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STEM CELL SOLUTION TO GAD65 ANTIBODIES INDUCED HURRICANE OF TYPE 1 DIABETES AND NEUROLOGICAL DISORDERS

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ABSTRACT

The encounter of disorders associated with antibodies to neuronal enzymes caused a paradigm shift in understanding the CNS autoimmunity. The autoimmune disorders targeting the 65kDa isoform of glutamic acid decarboxylase (GAD65) not only comprehends type 1 diabetes mellitus (T1DM), but also rather rare neurological disorders, including stiff-person syndrome (SPS), cerebellar ataxia, limbic encephalitis, and epilepsy. The patients with these autoimmune neurological disorders usually present with T1DM and suggests the presence of GAD65 antibodies. This is suggestive of autoimmune mechanisms for the development and worsening of these disorders. For better prognosis, its advanced screening and swift treatment are essential. Mesenchymal stem cells (MSCs) can be a promising salvage to these autoimmune disorders as they have proven hypoimmunogenic and immunomodulatory properties along with excellent regenerative ability. These self-renewing progenitor cells can differentiate into numerous cell types under explicit conditions, which includes neurons and pancreatic beta cells. MSCs annul the proinflammatory response in autoimmune disorders, may be through paracrine secretions, and hence, can help managing the hurricane of disturbed immunity. We present a link between the mechanisms driving autoimmune neurological diseases and T1DM in this review, based on the existence of GAD65 antibodies and an MSC-mediated solution for their treatment.

Keywords: GAD65 antibodies; Autoimmune neurological disorders; Type 1 diabetes; Disease correlation; Stem cell therapy; Immunization

INTRODUCTION

Antibodies to GAD, the rate-limiting enzyme in the manufacture of the inhibitory neurotransmitter GABA, have been detected in a range of neurological illnesses as well as type 1 diabetes (T1DM) (Graus, Saiz, and Dalmau, 2020). GAD expression is highly restricted to neurons, β-cells of pancreatic islets, mucosal epithelial cells of the oviduct, and spermatocytes and spermatids in the testes (Tillakaratne *et al*., 1992) (Vincent *et al*. 1983). GAD is made up of two distinct isoforms, GAD65 and GAD67, which are encoded by two separate genes on chromosomes 10 and 2, respectively. The middle segment and C terminus of these isoforms are 75% homologous, however the N-terminal portion is only 25% homologous (Erlander and Tobin 1991). GAD67 is located mostly in the cytoplasm of neurons, whereas GAD65 is found primarily in axon terminals, where the N-terminal domain binds it to synaptic vesicle membranes (Christgau *et al*. 1992; Kaufman, Houser, and Tobin 1991). Both isoforms are involved in the production of GABA from glutamate, which necessitates the addition of the cofactor pyridoxal 5-phosphate (PLP). GAD67 is a PLP-bound active holoenzyme that produces stable levels of neuronal cytosolic GABA, whereas GAD65 is a PLP-dissociated apoenzyme that mediates activity-dependent GABA synthesis when rapid postsynaptic inhibition is required (Kaufman *et al*. 1991; Patel *et al*. 2006).

GAD 65 antibody and autoimmune neurological disorders

Neurological diseases such as SPS, cerebellar ataxia, and temporal lobe epilepsy linked to high levels of GAD antibodies. Antibodies to the glutamic acid decarboxylase 65-kilodalton isoform (GAD65) are used to diagnose autoimmune CNS disorders and, more typically, non-neurological autoimmune diseases (Kaufman *et al*. 1991). Examples of CNS-localized neurological disorders (SPS) are Limbic encephalitis, epilepsy, cerebellar ataxia, and stiff-person syndrome. Classic SPS is a manifestation of CNS hyperexcitability that comprises characteristics that are either more confined (stiff-limb syndrome) or more extensive (progressive encephalomyelitis with rigidity and myoclonus). Although GAD65 antibody is not strongly predictive of a paraneoplastic etiology for neurological disorders, it has been linked to a variety of cancer types in the past. Immunotherapy responses are heterogeneous across all phenotypes (approximately 50 percent improve). GAD65 autoimmunity is critical to recognize for both non-neurological autoimmune connections and immunotherapy response (Graus, Saiz, and Dalmau 2020).

Autoimmune targets at the inhibitory synapse as well as the neurological diseases that accompany them. GAD, amphiphysin, GABA receptors, and glycine receptors are the principal autoimmune targets. Antibodies to GAD are most commonly found in stiff-person syndrome, cerebellar ataxia, and epilepsy, as well as encephalitis. Antibodies to amphiphysin are used to diagnose paraneoplastic stiff-person syndrome and breast cancer. Antibodies to glycine receptors are frequently linked to stiffness and myoclonus in progressive encephalomyelitis (PERM). Antibodies to GABAA receptors are found in autoimmune encephalitis, a kind of encephalitis that is frequently linked to refractory seizures and status epilepticus. The VIAAT is a protein that transports inhibitory amino acids through the cell membrane (Graus *et al*. 2020).

Stiff-person syndrome and variants

Stiff-man syndrome was described by Moersch and Woltman in 1956, and it is characterised by rigidity, spasms, and continuous motor unit activity of central origin (Clardy *et al*. 2013). This name was later replaced with SPS, and a wide range of phenotypic presentations were documented, ranging from limited involvement of one leg (stiff-leg syndrome) (Brown, Rothwell, and Marsden 1997; Saiz *et al*. 1998) to PERM, a complex disorder characterised by rigidity and spasms, as well as spontaneous and stimulus-sensitive myoclonus, brainstem symptoms, long tract signs, and dysautonomia (Brown *et al*. 1997; Saiz *et al*. 1998). These clinical characteristics are commonly referred to as SPS variants, and they are grouped along with classic SPS to form stiff-person spectrum diseases (Martinez-Hernandez *et al*. 2016). GAD antibodies are commonly found in people with SPS or stiff-leg syndrome, but they are uncommon in patients with PERM, who typically have antibodies to the glycine receptor's α1 subunit (Carvajal-González *et al*. 2014; Martinez-Hernandez *et al*. 2016).

SPS is expected to affect 1–2 million people, but no rigorous epidemiological investigations have been conducted (Meinck and Thompson 2002). The majority of patients (70 percent) are women, and the initial signs and symptoms usually appear between the ages of 30 and 50 (Meinck and Thompson 2002; Rakocevic, Alexopoulos, and Dalakas 2019; Saiz *et al*. 2008). Around 70% of patients have comorbidities, such as late-onset T1DM or other organ-specific autoimmune disorders (Hashimo to thyroiditis, Graves disease, pernicious anaemia, or vitiligo), and 10%–15% have epilepsy (Dalakas *et al*. 2000; Gary 1990; Saiz *et al*. 2008). SPS is diagnosed by recognising the typical clinical and electromyographic features, which requires a high level of suspicion. Stiffness begins in the axial muscles and progresses from the

trunk to the proximal limbs gradually. At first, the symptoms are only present for a short period of time. Sustained co-contraction of paraspinal and abdominal muscles occurs as the condition advances, resulting in lumbar hyperlordosis, a board-like look of the abdominal wall, truncal flexion restriction, and a stiff gait. Intermittent spasms, which can happen in response to a range of stimuli and are frequently associated with severe pain, are superimposed over the muscle rigidity. During sleep, muscle stiffness and spasms lessen or vanish (Blum and Jankovic 1991; Meinck and Thompson 2002) . In SPS, anxiety and task-specific phobia are very common (Henningsen and Meinck 2003). For example, patients may avoid crossing roadways or walking in open spaces, since they are scared of falling or experiencing other issues as a result of their motor limitations. Misdiagnosis as a psychogenic condition can result from these behaviors (Ameli *et al*. 2005; Henningsen and Meinck 2003). A fluctuating pattern of increasing stiffness and spasms restricted to one leg may present in certain patients, leading to permanent rigidity, aberrant posture, and gait impairment. The gait appears to be altered disproportionally in relation to the leg stiffness. Symptoms may be limited to the leg, or they may progress to the trunk and other limbs over time (Brown and Marsden 1999). A paraneoplastic etiology associated with amphiphilic antibodies should be considered if the affected limb is an arm (McKeon, Pittock, and Lennon 2009).

Cerebellar ataxia

A progressive cerebellar ataxia associated with high levels of GAD antibodies was discovered in three patients with polyendocrine autoimmunity in a 1997 investigation (McKeon *et al*. 2009). GAD antibodies were found in only 6 (2%) of 320 patients with sporadic cerebellar ataxia in a prospective analysis of 320 patients with sporadic cerebellar ataxia (Hadjivassiliou *et al*. 2008). Despite this, around 80% of patients with this ailment are women, and symptoms often appear in the fifth decade of life, despite the age range of 33–80 years. Approximately 80% of patients have concurrent organ-specific autoimmune disorders, like as T1DM, thyroiditis, pernicious anaemia, or vitiligo, which typically occur several years before the beginning of ataxia (Andres 2007; Ariño *et al*. 2014). The clinical course of cerebellar ataxia caused by GAD antibodies normally progresses over months or years, however up to 40% of patients have a subacute presentation that lasts weeks to less than six months. One of most frequently symptom is gait ataxia also limb ataxia, dysarthria, and nystagmus. Cerebellar ataxia is characterized by episodes of vertigo that last several months or, less frequently, diplopia or dysarthria (Ariño *et al*. 2014; Baizabal-

Carvallo and Alonso-Juarez 2017). Patients with predominant or isolated opsoclonus, various forms of nystagmus, or palatal tremor (Baizabal-Carvallo and Alonso-Juarez 2018; Markakis *et al*. 2008; Shaikh and Wilmot 2016; Tilikete *et al*. 2005; Vianello *et al*. 2003) may appear. Several years following the onset of cerebellar ataxia, overlap with SPS symptoms, sometimes limited to one leg, occurs in up to 25% of patients (Ariño *et al*. 2014; Rakocevic *et al*. 2006). The cerebellum frequently shows gradual atrophy on MRI (Saiz *et al*. 2008). Other ancillary investigations, aside from the detection of GAD antibodies and CSF oligoclonal bands in 70% of individuals, are negative or normal.

Epilepsy and limbic encephalitis

In 1998, GAD antibodies were linked to drug-refractory temporal lobe epilepsy for the first time (Giometto *et al*. 1998). Only about 200 cases have been recorded since then, most of them in individuals with persistent pharmacoresistant epilepsy involving the temporal lobes and no signs of inflammation in CSF or MRI scans. Patients with this illness are more likely to be female, are younger than those with SPS or cerebellar ataxia (median age 26 years at onset of seizures), and have less autoimmune comorbidities (40 percent of patients) 66–70. Because T2-weighted and/or fluid-attenuated inversion recovery (FLAIR) MRI indicates bilateral or unilateral hyperintensities involving the medial temporal Lobes at the onset of seizures or during the clinical course, some of these individuals are diagnosed with limbic encephalitis (Malter *et al*. 2010). In our opinion, the label limbic encephalitis is unsuitable for these patients, because their state does not meet the clinical and radiological criteria that identify this disorder (Graus *et al*. 2016). Indeed, the term limbic encephalitis was coined to characterise a disease that meets clinical and radiological criteria that are not met by all limbic inflammatory disorders. Patients with actual limbic encephalitis experience subacute confusion, an inability to acquire new memories, and behavioural abnormalities that are accompanied by seizures. Regardless of the MRI findings, patients with severe seizures but little or no memory loss and/or no personality changes should not be diagnosed with limbic encephalitis. The recently developed idea of temporal lobe epilepsy with autoimmune amygdala hypertrophy appears to be more relevant for these patients (Malter *et al*. 2016). This distinction is crucial because limbic encephalitis involves a different work-up (more comprehensive antibody investigations and, in certain cases, a tumour search), as well as a different prognosis (Graus *et al*. 2016). Although a small percentage of patients with GAD antibodies

meet the criteria for limbic encephalitis, this finding could indicate the presence of other, more pathogenically relevant antibodies, such as antibodies to the GABA B receptor, which are associated to all of the criteria for limbic encephalitis (Blanc *et al*. 2009; Markakis *et al*. 2014; Mirabelli-badenier *et al*. 2015; Sharma *et al*. 2012). However, this finding could indicate the existence of additional, more pathogenically relevant antibodies, such as antibodies to the GABA B receptor, which are related to all limbic encephalitis criteria (Boronat 2011).

Paraneoplastic neurological syndromes

The presence of an underlying cancer in patients with GAD antibody-associated neurological disorders is infrequent, and in some cases, it is most likely coincidental. However, patients with SPS or cerebellar ataxia who have a very highly prevalent tumour (for example, breast cancer) as well as T1DM or other autoimmune comorbidities should be examined. Demonstration that cancer cells express GAD is required in this situation to establish a causal relationship between the tumor and GAD autoimmunity (Report 2009). Some patients with SPS have an underlying thymoma, which is likely due to thymomas' propensity for causing autoimmune diseases, frequently with several autoantibodies present (Iwata *et al*. 2006; Vernino *et al*. 2004). In a group of 15 patients with neurological syndromes related to GAD antibodies and thought to be paraneoplastic due to the close temporal relationship between the two diseases, 8 (53%) had antibodies to neuronal surface proteins and 10 (66%) developed neurological syndromes that can be linked to GAD antibodies in rare cases (such as limbic or brainstem encephalitis or opsoclonus–myoclonus syndrome) (Investigation 2015). Lung cancer (six patients) and thymoma (four individuals) were the most common tumors. Age, male sex, the presence of a neurological disease not generally associated with GAD antibodies (that is, not SPS or cerebellar ataxia), and the presence of neuronal cell-surface antibodies all elevated the chance of a paraneoplastic etiology.

GAD 65 antibody and T1DM

GAD65 autoantibodies and its role as biomarker of Type 1 diabetes

The plasticity of interest in GAD over time is matched only by its relevance in human health and the interest it continues to elicit as a tool for predicting or possibly treating diabetes (Towns and Pietropaolo 2012). Another molecule with such critical biochemical relevance in a fundamental pathway of neuronal physiology is difficult to imagine. Its tissue distribution enables the identification of at-risk populations and the development of potential therapeutic approaches, and its role as a self-antigen in T1D enables the regulation of significant

portions of physiology in nerve function and metabolic regulation (Towns and Pietropaolo 2012).

GAD as Clinical Relevance for T1D Diagnosis

Estimation of Risk

Autoantibodies against GAD are a good predictor of autoimmune diabetes risk and development. In the determination of T1D autoantibodies, coupled positivity to GAD65 and other islet autoantigens provides a reliable and accurate predictive use (Achenbach *et al*. 2004; Eisenbarth 2007; Morran *et al*. 2010). Interestingly, the intermolecular increase in antigenic determinants described for GAD in the previous section has an intermolecular counterpart in the spread of islet autoantigenic proteins during autoimmune diabetes development, where the number of autoantibodies against different islet autoantigens generally rises sequentially (Yu *et al*. 2000). While anti-insulin autoantibodies have been reported to be among the first to appear in both animal models and humans, and appear to be the more relevant single autoantibody in the prediction of risk and progression to clinical type 1 diabetes. Anti-GAD autoantibodies are also frequently found at early preclinical stages. Anti-GAD autoantibodies are the most often utilised first screen to assess risk or progression to the insulin-requiring stage of the disease due to their early presence and relative ease of assaying. Furthermore, given the amount of autoantibodies against various islet autoantigens has been demonstrated to correlate with the risk and rate of development to overt diabetes, an increase in intermolecular islet antigen spreading has enormous prognostic value (Brooks-Worrell *et al*. 2001; Krischer *et al*. 2003; Morran *et al*. 2015; Steck *et al*. 2011; Verge *et al*. 1996). As a result, a consensus has emerged from the body of knowledge that has developed from the initial key studies on single and multiple humoral autoimmunity and T1D risk prediction that the identification of a single autoantibody against islet proteins has a modest predictive potential. However, screening for a single autoantibody, such as GAD65 autoantibodies, gives a useful warning sign if the screen is positive, and this single positivity indicates that more islet autoantibody assays are needed to accurately assess risk and forecast T1D development. There are many similar research and various prediction models have been developed. According to some models triple autoantibody positive against distinct islet proteins, for example, is associated with a risk of developing diabetes ranging from 48 to 86 percent over five years and 64 to 86 percent over ten years, according to some models (Achenbach *et al*. 2004; Morran *et al*. 2015). While other models have estimated that the risk of T1D onset with

triple autoantibody positivity approaches 100% after 5 years, other models have estimated that the risk of T1D onset with triple autoantibody positivity approaches 100% after 5 years (Verge *et al*. 1996). Specific procedures for testing for islet autoantibodies have become common place in clinical practice, and they are based on the tests' predictive value as well as their relative methodological accessibility. Apart from this trio of assays as risk determinants, the requirement for easier-to-implement and easier-to-monitor assays has led to T1D autoantibody assays becoming standardized in current clinical practice. As a result, the most often utilized strategy for screening at-risk groups for T1DM is to assess for autoantibodies against the insulin, GAD65, and IA-2 islet antigens (Achenbach *et al*. 2004). Antibody tests against the zinc transporter ZnT8 have lately been suggested as an additional screen (Pietropaolo, Towns, and Eisenbarth 2012). In either instance, GAD is clearly a "common denominator" assay in both strategies for multiple autoantibody testing in order to predict risk and the rate of progression to the insulin-requiring stage in T1D. In fact, in routine clinical health-care practice, GAD autoantibody measurements are the initial assay in the assessment of autoantibody reactivity in T1D. While GAD autoantibodies are a first-line tool for assessing T1D risk, and the combination of GAD and other islet autoantibodies yields a highly accurate predictor of risk, the determination of GAD autoantibodies has some unique clinical features due to its age-related incidence in susceptible populations. Other islet autoantibodies, such as insulin autoantibodies and phogrin, have long been known to be highly accurate T1D risk predictors in children, and the age of insulin autoantibody detection, in particular, has been linked to the age of T1D diagnosis in children (Steck *et al*. 2011). In contrast, the presence of autoantibodies against GAD65 appears to be associated to adult ages, and high GAD65 antibody titers have been linked to longer-term diabetes problems such as retinopathy 15 years after T1D onset (Jensen *et al*. 2011).

Link between Autoimmune neurological disorder and T1DM with immunological mechanisms caused due to antibody

T1DM is a chronic condition that affects adolescences and is becoming more common around the world, particularly among children under the age of five. The condition still has no cure, and therapy goals and recommendations are difficult to come by. Currently, the majority of juvenile patients with T1DM do not meet glycemic control targets, despite extensive care and technological advances in therapy. This can lead to long-term diabetes problems such as nephrological, cardiac, ophthalmological, and neurological difficulties. Unfortunately,

neurological indications, such as neurocognitive and behavioral problems, can appear quickly after the onset of the disease, in infancy and adolescence (Litmanovitch 2015).

Limbic encephalitis is a rare neurological condition that can be challenging to diagnose. Memory loss, temporal lobe seizures, and emotional dysfunction are all symptoms. A 10-year-old girl with type 1 diabetes mellitus presented with seizures, depression, and memory loss in one case. GAD65-mediated limbic encephalitis was diagnosed using cerebral magnetic resonance imaging lesions and elevated GAD65-antibody titers in the cerebrospinal fluid. With clinical improvement, high-dose steroidal therapy was commenced. Following a relapse, a second high-dose steroid treatment was administered, followed by rituximab, which resulted in remission. There was a link discovered between serum GAD65-antibodies levels and symptoms, indicating that GAD65-antibodies titers could be relevant for clinical follow-up and immunotherapy counseling. This report promotes awareness of this critical neurological illness that is linked to type 1 diabetes, emphasizing the significance of early detection and treatment for a better prognosis (Grilo *et al*. 2016).

Role of stem cells in managing autoimmunity due to GAD 65

Immune system immunosuppression is a property of mesenchymal stem cells (MSCs). MSC do not express important costimulatory molecules B7-1, B7-2, CD40, or CD40L, and do not express major histocompatibility complex class II molecules (Zanone *et al*. 2010). The immunosuppressive effect of MSCs is not mediated by apoptosis or increased T cell anergy (Aggarwal and Pittenger 2005; Le Blanc *et al*. 2004). There is substantial evidence that GAD 65 antibodies are linked to the loss of immunological tolerance to self in people with type 1 diabetes. It has been discovered that auto reactive T lymphocytes that detect islet autoantigens have a direct role in disease immunopathogenesis (Arif *et al*. 2004). Given the continuing rise of type 1 diabetes, developing safe and effective techniques of preventing or reversing the disease is a top priority task. MSCs are interesting candidates because of their immunomodulator properties, however their influence on T cells in human type 1 diabetes has yet to be investigated (Zanone *et al*. 2010).

Mesenchymal stem cells have been shown to reduce the expression of lymphocyte activation markers, change the cytokine profile of dendritic cells, T cells, and NK cells, and increase the number of regulatory T cells. In preclinical studies autoimmune encephalomyelitis, shows multiple sclerosis

caused by autoreactive T cells, Mesenchymal stem cells are infused and move to lymphoid organs, where they cluster around T cells, preventing disease. In this situation (Gerdoni *et al*. 2007), MSCs induce peripheral tolerance, lowering both the cellular and humoral arms of the encephalitogenic immune response without causing Trans differentiation into brain cells (Gerdoni *et al*. 2007; Zappia *et al*. 2005). MSCs were found to be capable of suppressing antigen-specific T cell activation by inhibiting IFN gamma production and inducing anti-inflammatory IL-4 production in type 1 diabetic patients' PBMCs (Zanone *et al*. 2010). MSCs may be used to preserve or minimize beta cell loss in people at risk of type 1 diabetes or at the outset of the disease. Given the dangers associated with transplanting multipotent stem cells, secretome derivatives of these cells could be regarded viable practical treatments in regenerative medicine for the treatment of T1DM (Zanone *et al*. 2010).

CONCLUSION

Glutamic acid decarboxylase (GAD65) is a powerful autoantigens that causes beta cell-specific autoimmunity in genetically predisposed people. In people with type 1 diabetes, stiff-person syndrome, and latent autoimmune diabetes, autoantibodies against GAD65 (GADA) are common. GADA in combination with other islet cell autoantibodies or single high-titre GADA predict type 1 diabetes in both first-degree relatives and the general population, and are linked to an enhanced HLA-determined diabetes risk. Although the formation of islet cell antibodies is the first evidence of beta cell autoimmunity, their role in the pathophysiology of type 1 diabetes is still being contested. Because GAD65 can induce significant neurological problems in people with T1DM, it's important to know what level it's at for a better prognosis and treatment.

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