impairs the function of immune cells including granulocytes, monocytes and lymphocytes, which elevates the risk of infections, particularly bacterial ones[3].

Compromised immune function is a key factor that can lead to more serious influenza virus infections[4]. While flu vaccinations are the top preventative strategy, their success can be influenced by individual factors including the person's age, health condition and the match between the vaccine strains and the circulating virus. The effectiveness of the inactivated flu vaccine in eliciting an immune response is around 60%[5].

Probiotics such as *S. cerevisiae* are widely recognized for their safety profile and therapeutic potential in managing ailments of the digestive and respiratory systems, as well as immune-related disorders[6]. Previous research indicated that probiotics could enhance immune defense by promoting phagocytic activity and the production of immunoglobulin-secreting cells[7].

Specifically, *S. cerevisiae* has been noted for its ability to modulate the immune system by stimulating cytokine and immunoglobulin production via Toll-like receptors. It can also influence anti-inflammatory cytokine signaling pathways, potentially reducing inflammation[8]. Studies also suggest that *S. cerevisiae* may improve glucose metabolism in type 2 diabetes patients[9]. Its mucosal immunomodulatory impact has been observed in the intestinal cytokine secretion in mice, a process that depends on the expression of specific Toll-like receptors and affects the regulation of pro- and anti-inflammatory cytokines, including the neutrophil chemokine KC[10]. Ultimately, *S. cerevisiae* is thought to favor a Th1 immune response, which could be advantageous in treating various immunological conditions[11].

Cytokines like interleukins and interferons play a pivotal role in the regulation of both innate and adaptive immunity[12]. The IL-15, a cytokine produced by monocytes and macrophages, has effects akin to IL-2 and is crucial for the proliferation and migration of $CD4^+$ and $CD8^+$ T cells, B-cell differentiation and the stimulation of TNF-α and IFN-γ production by natural killer cells[13]. The IL-18, part of the IL-1 superfamily and formerly called an interferon-inducing factor, is integral to Th1 cell, macrophage and dendritic cell activation facilitating the secretion of Th1 cytokines and possessing proinflammatory capabilities by initiating IFN-γ, chemokines and NF-κB while suppressing anti-inflammatory IL-10[14]. The IL-23, from the IL-12 family and secreted by cells including macrophages, dendritic cells, B cells and endothelial cells, is essential for Th17 cell development and differentiation through STAT3 activation and promote the release of proinflammatory cytokines such as IFN-γ, TNF-α, IL-1 and IL-6[15]. Lastly, IL-25 or IL-17E, produced by various cells like alveolar macrophages and Th2 cells, curtails Th1 and Th17 responses while encouraging Th2 responses, inducing the production of cytokines like IL-4, IL-5, and IL-13[16].

This research aimed to examine the effects of *Saccharomyces cerevisiae* on the secretion levels of cytokines IL-15, IL-18, IL-23, IL-25 and various immunoglobulin isotypes in the blood serum of diabetic rats vaccinated against influenza. This was in comparison to diabetic rats that did not receive any treatment and a control group of healthy rats.

MATERIALS AND METHODS

Study area: This research was undertaken from 2019 to 2021 at King Abdulaziz University's Biological Science Department, located within the Faculty of Science in Jeddah, Saudi Arabia.

Resources and acquisition of vaccines, ELISA kits and chemicals used in the study: For the research conducted, influenza vaccines were supplied by the Saudi Ministry of Health and Streptozotocin (STZ) were purchased from Sigma Aldrich (CAS NO, 1883-66-4). The ELISA kits for measuring IgG, IgM, IgA, IgG1 and IgG4 levels came from MyBioSource in the US, specifically from San Diego, with product numbers MBS2513365, MBS251063, MBS2500774, MBS745389 and MBS753686, respectively. The ELISA kit for IgG2 was acquired from Thermofisher in the US (Cat. NO. 88-50510) and the one for IgG3 was sourced from Abnova in Taiwan (Cat. No. KA2471). Additionally, MyBioSource in San Diego provided the ELISA kits for detecting IL-15, IL-18, IL-23 and IL-25, with catalog numbers MBS701942, MBS260091, MBS704680 and MBS2515787, respectively. All other utilized chemicals and reagents were of analytical grade and met the necessary quality specifications.

Yeast probiotic formulation: The *Saccharomyces cerevisiae* strain used as a probiotic in the study was obtained as Saf-instant yeast from a commercial supplier in Turkey. To prepare the probiotic for administration, dried *Saccharomyces*

cerevisiae, at a dosage of 11.2 mg/kg of body weight, was mixed into 1 mL of distilled water. This solution was then given orally to the rats with diabetes.

Experimental design and rat grouping: Male albino rats weighing between 200-300 g were sourced and housed at the animal facility of the Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia. They were granted unlimited access to food and water. Forty rats were placed in wired cages with consistent temperature maintained at 22°C (±2) and subjected to a 12 hrs light-dark cycle.

Ethical consideration: The care provided to these animals was in line with Saudi animal welfare regulations and the study received ethical clearance from the Scientific Research Ethics Committee at the Faculty of Science, King Abdulaziz University, Saudi Arabia.

The rats were divided into four distinct groups: Group UN served as the control (no diabetes, no flu vaccine, no *Saccharomyces* treatment); Group UD was the diabetes control (diabetes induced with a single dose of 40 mg/kg STZ, no flu vaccine, no *Saccharomyces* treatment); Group DF included diabetic rats vaccinated for flu (diabetes induced as in UD, flu vaccine administered intramuscularly at 0 and 7 days, no *Saccharomyces* treatment); Group DFS comprised diabetic rats receiving both the flu vaccine and oral *Saccharomyces cerevisiae* treatment three times weekly from day -1 to day 14.

To confirm the induction of diabetes, blood glucose levels were measured on day -4 via tail tip amputation using an Accu-Chek glucometer (Roche, Basel, Switzerland) and Glucophage was administered to ensure the rats' survival. On day 15, the animals were euthanized via cardiac punctures under anesthesia with 50 mg/kg of thiopental sodium intraperitoneally. Their blood was collected and preserved at -20°C for subsequent analysis of humoral immune responses.

Quantification of immunoglobulins and cytokines

ELISA assays and statistical analysis: The measurement of rat immunoglobulin isotypes, subclasses and specific cytokines in serum was performed using commercially available capture ELISA kits, following the manufacturer's protocols. In brief, serum samples from the rat groups and standards for immunoglobulins were added to designated wells on pre-coated 96-well plates specific to each assay and incubated for a specified time. Biotinylated antibody reagents for detection and enzyme conjugates of horseradish peroxidase (HRP) were subsequently added. The reactions were initiated using appropriate substrates for the kits and stopped after 15 min with a stop solution. The optical density of each well was measured at 450 nm using an ELISA plate reader, allowing for the determination of immunoglobulin and cytokine concentrations.

Statistical analysis: Data obtained from the study were analyzed using the Mega Stat Statistical Software (version 10.2 release 2.1). Statistical significance was determined using the One-way Analysis of Variance (ANOVA) test, with p-value less than 0.05 indicating significance.

RESULTS

Impact of *Saccharomyces* probiotics on proinflammatory cytokine levels in diabetic rats following influenza vaccination: In the serum of diabetic rats, the levels of cytokines IL-15, IL-18, IL-23 and IL-25 were significantly elevated compared to nondiabetic rats ($p = 0.0000$ per cytokine) (Fig. 1). However, when diabetic rats were administered both *S. cerevisiae* and the flu vaccine, there was a highly significant decrease in the levels of IL-15, IL-18, IL-23 and IL-25 compared to untreated diabetic rats ($p = 0.0000$ per cytokine) (Fig. 2). Furthermore, the decreases in IL-15, IL-18 and IL-23 were significant in the group that received both *S. cerevisiae* and the flu vaccine compared to the group that received only the flu vaccine ($p<0.05$ per cytokine), although no significant changes were observed in the IL-25 cytokine (Fig. 2). The group that received only the flu vaccine also exhibited significant reductions in IL-18, IL-23 and IL-25 compared to untreated diabetic rats ($p = 0.0000$ per cytokine) (Fig. 2).

***Saccharomyces* impacts on immunoglobulins levels in diabetic rats:** The study observed that untreated diabetic rats exhibited a significant decrease in the levels of polyclonal IgG and IgM compared to non-diabetic control rats ($p = 0.0000$ for each; Fig. 3). Conversely, the levels of polyclonal IgA were significantly elevated in untreated diabetic rats compared to the non-diabetic group ($p = 0.0009$; Fig. 3). In the diabetic rat groups that received either the FLU vaccine or both *Saccharomyces* and the FLU vaccine, the levels of polyclonal IgG and IgM were significantly higher than in untreated diabetic rats ($p = 0.0000$ for each group/each Ig; Fig. 4). Additionally, the levels of total IgG and IgM were significantly lower in diabetic rats treated with *Saccharomyces* and the FLU vaccine compared to those vaccinated with just the FLU vaccine ($p = 0.0000$; Fig. 4). Notably, the level of polyclonal IgA

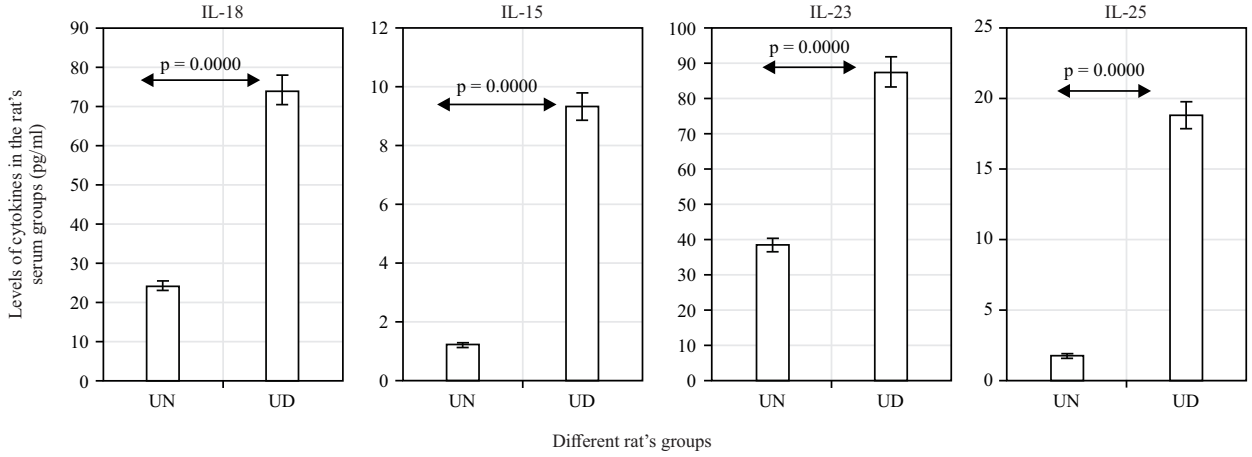

Fig. 1: Levels of IL-15, IL-18, IL-23 and IL-25 cytokines in the serum of both diabetic and non-diabetic rats' groups

UN: Serum from the standard untreated rat group and UD: Serum from rats with diabetes. A p-value less than 0.05 was considered statistically significant according to variance analysis using one-way ANOVA. Each bar represents the average of three independent experiments and the error bars represent a 5% standard error of the mean

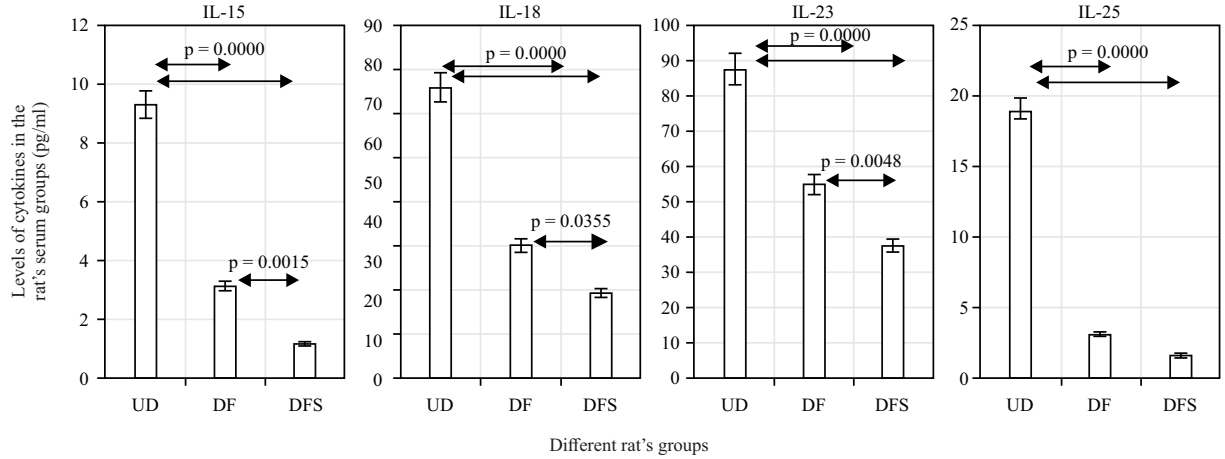

Fig. 2: Levels of IL-15, IL-18, IL-23 and IL-25 cytokines in serum from various diabetic rat treatment groups

UD: Serum from rats with diabetes, DF: Serum from diabetic rats that received the influenza vaccine and DFS: Serum from diabetic rats that received both the influenza vaccine and *Saccharomyces cerevisiae* probiotics. A p-value less than 0.05 was considered statistically significant according to variance analysis using one-way ANOVA. Each bar represents the average of three independent experiments and the error bars represent a 5% standard error of the mean

decreased in the serum of the diabetic group treated with *Saccharomyces* and the FLU vaccine, with a significant reduction compared to diabetic rats immunized with the FLU vaccine alone ($p = 0.0002$), while showing a non-significant difference from untreated diabetic rats. Meanwhile, the levels of total IgA were significantly higher in diabetic rats vaccinated with just the FLU vaccine compared to non-diabetic rats ($p = 0.0025$; Fig. 4).

Effects of FLU vaccine and *Saccharomyces* treatment on IgG subclasses in diabetic rats: In the untreated diabetic rat group, there was a significant decrease in the levels of all IgG subclasses (IgG1, IgG2, IgG3 and IgG4) compared to non-diabetic rats ($p = 0.0001$ for IgG1, IgG2, IgG3; $p = 0.0043$ for IgG4; Fig. 5). However, administering the FLU vaccine to diabetic rats resulted in a significant increase in IgG subclass levels and this effect was further amplified when combining *Saccharomyces* probiotics with the FLU vaccine, compared to untreated diabetic rats ($p<0.0001$ for each IgG subclass; Fig. 5). Notably, diabetic rats immunized with the FLU vaccine alone and those treated with both *Saccharomyces* and the FLU vaccine exhibited significantly elevated IgG1 and IgG2 subclass levels compared to non-diabetic rats ($p<0.0001$; Fig. 6). Interestingly, diabetic rats treated with both the FLU

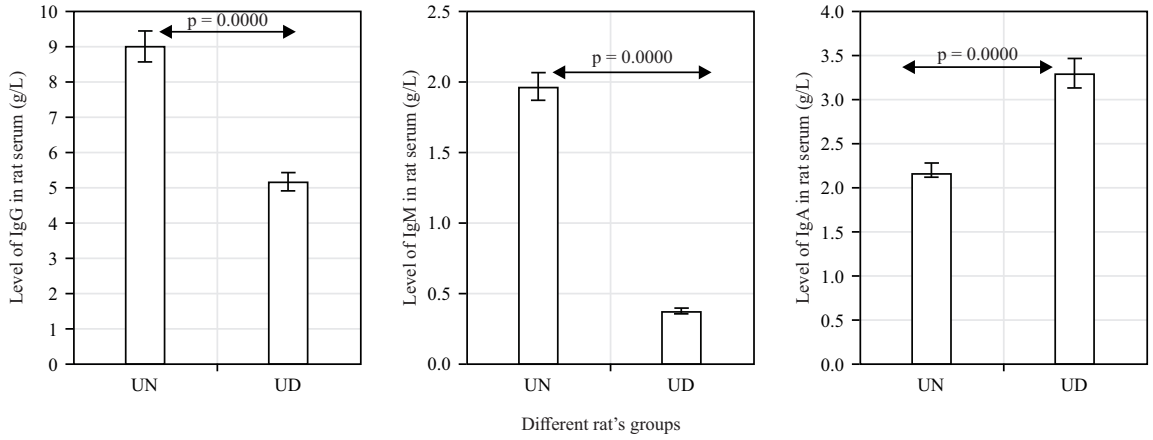

Fig. 3: Distribution of polyclonal IgG, IgM and IgA levels in the serum from both diabetic and non-diabetic rats
UN: Serum samples from the standard untreated control group and UD: Serum samples from diabetic rats. A p-value less than 0.05 was considered statistically significant based on the analysis of variance using a one-way ANOVA test. Each bar in the graph represents the mean of three independent experiments and the error bars indicate a 5% standard deviation from the mean

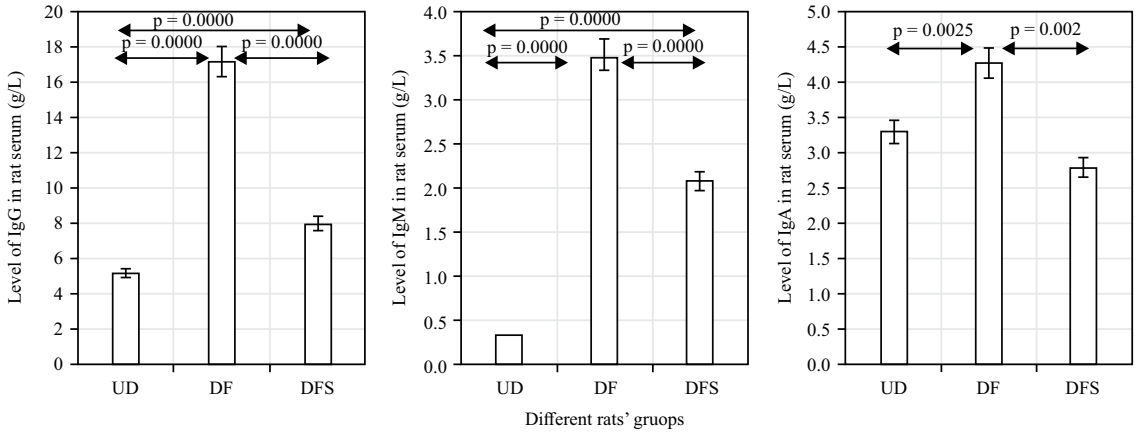

Fig. 4: Distribution of polyclonal IgG, IgM and IgA levels in serum from differently treated groups of diabetic rats
UD: Serum samples from diabetic rats, DF: Serum from diabetic rats that were administered the influenza virus vaccine and DFS: Signifies serum from diabetic rats that received a combination treatment of the influenza virus vaccine and *Saccharomyces cerevisiae* probiotics. A p-value less than 0.05 was considered statistically significant based on the analysis of variance using a one-way ANOVA test. Each bar in the graph represents the mean of three independent experiments and the error bars indicate a 5% standard deviation from the mean

vaccine and *Saccharomyces* showed a significant reduction in IgG1 compared to those immunized with the FLU vaccine alone ($p<0.0001$), while changes in IgG2 levels were not significant. Conversely, the group treated with both the FLU vaccine and *Saccharomyces* demonstrated significant increases in IgG3 and IgG4 levels compared to non-diabetic rats ($p = 0.0000$ and 0.0174, respectively). In contrast, the group receiving the FLU vaccine alone exhibited a significant reduction in IgG3 ($p = 0.0022$) and a significant elevation in IgG4 ($p = 0.0118$) compared to non-diabetic rats. Furthermore, the group treated with both the FLU vaccine and *Saccharomyces* displayed significant increases in IgG3 and IgG4 levels compared to the group receiving the FLU vaccine alone ($p = 0.0000$ and 0.0174, respectively; Fig. 6).

DISCUSSION

Immune dysfunction in diabetic patients increases the risk of infections and complications. Diabetic individuals are more likely to be hospitalized and experience higher mortality during influenza epidemics[17]. Vaccination provides partial protection and reduces hospital admissions[18]. *Saccharomyces cerevisiae* has emerged as a beneficial probiotic for enhancing immune response. It stimulates innate and adaptive immunity, including immune cell activation[19].

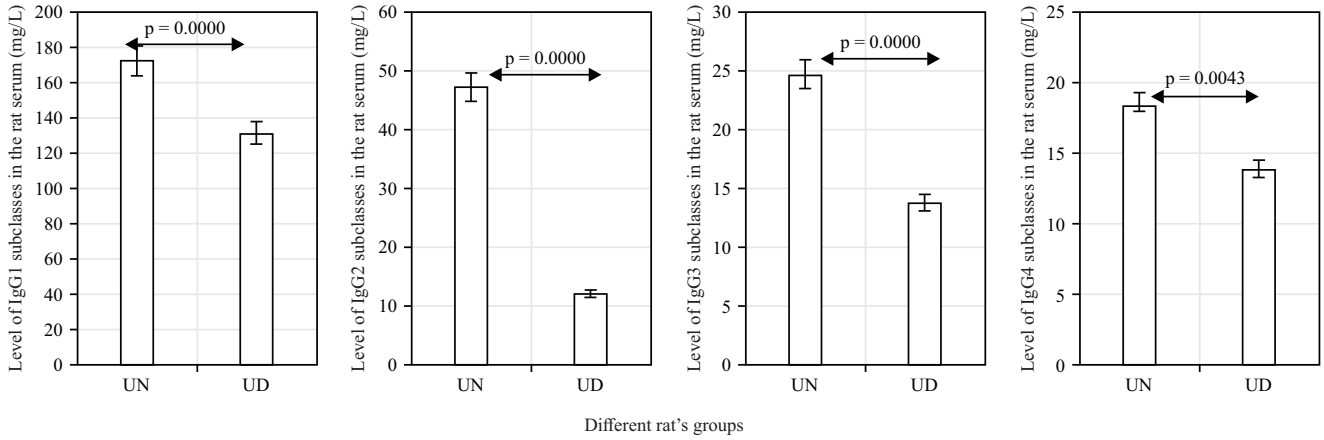

Fig. 5: Illustrates the concentrations of total IgG subclasses in two scenarios compares serum from diabetic rats to non-diabetic rats

UN: Serum from the control group of rats that did not receive any treatment and UD: Serum from rats with diabetes. A p-value lower than 0.05 was considered statistically significant according to the analysis of variance using a one-way ANOVA test. Each bar on the graph represents the average of three independent experiments, with error bars showing a deviation of 5% from this mean value

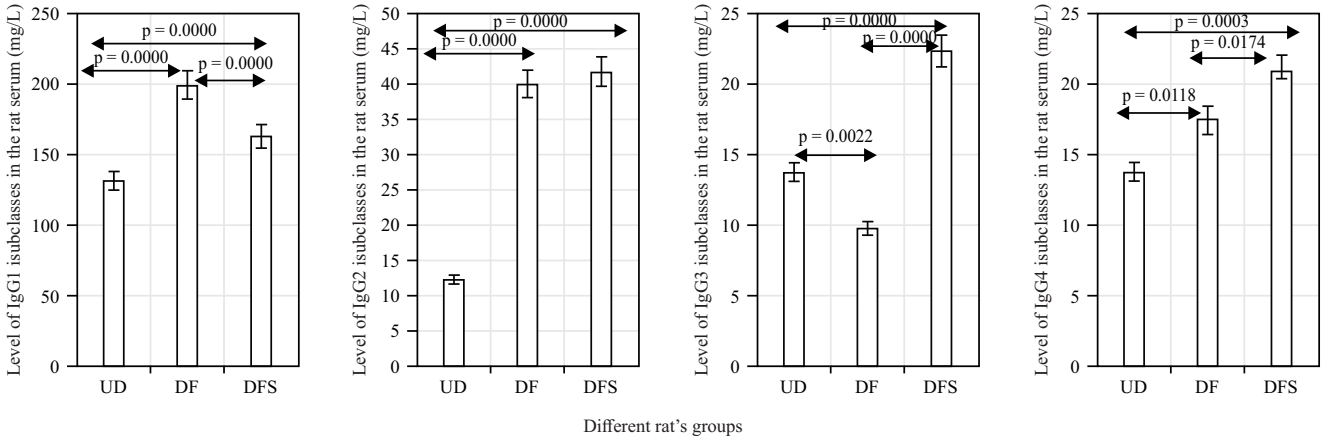

Fig. 6: Illustrates the concentrations of total IgG subclasses in two scenarios examines serum from diabetic rats subjected to various treatments

UD: Serum from rats with diabetes, DF: Serum from diabetic rats that were given the influenza virus vaccine and DFS: Serum from diabetic rats treated with a combination of the influenza virus vaccine and *Saccharomyces cerevisiae* probiotics. A p-value lower than 0.05 was considered statistically significant according to the analysis of variance using a one-way ANOVA test. Each bar on the graph represents the average of three independent experiments, with error bars showing a deviation of 5% from this mean value

The serum of the current diabetic rats exhibited elevated levels of cytokines, specifically IL-15, IL-18, IL-23 and IL-25, compared to non-diabetic rats. The increased cytokine levels, particularly IL-15, IL-18, IL-23 and IL-25, suggest an inflammatory response in diabetic rats, which may contribute to the chronic inflammation associated with diabetes[20]. Moreover, this present study revealed that untreated diabetic rats had significantly lower levels of polyclonal IgG and IgM compared to non-diabetic control rats. The reduced levels of polyclonal IgG and IgM in untreated diabetic rats indicate impaired antibody production, potentially compromising their ability to mount an effective immune response against infections[21]. Conversely, the levels of polyclonal IgA were significantly higher in the present untreated diabetic rats compared to the non-diabetic group. Conversely, the elevated levels of polyclonal IgA in untreated diabetic rats could reflect a compensatory response to the inflammatory environment associated with diabetes[22]. In diabetic patients, particularly those with type 1 diabetes, the humoral immune response, which includes various immunoglobulin isotypes in the

bloodstream is notably altered and plays a critical role in the complex process that leads to the destruction of β-cells[2]. The presence of human antibodies reacting in such a manner has been described as anti-ruminant antibodies[23]. These changes in IgG, IgM and IgA levels could potentially serve as indicators for type 2 diabetes[24]. Additionally, in the present study, when examining the IgG subclasses, all subclasses (IgG1, IgG2, IgG3 and IgG4) showed a significant decrease in the untreated diabetic rat group compared to non-diabetic rats. Our findings indicate that diabetes leads to immune dysregulation characterized by altered cytokine levels and antibody production[25]. Furthermore, the significant decrease in all IgG subclasses (IgG1, IgG2, IgG3 and IgG4) in untreated diabetic rats compared to non-diabetic rats suggests a global impairment in IgG subclass production. The IgG subclasses play important roles in immune defense and targeting specific pathogens[26]. The decrease in these subclasses in diabetic rats may further contribute to their increased susceptibility to infections and complications[27].

The use of probiotics and postbiotics as novel mucosal aids have gained increased attention for their role in enhancing the immune response to vaccinations[28]. This current study observed a significant decrease in the serum levels of IL-15, IL-18, IL-23 and IL-25 cytokines in diabetic rats after receiving both the influenza vaccine and oral treatment with *Saccharomyces cerevisiae* probiotics, compared to the untreated diabetic group. Generally, these cytokines play a crucial role in enhancing the immune response to the influenza vaccine by promoting specific T-helper cell responses[29]. This reduction in cytokine levels indicates potential benefits in regulating the immune system, reducing inflammation and modifying immune cell function[30]. Notably, IL-15, IL-18 and IL-23 showed significant decreases in the current diabetic rats that received both the flu vaccine and probiotic treatment compared to those who only received the vaccine. The study suggested that *Saccharomyces* probiotics can effectively suppress autoimmune proinflammatory cytokines[31], which have a significant impact on the humoral immune response to influenza vaccines in diabetic rats[32]. However, this current IL-25 did not exhibit a significant change in diabetic rats that received both the flu vaccine and probiotic treatment compared to those who only received the vaccine. The IL-25 is crucial for the development of Th2 cells which play a vital role in the response to influenza vaccination. Therefore, *Saccharomyces* might impact the vaccine's efficacy[33].

In the current study, diabetic rats that received the FLU vaccine alone or in combination with *Saccharomyces* probiotics showed a significant increase in polyclonal IgG and IgM levels compared to untreated diabetic rats. However, current rats treated with both *Saccharomyces* probiotics and the FLU vaccine exhibited lower levels of total IgG and IgM compared to those given only the FLU vaccine. Generally, probiotics are known to enhance polyclonal antibody production[34], but the current findings suggest that *Saccharomyces* in combination with the FLU vaccine reduces the production of IgG and IgM. It is important to note that IgA possesses potent anti-inflammatory properties and its FcαRI receptors facilitate the transmission of inhibitory immune signals[35]. Significantly, the current diabetic rats treated with both *Saccharomyces* probiotics and the FLU vaccine experienced a notable decrease in polyclonal IgA levels in their serum, showing a significant reduction compared to diabetic rats immunized with the FLU vaccine alone. However, this reduction did not differ significantly from untreated diabetic rats. In contrast, diabetic rats vaccinated with only the FLU vaccine had significantly higher levels of total IgA compared to non-diabetic rats. This reduction could potentially be attributed to a decrease in IL-5, which affects the proliferation and differentiation of B lymphocytes[36], as well as lower levels of IL-4 cytokine, resulting in a decline in primary extracellular defense antibodies, namely IgG and IgM[37,38]. The decrease in immunoglobulin isotypes observed in diabetic rats treated with *Saccharomyces* probiotics alongside the FLU vaccine suggests that reduced anti-inflammatory cytokines may impact the stimulation of immunoglobulin production[39].

In the current untreated diabetic rat group, there was a significant decrease in the levels of all IgG subclasses (IgG1, IgG2, IgG3 and IgG4) compared to non-diabetic rats. The reduction of immunoglobulins in diabetic individuals is a noteworthy observation with potential implications. Immunoglobulins, such as IgG subclasses (IgG1, IgG2, IgG3 and IgG4), play a critical role in immune defense and maintaining overall immune function[40]. The significant decrease in the levels of these immunoglobulins in untreated diabetic individuals compared to non-diabetic individuals suggests an impaired immune response in diabetes. This reduction could compromise the ability of the immune system to effectively combat infections and protect against diseases[36].

In this study, it was observed that diabetic rats receiving the FLU vaccine along with *Saccharomyces* probiotics exhibited a deficiency in IgG1 levels compared to those receiving either the FLU vaccine alone. This reduction in IgG1, an important subclass of immunoglobulins, suggests an impaired immune response in diabetes. The IgG1 plays a crucial role in immune defense and is known for its ability to activate various immune cells, neutralize pathogens and promote antibody-mediated clearance of infections. The

decrease in IgG1 levels in diabetic rats receiving FLU vaccine along with *Saccharomyces* probiotics highlights a potential vulnerability in their immune system, as it may compromise their ability to effectively combat infections and protect against diseases[41]. Excitingly, the current combination of the FLU vaccine and *Saccharomyces* probiotics resulted in a significant boost in IgG3 and IgG4 subclass levels in diabetic rats, surpassing even the levels seen in non-diabetic rats and those receiving the FLU vaccine alone. This indicates a remarkable improvement in the rats' immune response, as IgG3 and IgG4 antibodies are vital for immune defense and regulation[42]. The IgG3 helps control excessive inflammation[43], while IgG4 is associated with allergens, parasites and immune tolerance[42]. However, the heightened IgG4 levels raise concerns about potential allergic reactions[42]. These findings have important implications for the FLU vaccine's effectiveness, as the modulation of IgG3 and IgG4 levels could directly impact its ability to protect against the FLU. Furthermore, the specific influence of *Saccharomyces* probiotics on different IgG subclasses may depend on the nature of the antigen and the overall responsiveness of the immune system[7].

CONCLUSION

This study highlighted that diabetes leads to chronic inflammation and immune dysfunction, contributing to complications. Untreated diabetic rats exhibited elevated inflammatory cytokines and impaired antibody production, compromising their ability to fight infections. However, when diabetic rats received both *Saccharomyces* probiotics and the FLU vaccine, there was a significant reduction in inflammation, indicating an anti-inflammatory effect. Although the combination treatment increased overall antibody levels, they were lower compared to rats receiving only the FLU vaccine. Notably, the combination treatment resulted in decreased levels of IgA, possibly influenced by the inflammatory diabetic environment. Furthermore, untreated diabetic rats had reduced production of all IgG subclasses, weakening their immune defense. However, the combination treatment boosted levels of IgG3 and IgG4 subclasses, crucial for immune regulation and defense. It is important to consider the impact on the immune response to the FLU vaccine in diabetic patients. While *Saccharomyces* probiotics are generally safe, there are potential risks such as rare allergic reactions and invasive infections, especially in those with weakened immune systems. Ensuring probiotic quality, monitoring for infections and considering interactions with diabetes medications are essential precautions.

SIGNIFICANCE STATEMENT

The study aimed to explore the effects of *Saccharomyces cerevisiae* on immune responses when administered alongside influenza vaccinations in diabetic rats. The key findings revealed that while *S. cerevisiae* has immune-modulating potential, it reduced cytokine and antibody levels in vaccinated diabetic rats compared to those receiving only the vaccine. This suggests that *S. cerevisiae* might impair the immune response to influenza vaccination in diabetic rats. These results underscore the importance of understanding the interactions between probiotics and the immune system, particularly in diabetic and immune-compromised individuals, to optimize vaccination strategies and ensure effective immune responses.

ACKNOWLEDGMENT

All authors would like to acknowledge King Fahd Center for Medical Research for conducting this study.

REFERENCES

1. Burrack, A.L., T. Martinov and B.T. Fife, 2017. T cell-mediated beta cell destruction: Autoimmunity and alloimmunity in the context of type 1 diabetes. Front. Endocrinol., Vol. 8. 10.3389/fendo.2017.00343.
2. Roep, B.O., S. Thomaidou, R. van Tienhoven and A. Zaldumbide, 2021. Type 1 diabetes mellitus as a disease of the β-cell (do not blame the immune system?). Nat. Rev. Endocrinol., 17: 150-161.
3. Daryabor, G., M.R. Atashzar, D. Kabelitz, S. Meri and K. Kalantar, 2020. The effects of type 2 diabetes mellitus on organ metabolism and the immune system. Front. Immunol., Vol. 11. 10.3389/fimmu.2020.01582.
4. Mifsud, K.R., C.L.M. Kennedy, S. Salatino, E. Sharma and E.M. Price *et al*., 2021. Distinct regulation of hippocampal neuroplasticity and ciliary genes by corticosteroid receptors. Nat. Commun., Vol. 12. 10.1038/s41467-021-24967-z.
5. Trombetta, C.M., O. Kistner, E. Montomoli, S. Viviani and S. Marchi, 2022. Influenza viruses and vaccines: The role of vaccine effectiveness studies for evaluation of the benefits of influenza vaccines. Vaccines, Vol. 10. 10.3390/vaccines10050714.
6. Tegegne, B.A. and B. Kebede, 2022. Probiotics, their prophylactic and therapeutic applications in human health development: A review of the literature. Heliyon, Vol. 8. 10.1016/j.heliyon.2022.e09725.
7. Mazziotta, C., M. Tognon, F. Martini, E. Torreggiani and J.C. Rotondo, 2023. Probiotics mechanism of action on immune cells and beneficial effects on human health. Cells, Vol. 12. 10.3390/cells12010184.

8. Guo, N. and L.L. Lv, 2023. Mechanistic insights into the role of probiotics in modulating immune cells in ulcerative colitis. Immun. Inflammation Dis., Vol. 11. 10.1002/iid3.1045.
9. Dilworth, L., A. Facey and F. Omoruyi, 2021. Diabetes mellitus and its metabolic complications: The role of adipose tissues. Int. J. Mol. Sci., Vol. 22. 10.3390/ijms22147644.
10. Vijay, K., 2018. Toll-like receptors in immunity and inflammatory diseases: Past, present, and future. Int. Immunopharmacol., 59: 391-412.
11. Dagenais, A., C. Villalba-Guerrero and M. Olivier, 2023. Trained immunity: A "new" weapon in the fight against infectious diseases. Front. Immunol., Vol. 14. 10.3389/fimmu.2023.1147476.
12. Mantovani, A., C.A. Dinarello, M. Molgora and C. Garlanda, 2019. Interleukin-1 and related cytokines in the regulation of inflammation and immunity. Immunity, 50: 778-795.
13. Carlini, V., D.M. Noonan, E. Abdalalem, D. Goletti, C. Sansone, L. Calabrone and A. Albini, 2023. The multifaceted nature of IL-10: Regulation, role in immunological homeostasis and its relevance to cancer, COVID-19 and post-COVID conditions. Front. Immunol., Vol. 14. 10.3389/fimmu.2023.1161067.
14. Ihim, S.A., S.D. Abubakar, Z. Zian, T. Sasaki, M. Saffarioun, S. Maleknia and G. Azizi, 2022. Interleukin-18 cytokine in immunity, inflammation, and autoimmunity: Biological role in induction, regulation, and treatment. Front. Immunol., Vol. 13. 10.3389/fimmu.2022.919973.
15. Bunte, K. and T. Beikler, 2019. Th17 cells and the IL-23/IL-17 axis in the pathogenesis of periodontitis and immune-mediated inflammatory diseases. Int. J. Mol. Sci., Vol. 20. 10.3390/ijms20143394.
16. Kuen, D.S., B.S. Kim and Y. Chung, 2020. IL-17-producing cells in tumor immunity: Friends or foes? Immune Netw., Vol. 20. 10.4110/in.2020.20.e6.
17. Al-Sayyar, A., K.D. Hulme, R. Thibaut, J. Bayry, F.J. Sheedy, K.R. Short and F. Alzaid, 2022. Respiratory tract infections in diabetes-lessons from tuberculosis and influenza to guide understanding of COVID-19 severity. Front. Endocrinol., Vol. 13. 10.3389/fendo.2022.919223.
18. Niessen, A., A.C. Teirlinck, S.A. McDonald, W. van der Hoek, R. van Gageldonk-Lafeber and M.J. Knol, 2022. Sex differences in COVID-19 mortality in the Netherlands. Infection, 50: 709-717.
19. Paola, M.D., N. Meriggi and D. Cavalieri, 2020. Applications of wild isolates of saccharomyces yeast for industrial fermentation: The gut of social insects as niche for yeast hybrids' production. Front. Microbiol., Vol. 11. 10.3389/fmicb.2020.578425.
20. Nirenjen, S., J. Narayanan, T. Tamilanban, V. Subramaniyan and V. Chitra *et al*., 2023. Exploring the contribution of pro-inflammatory cytokines to impaired wound healing in diabetes. Front. Immunol., Vol. 14. 10.3389/fimmu.2023.1216321.
21. Brown, B., V. Ojha, I. Fricke, S.A. Al-Sheboul and C. Imarogbe *et al*., 2023. Innate and adaptive immunity during SARS-CoV-2 Infection: Biomolecular cellular markers and mechanisms. Vaccines, Vol. 11. 10.3390/vaccines11020408.
22. Farrag, E.A.E., M.O. Hammad, S.M. Safwat, S. Hamed and D. Hellal, 2023. Artemisinin attenuates type 2 diabetic cardiomyopathy in rats through modulation of AGE-RAGE/HMGB-1 signaling pathway. Sci. Rep., Vol. 13. 10.1038/s41598-023-37678-w.
23. Avril, A., J.N. Tournier, J.C. Paucod, B. Fournes, P. Thullier and T. Pelat, 2022. Antibodies against anthrax toxins: A long way from benchlab to the bedside. Toxins, Vol. 14. 10.3390/toxins14030172.
24. Alhalwani, A.Y., K. Abudawood, A.B.E.A. Qadizadah, S. Jambi and N.S. Sannan, 2023. Immunoglobulin A levels and its correlation with neutrophil-to-lymphocyte ratio as inflammatory biomarkers for dry eye disease in type 2 diabetes: A retrospective study. Front. Immunol., Vol. 14. 10.3389/fimmu.2023.1184862.
25. Berbudi, A., N. Rahmadika, A.I. Tjahjadi and R. Ruslami, 2020. Type 2 diabetes and its impact on the immune system. Curr. Diabetes Rev., 16: 442-449.
26. MacGlashan Jr., D., S. Alvarez-Arango and J. Tversky, 2021. Subclasses of allergen-specific IgG: Serum IgG2 and IgG3 levels are not predicted by IgG1/IgG4 levels. Clin. Exp. Allergy, 51: 1093-1095.
27. Jin, Y. and R. Arroo, 2023. The protective effects of flavonoids and carotenoids against diabetic complications-A review of *in vivo* evidence. Front. Nutr., Vol. 10. 10.3389/fnut.2023.1020950.
28. El Hadad, S., M. Alsolami, A. Aldahlawi, J. Alrahimi, F. Basingab, S. Hassoubah and H. Alothaid, 2021. *In vivo* evidence: Repression of mucosal immune responses in mice with colon cancer following sustained administration of *Streptococcus thermophiles*. Saudi J. Biol. Sci., 28: 4751-4761.
29. Janssens, Y., J. Joye, G. Waerlop, F. Clement, G. Leroux-Roels and I. Leroux-Roels, 2022. The role of cell-mediated immunity against influenza and its implications for vaccine evaluation. Front. Immunol., Vol. 13. 10.3389/fimmu.2022.959379.
30. Liu, C., D. Chu, K. Kalantar-Zadeh, J. George, H.A. Young and G. Liu, 2021. Cytokines: From clinical significance to quantification. Adv. Sci., Vol. 8. 10.1002/advs.202004433.
31. Cristofori, F., V.N. Dargenio, C. Dargenio, V.L. Miniello, M. Barone and R. Francavilla, 2021. Anti-inflammatory and immunomodulatory effects of probiotics in gut inflammation: A door to the body. Front. Immunol., Vol. 12. 10.3389/fimmu.2021.578386.
32. Rasaei, D., S.A. Hosseinian, K. Asasi, S.S. Shekarforoush and A. Khodakaram-Tafti, 2023. The beneficial effects of spraying of probiotic *Bacillus* and *Lactobacillus* bacteria on broiler chickens experimentally infected with avian influenza virus H9N2. Poult. Sci., Vol. 102. 10.1016/j.psj.2023.102669.

33. Kazemifard, N., A. Dehkohneh and S.B. Ghavami, 2022. Probiotics and probiotic-based vaccines: A novel approach for improving vaccine efficacy. Front. Med., Vol. 9. 10.3389/fmed.2022.940454.
34. Darma, A., A.F. Athiyyah, R.G. Ranuh, A. Endaryanto, Budiono and S.M. Sudarmo, 2020. Effects of probiotics on the enhancement of the innate mucosal immune response against pathogenic bacteria. Iran. J. Microbiol., 12: 445-450.
35. Breedveld, A. and M. van Egmond, 2019. IgA and FcαRI: Pathological roles and therapeutic opportunities. Front. Immunol., Vol. 10. 10.3389/fimmu.2019.00553.
36. Munteanu, C. and B. Schwartz, 2022. The relationship between nutrition and the immune system. Front. Nutr., Vol. 9. 10.3389/fnut.2022.1082500.
37. Junttila, I.S., 2018. Tuning the cytokine responses: An update on interleukin (IL)-4 and IL-13 receptor complexes. Front. Immunol., Vol. 9. 10.3389/fimmu.2018.00888.
38. El Hadad, S., A. Zakareya, A. Al-Hejin, A. Aldahlawi and M. Alharbi, 2019. Sustaining exposure to high concentrations of bifidobacteria inhibits gene expression of Mouse's mucosal immunity. Heliyon, Vol. 5. 10.1016/j.heliyon.2019.e02866.
39. Rio, P., M. Caldarelli, M. Chiantore, F. Ocarino and M. Candelli *et al*., 2024. Immune cells, gut microbiota, and vaccines: A gender perspective. Cells, Vol. 13. 10.3390/cells13060526.
40. Moura, A.D., H.H.M. da Costa, V.A. Correa, A.K. de S. Lima and J.A.L. Lindoso *et al*., 2021. Assessment of avidity related to IgG subclasses in SARS-CoV-2 Brazilian infected patients. Sci. Rep., Vol. 11. 10.1038/s41598-021-95045-z.
41. Helmy, Y.A., K. Taha-Abdelaziz, H.A.E.H. Hawwas, S. Ghosh and S.S. AlKafaas *et al*., 2023. Antimicrobial resistance and recent alternatives to antibiotics for the control of bacterial pathogens with an emphasis on foodborne pathogens. Antibiotics, Vol. 12. 10.3390/antibiotics12020274.
42. Uversky, V.N., E.M. Redwan, W. Makis and A. Rubio-Casillas, 2023. IgG4 antibodies induced by repeated vaccination may generate immune tolerance to the SARS-CoV-2 spike protein. Vaccines, Vol. 11. 10.3390/vaccines11050991.
43. Chu, T.H., E.F. Patz Jr and M.E. Ackerman, 2021. Coming together at the hinges: Therapeutic prospects of IgG3. mAbs, Vol. 13. 10.1080/19420862.2021.1882028.