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3G11 expression in CD4⁺ T cell-mediated autoimmunity and immune tolerance

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Abstract

3G11 is a sialylated carbohydrate epitope of the disialoganglioside molecule expressed on mouse CD4⁺ T cells. Recent research showed that 3G11 expression is related to the modulation of T cell function, i.e., 3G11⁺ T cells exhibit anergic/Treg characteristics and efficiently inhibit autoimmunity in the central nervous system. The relationship between 3G11 expression and immune tolerance is summarized in this literature review.

Keywords

3G11; regulatory T cells; T helper cells; autoimmunity; peripheral tolerance

1. Introduction

Multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE), are chronic inflammatory demyelinating diseases resulting from an autoimmune reaction against CNS myelin [1]. The etiology of MS is unknown; however, its symptoms become evident following the infiltration of peripherally activated myelin-reactive T cells into the CNS. Subsequently these cells are presented with myelin antigens and become activated/reactivated, thereby triggering an immunologic cascade that results in myelin damage. This process occurs in the initial phase of the disease and continues to some extent during the chronic and relapse phases [1–3]. In the CNS, autoreactive T cells and antigen presenting cells (APCs), including activated microglia and astrocytes, as well as infiltrating macrophages and dendritic cells, produce proinflammatory cytokines IFN- γ , TNF- α , IL-17, and IL-23, and promote cell-mediated immunity in the CNS [4–6]. Conversely, immunoregulatory cytokines IL-4, IL-10, TGF- β and IL-27 may be protective [6–9]. In spite of extensive research to develop pharmacotherapeutic agents to reduce myelin damage, only a few therapies are available, e.g., IFN- β , glatiramer acetate (GA) and mitoxantrone, all with potential side effects and only mild to moderate efficacy [10,11].

Regulatory T cells (Tregs) have been defined as CD4⁺ T cells that inhibit immunopathology or autoimmune disease *in vivo* [12,13]. Thus far two major Treg populations have been

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described: naturally occurring CD4⁺CD25⁺ Tregs (nTregs) and adaptive (induced) Tregs (iTregs). Naturally occurring CD4⁺CD25⁺ Tregs are generated in the thymus and represent 5–10% of CD4⁺ T lymphocytes in healthy adult mice and humans. These cells constitutively express the forkhead/winged helix transcription factor Foxp3, and have a specialized role in controlling both the innate and the adaptive immune system [14,15]. It has also been found that CD4⁺CD25⁺ Tregs can be converted from CD4⁺CD25⁻ cells *in vitro* [9,16] and *in vivo* [17]. Tregs play an important role in immune tolerance and anti-inflammation [18–22].

Immune tolerance can be divided into central tolerance and peripheral tolerance [23]. Auto-reactive T cells that target self-antigen can be depleted through negative selection in the thymus so that autoimmune responses induced by auto-reactive T cells can be avoided [24]. Nevertheless, some auto-reactive T cells can migrate to the local environment. The activity of these T cells can be inhibited by antigen presenting cells such as dendritic cells (DCs) and by Tregs, resulting in peripheral tolerance [24–29].

The molecular mechanisms of immune tolerance have not been fully elucidated. It is known that certain co-stimulatory molecules expressed on DCs, such as CD80, CD86 and other B7-family members including B7-H1, -H2, -H3, -H4 and B7-DC, can bind to specific ligands expressed on T cells and lead to apoptosis of auto-reactive T cells [30,31]. In addition, Tregs can secrete anti-inflammatory cytokines such as IL-10 to induce T cell anergy [29,32,33]. However, there are few markers to identify anergic T cells. It has been reported that 3G11 is a molecule whose expression is related to induction of T cell anergy.

2.3G11 expression on T cells

The 3G11 antigen, originally defined by the monoclonal antibody SM3G11, is a molecule that has been identified as a sialylated carbohydrate antigen expressed on the disialoganglioside, IV(NeuAc)2-GgOse4Cer [34]. The 3G11 molecule is expressed on both thymocytes and peripheral T cells, predominantly on the membranes of CD4⁺ T cells, but is not found on non-T cells [35]. 3G11⁺ T cells stimulated by mitogen produced a large amount of IL-2. In contrast, 3G11⁻ T cells did not produce IL-2; they were enriched for a CD45RB^{lo/-}, L-selectin⁻ and CD44^{hi} phenotype as compared to the 3G11⁺ subsets, indicating an activated/ memory T cell phenotype, and were capable of IL-4 production upon anti-CD3 stimulation [36]. Thus, we hypothesized that the level of 3G11 expression might reflect the functional state of CD4⁺ T cells in autoimmune disease, and could be a cell surface marker for the anergic state. In our previous studies we tested this possibility in autoantigen-induced i.v. tolerance in MBP TCR transgenic mice with EAE. Our data suggest that 3G11 expression can be downregulated by the induction of tolerance, and that loss of the 3G11 molecule on autoantigen-reactive CD4⁺ T cells represents an anergic/Treg state of these cells. These data encourage us to further characterize 3G11⁻ T cells, to study their role in tolerance, and to explore the possibility that 3G11⁻ T cells might be a unique cell population of anergic Tregs in autoimmune diseases [37,38].

Greene *et al.* investigated the expression of 6C10 and 3G11, which are surface markers of CD4⁺ T cells. Their results showed that 6C10^{lo}3G11^{lo} CD4⁺ T cells express low levels of CD45RB and CD62L. 6C10⁻3G11^{lo} CD4⁺ T cells may represent an anergic state by an unknown mechanism [39]. Additionally, Weigle *et al.* investigated the difference of CD45RB, PgP-1 and 3G11 expression on CD4⁺ T cells. Their results showed that the majority of CD4⁺ T cells in young mice are 3G11⁺CD45RB^{hi}PgP-1^{lo} phenotype. Reduction of 3G11 expression is related to a low level of CD45RB expression and a high level of PgP-1 expression. In contrast, the majority of CD4⁺ T cells in aged mice are 3G11^{lo}CD45RB^{lo}PgP-1^{hi}. These results indicate that the function of 3G11⁻ CD4⁺ T cells could be different from that of 3G11⁺ CD4⁺ T cells, given that they produced different

cytokines: 3G11⁺CD4⁺ T cells produced IL-2, while 3G11⁺CD4⁺ T cells generated IL-4. These results suggest that 3G11⁺ and 3G11⁺CD4⁺ T cells may regulate different immune responses mediated by IL-2 and IL-4, respectively [36]. In addition, these results are consistent with those of Hayakawa *et al.*, showing that IL-4 was produced by a 3G11⁺ subset with a CD45RB⁺, lectin⁺ and CD44^{hi} phenotype and that IL-4 producing 3G11⁺CD4⁺ T cells were not typical Th2 cells [34,36].

3.3G11⁺ Tregs can be induced by intravenous (i.v.) tolerance

Zhang *et al.* recently defined the correlation between 3G11 expression on MBP-specific T cells and i.v. tolerance induction against EAE. Mice transgenic for a TCR specific for the Ac1-11 peptide of myelin basic protein (MBP₁₋₁₁) immunized to induce EAE and MBP₁₋₁₁ peptide were i.v. injected to induce tolerance. While the clinical disease was significantly suppressed, 3G11 expression on CD4⁺19G⁺ cells (MBP-reactive T cells) was dramatically decreased in central and peripheral immune organs and the CNS. In lymph nodes, 72.2% of CD4⁺19G⁺ cells of PBS-i.v. mice expressed 3G11, whereas this percentage was 31.5 in MBP₁₋₁₁-i.v. mice. Similar results were shown in spleen and spinal cord. In the thymus, a similar level of 3G11 expression was found at the CD4⁺8⁺ stage, and 3G11 expression was dramatically down-regulated on CD4⁺8⁺ cells (70.6% in PBS i. v. mice vs. 12.7% in MBP₁₋₁₁-i.v. mice). 3G11⁺CD4⁺ T cells can secrete large amounts of IL-10, but cannot produce IL-2 and interferon-gamma (IFN- γ). In contrast, 3G11⁺CD4⁺ T cells produced IL-2 and IFN- γ , but did not secrete IL-10. Moreover, 3G11⁺CD4⁺ T cells inhibited proliferation of antigen-reactive T cells, which play an important role in EAE induction. In contrast, 3G11⁺CD4⁺ T cells did not affect proliferation of effector CD4⁺ T cells. The results of adoptive transfer studies showed that 3G11⁺CD4⁺ T cells suppressed EAE induction while transfer of 3G11⁺CD4⁺ T cells had no effect on inhibition of EAE development. These results suggest that 3G11⁺CD4⁺ T cells can be used in immunotherapy in for EAE in future clinical trials [37].

4. Correlation among naturally occurring 3G11⁺, Foxp3, and CD4⁺CD25⁺ nTregs

To test the hypothesis that 3G11⁺ T cells represent a naturally occurring Treg (nTreg) population, Zhao *et al.* [37,38] determined Foxp3 expression on 3G11⁺ T cells vs. 3G11⁺ T cells prepared from spleen of naïve C57BL/6 mice. While only $4.1 \pm 0.5\%$ of 3G11⁺CD4⁺ T cells expressed Foxp3, $34.9 \pm 1.5\%$ of 3G11⁺CD4⁺ T cells were Foxp3⁺. These cells produced a large amount of IL-10 and significantly suppressed proliferative responses of effector T cells *in vitro*. These results indicate that the 3G11⁺ population is enriched in Foxp3⁺ Treg cells.

Furthermore, the lack of 3G11 expression overlaps to a great extent with CD25 (CD25⁺3G11⁺) expression. While only 9.5% of CD25⁺ T cells were 3G11⁺, in CD25⁺ T cells the percentage of 3G11⁺ was 54.4. Similarly, only 3.5% of 3G11⁺ T cells were CD25⁺ but 29.4% of 3G11⁺ T cells were CD25⁺. The highest percentage of Foxp3-expressing cells was found in CD25⁺3G11⁺ T cells (nTregs) and the lowest percentage in CD25⁺3G11⁺ cells, indicating effector T cells. An intermediate percentage of Foxp3⁺ cells was observed in CD25⁺3G11⁺ and CD25⁺3G11⁺ cells, both of which exhibited lower immunoregulatory capacity than CD25⁺3G11⁺ T cells. As expected, CD25⁺3G11⁺ T cells expressed the highest levels of Foxp3 and IL-10 and most efficiently inhibited mitogenic and antigen-specific immune responses *in vitro* and clinical EAE *in vivo*. They concluded that 3G11⁺, combined with CD25 expression (CD25⁺3G11⁺), defines a cell surface phenotype of a highly potent Treg subpopulation [37,38].

5. Targeting 3G11 on CD4⁺ T cells alters the balance between Treg and Th17 cells and suppresses EAE

Given that 3G11⁺ T cells play an important role in inflammatory responses, anti-3G11 mAb was used to inhibit 3G11⁺ T cell-mediated autoimmune disease. For example, anti-3G11 mAb has been shown to inhibit induction of EAE at an early stage and to suppress disease development at a later stage. Also, the number of effector CD4⁺ T cells that can induce EAE was decreased after injection of anti-3G11 mAb *in vivo*. Interestingly, the results showed that there was an increase in 3G11[−] CD4⁺ T cells, but a decrease in 3G11⁺CD4⁺ T cells. Moreover, proliferation assays indicated that MOG-primed T lymphocyte proliferative responses were significantly inhibited after treatment with anti-3G11 antibody, leading to MOG-primed T lymphocyte apoptosis. In addition, Th1 and Th17-associated cytokine secretion was also suppressed by anti-3G11 antibody. These results suggest that anti-3G11 antibody can block effector T cell generation and their immune function *in vivo* [40].

Interestingly, anti-3G11 antibody does not suppress EAE in IL-10^{−/−} mice [40], which suggests that anti-3G11 antibody treatment-induced inhibition of EAE is IL-10-dependent; however, the mechanism has not been fully elucidated. Experimental results showed that only CD4⁺CD25⁺FoxP3⁺IL-10⁺3G11[−] Tregs inhibited EAE induction *in vivo* while CD4⁺3G11⁺ T cells did not protect mice from EAE, demonstrating that CD4⁺3G11⁺ T cells are effectors and CD4⁺3G11[−] T cells are Tregs with CD25 and FoxP3 positive expression. Furthermore, the results showed that the lack of 3G11 expression is a biological feature of anergic T cells, suggesting that this may be a useful marker of anergic/Treg subpopulations *in vivo* [37,38].

6. Conclusion

In summary, the above studies indicated that loss of 3G11 expression on CD4⁺ T cells might be a novel marker for Tregs. While a positive correlation has been observed between 3G11[−] and CD25⁺Foxp3⁺ Tregs, the relationship between this marker and other possible markers of Tregs (Table 1) remains to be defined. It is also necessary to undertake further studies to investigate the molecular mechanisms of 3G11 that regulate T cell activation *in vivo* so that an immunotherapy using CD4⁺3G11[−] Tregs to target autoimmune diseases can be developed in the future.

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Table 1Surface markers of regulatory CD4⁺ T cells

Surface markers	Expression /function	References
CD25	IL-2 receptor α chain that is expressed on naturally occurring Tregs and activated T lymphocytes.	[41–44]
CD127	A type I cytokine receptor that interacts with CD132 to form IL-7 receptor; its expression is down-regulated on CD4 ⁺ CD25 ⁺ Tregs.	[45–49]
CD122	IL-2 receptor β chain that interacts with CD25 and CD132 to form high affinity of IL-2 receptor.	[50–53]
3G11	A sialylated carbohydrate antigen that is primarily expressed on CD4 ⁺ T cells. Loss of 3G11 on CD4 ⁺ T cells leads to an anergic/Treg phenotype.	[35,37,38,40]
Glucocorticoid-induced TNFR-related (GITR) protein	One of the TNF receptor super-family members; expressed on CD4 ⁺ CD25 ⁺ Tregs and is a negative regulator for inhibitory function of CD4 ⁺ CD25 ⁺ Tregs.	[54–58].
Latency-associated peptide (LAP)	The N-terminal propeptide of TGF- β precursor peptide that is expressed on the surface of activated Tregs; binds to TGF- β ₁ and then secretes as a complex of TGF- β and LAP.	[59–62]
CD152	A member of the immunoglobulin superfamily; is expressed on activated T cells; plays an important role in induction and maintenance of immune tolerance as a negative regulator of T cell-mediated immune responses.	[63–67]
Glycoprotein A repetitions predominant (GARP)	A transmembrane protein that is expressed on the surface of activated Tregs; plays an important role in trafficking of TGF- β to plasma membrane of Tregs.	[68–72]