Semaphorin3A: A potential therapeutic tool in immune-mediated diseases

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Abstract

The significance of semaphorin3A (sema3A) in regulating immune-mediated inflammation is widely reported. There are multiple mechanisms involved in the process of sema3A-mediated regulation. One of them is the ability of sema3A to maintain a sufficient regulation of both T-cell and B-cell activation. Because it is involved in the pathogenesis of many autoimmune, infectious, and malignant diseases, sema3A turns to be a promising therapeutic tool to be studied and applied in these diseases.

Keywords: Semaphorin 3A, immune mediated diseases, atopic diseases

Introduction

Semaphorins are a large family of secreted and membrane-bound proteins, characterized by a homologous cysteine-rich domain of approximately 500 amino acids in an N-terminal extracellular domain, called the semaphorin domain. Classes 2 and 3 are secreted proteins, whereas classes 1, 4, 5, and 6 are membrane bound (1, 2). In addition to being involved in tumor angiogenesis, metastasis, and tumor survival, semaphorins are continuously reported to be important regulators of immune-mediated responses, thus also called immune semaphorins. They are expressed on most immune cells, such as T, B, and macrophages. Semaphorins were shown to be involved in all phases of both normal and pathological immune responses (3-5). In the last decade, semaphorin3A (sema3A) was recognized as one of the most active semaphorins in the modulation of inflammatory conditions, therefore receiving special attention. The expression of sema3A and its receptors, neuropilin-1 (NP-1), neuropilin-2 (NP-2), and plexins, were found to be increased on differentiating macrophages and activated T cells, suggesting that they may play the key role in immune-mediated diseases (5, 6).

When sema3A was added to co-culture of dendritic and T cells, it significantly inhibited allogeneic T-cell proliferation. In addition, by acting directly on T cells, sema3A blocked anti-CD3/CD28-induced T-cell proliferation. In contrast, when endogenous sema3A was neutralized by blocking antibodies, one could see the reverse in T-cell proliferation, suggesting that the inhibition of T-cell proliferation is sema3A dependent. Similarly, sema3A was also found to be involved in the regulation of human thymocyte migration and proven to inhibit triggered chemotaxis by CXCL12 (7, 8). Of the many immune-regulatory functions of sema3A, one should mention its involvement in the maintenance of self-tolerance. Semaphorin3A triggers a pro-apoptotic process by sensitizing leukemic T cells to Fas (CD95)-mediated apoptosis. It provokes Fas translocation into lipid raft micro domains before binding with agonistic antibody or FasL (CD95L). Disruption of lipid rafts reduces sensitivity to Fas-mediated apoptosis in the presence of sema3A. The present study emphasizes the role of sema3A in controlling Fas-mediated apoptosis and the prevention of immune-mediated inflammation (9). Because of its above-noted characteristics, sema3A can be used in the treatment of immune-mediated diseases. Below, we discuss sema3A involvement in specific immune-mediated diseases (Figure 1).

Semaphorin3A and rheumatoid arthritis (RA)

The levels of sema3A (a repellent of sympathetic nerve fibers) were found to be increased in the synovial tissue of patients with RA and in association with synovial damage (10). Taking into consideration the regulatory function of sema3A, researchers looked to sema3A as a good candidate in treating RA. In a study by M. Catalano, the effect of injected sema3A was examined in a mouse model of collagen-induced arthritis (CIA). The administration of plasmid DNA encoding sema3A significantly reduced the incidence, disease severity, and articular damage when compared with mice injected with empty plasmid. On the one hand, sema3A reduced anti-collagen IgG levels and decreased the release of relevant pro-inflamma-
Sema3A is a unique regulatory molecule playing a role in the management of rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), systemic sclerosis (SSc), and allergic rhinitis (AR).

**Figure 1.** Semaphorin 3A is a unique regulatory molecule playing a role in the management of rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), systemic sclerosis (SSc), and allergic rhinitis (AR).

**Table 1.** The expression of sema3A in serum and regulatory cells in immune mediated diseases

| Disease                        | Sema3A in Serum | Sema3A in Treg Cells | Sema3A in Breg Cells |
|--------------------------------|-----------------|----------------------|---------------------|
| Rheumatoid arthritis          | Reduced         | Reduced              | ND                  |
| Systemic lupus erythematosus  | Reduced         | Equal to normal controls | Reduced         |
| Familial Mediterranean fever  | Reduced during attacks | Reduced during attacks | Reduced during attacks |
| Systemic sclerosis            | Reduced         | Reduced              | Reduced             |
| Inflammatory bowel disease    | Equal to normal controls | Reduced during attacks | ND                  |
| Atopic dermatitis             | ND              | ND                   | ND                  |
| Atopic diseases               | ND              | ND                   | ND                  |

The expression of sema3A in serum and regulatory cells in immune mediated diseases was evaluated. The expression of sema3A on B regulatory (Breg) cells, namely CD19+CD25+ and NP-1+, was analyzed. As expected sema3A expression on these cells was significantly lower in SLE patients compared to B cells from normal individuals, suggesting this to be in part responsible for B-cell auto-reactivity in SLE. Our other finding was the down-regulation of NP-1 expression on B cells from SLE patients, suggesting that both sema3A and NP-1 are essential in the process of regulation autoimmunity in SLE. This increased expression was found to be in positive association with SLE disease severity and with anti-dsDNA antibody production. Considering sema3A to be an important regulator in autoimmune diseases, we analyzed the possibility of lowering TLR-9 expression on B cells by co-culturing them with sema3A. We found that the addition of sema3A to activated B cells of SLE patients decreased the expression of TLR-9, strengthening the idea of using sema3A as a therapeutic agent for SLE (13). The expression of CD72 (a regulatory molecule) on B cells is critical in the process of regulating/ suppressing B-cell over-activity. The ligation of CD72 on B cells was found to down-regulate B-cell receptor-related signaling, thereby maintaining self-tolerance (14). This is in line with previous reports of CD72 expression on activated B cells from SLE patients.

Real-time polymerase chain reaction (qPCR). The protein expression of sema3A in synovial lining cells was decreased in RA tissues compared with that in OA samples. Sema3A mRNA levels correlated with the inflammation score, namely with the extent of lymphocyte infiltration (R=0.50, p=0.004). Thus, the correlation of sema3A expression in RA synovial tissues may become a therapeutic target in RA (12).

**Semaphorin 3A and systemic lupus erythematosus (SLE)**

Hypothesizing that sema3A may have a similar role in SLE, we assessed its serum levels in SLE patients. Sema3A serum levels in SLE patients were found to be significantly lower than in RA patients (55.04±16.30 ng/mL vs. 65.54±14.82 ng/mL, p=0.018) and lower yet than in normal individuals (55.04±16.30 ng/mL vs. 74.41±17.60 ng/mL, p<0.0001). Serum sema3A levels in SLE patients were low in negative correlation with SLE disease activity, renal damage, and the presence of relevant autoantibodies (R=−0.89, p<0.0001) (13). Being the source of autoantibodies and pro-inflammatory cytokines, the regulatory status of B cells was evaluated. The expression of sema3A on B regulatory (Breg) cells, namely CD19+CD25+ and NP-1+, was analyzed. As expected sema3A expression on these cells was significantly lower in SLE patients compared to B cells from normal individuals, suggesting this to be in part responsible for B-cell auto-reactivity in SLE. Our other finding was the down-regulation of NP-1 expression on B cells from SLE patients, suggesting that both sema3A and NP-1 are essential in the process of regulation autoimmunity in SLE (13). Auto-reactive B cells in SLE are characterized by the over-expression of TLR-9 and increased production of IL-6 and IFN-gamma. This increased expression was found to be in positive association with SLE disease severity and with anti-dsDNA antibody production. Considering sema3A to be an important regulator in autoimmune diseases, we analyzed the possibility of lowering TLR-9 expression on B cells by co-culturing them with sema3A. We found that the addition of sema3A to activated B cells of SLE patients decreased the expression of TLR-9, strengthening the idea of using sema3A as a therapeutic agent for SLE (13). The expression of CD72 (a regulatory molecule) on B cells is critical in the process of regulating/ suppressing B-cell over-activity. The ligation of CD72 on B cells was found to down-regulate B-cell receptor-related signaling, thereby maintaining self-tolerance (14). This is in line with previous reports of CD72 expression on activated B cells from SLE patients.

**Semaphorin 3A and other rheumatic diseases**

The possible involvement of Treg and Breg cells in the pathogenesis of familial Mediterranean fever (FMF) was previously suggested. Being a marker of both Treg and Breg cells, sol-
Sema3A a treatment for immune mediated diseases

Semaphorin3A and inflammatory bowel disease (IBD)
The possible involvement of semaphorins in peripheral immune responses and bowel tissue inflammation of patients suffering from Crohn’s disease (CD) and ulcerative colitis (UC) was also evaluated. Serum levels of sema3A and sema4A, their expression on Treg cells, and their tissue expression in bowel biopsies were assessed in CD, UC, acute diverticulitis (a disease control), and in healthy individuals as normal controls. The percentage (%) of sema3A expressing Treg cells with active CD (64.5±14.49%) and active UC (49.8±16.45%) was significantly lower compared to that of healthy controls (88.7±3.6%) p<0.001 and 0.0001, respectively. This finding was observed to be in negative correlation with CD activity. Serum levels of sema4A were significantly lower in patients with CD and UC compared to that of both control groups. In addition, sema4A was highly expressed in lymphocytes of lamina propria of CD and UC patients, but was absent in patients in acute diverticulitis or in normal individuals. An altered percentage of sema3A expressing Treg cells in patients with inflammatory bowel disease (IBD) is suggested to be partially responsible for their failure in suppressing T-cell-induced inflammation in IBD, and therefore sema3A could become a therapeutic candidate for improving immune-mediated bowel inflammation. Sema4A expression was found to be increased in bowel biopsies of CD and UC, but not in normal bowel tissues. Accordingly, it was suggested that it acts as a regulatory factor in preventing local tissue inflammation in IBD.

Semaphorin3A in atopic dermatitis (AD)
The involvement of regulatory cells such as Treg cells in allergic rhinitis (AR) and asthma is well recorded, making sema3A a regulatory molecule and a good candidate to be assessed in atopic diseases (19). Sema3A, previously shown to restrict innervation of sensory neurons, was presumed to play a role in the pathogenesis of AR. Namely it was hypothesized that an alteration in the expression of sema3A in the nasal mucosa might contribute to the hyper-innervation of nasal neurons that we see in nasal epithelium. Indeed, the expression of sema3A in the nasal epithelium of ovalbumin-sensitized AR in a mouse model was found to be significantly decreased. This was observed to be in association with sneezing and nasal rubbing in this murine model, along with an increased nerve fiber density in the lamina propria of the turbinate. Furthermore, when recombinant sema3A was administered intra-nasally to these mice, sneezing and nasal rubbing symptoms were alleviated. This was an additional study showing evidence that sema3A may provide a novel therapy for AR and other atopic disorders such as conjunctivitis and bronchial asthma (21, 22). Semaphorins are well reported to be negatively correlated with CD activity. Serum levels of sema4A were significantly lower in patients with CD and UC compared to that of both control groups. In addition, sema4A was highly expressed in lymphocytes of lamina propria of CD and UC patients, but was absent in patients in acute diverticulitis or in normal individuals. An altered percentage of sema3A expressing Treg cells in patients with inflammatory bowel disease (IBD) is suggested to be partially responsible for their failure in suppressing T-cell-induced inflammation in IBD, and therefore sema3A could become a therapeutic candidate for improving immune-mediated bowel inflammation. Sema4A expression was found to be increased in bowel biopsies of CD and UC, but not in normal bowel tissues. Accordingly, it was suggested that it acts as a regulatory factor in preventing local tissue inflammation in IBD (18).

The above results are summarized in Table 1.

Conclusion
Semaphorin 3A is well known for its suppressive/regulatory effect on many inflammatory processes. Its low level of expression on both T and B regulatory cells in autoimmune diseases, such as SLE and RA, contributes to the failure of self-tolerance in these diseases. Thus, sema3A should be considered a promising therapeutic tool that could be applied in a wide spectrum of immune-mediated diseases.

Peer review: Externally peer-reviewed.

Author Contributions: Concept - VZ, TE; Design - VZ, TE; Supervision - VZ, TE; Resources - VZ, TE; Materials - VZ, TE; Data Collection and/or Processing - VZ, TE; Analysis and/or Interpretation - VZ, TE; Literature Search - VZ, TE; Writing Manuscript - VZ, TE; Critical Review - VZ, TE.

Conflict of Interest: No conflict of interest was declared by the authors.
Financial Disclosure: The authors declared that this study has received no financial support.

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