Pathogenic role of anti-M3 muscarinic acetylcholine receptor immune response in Sjögren’s syndrome

Takayuki Sumida, Mana Iizuka, Hiromitsu Asashima, Hiroto Tsuboi, Isao Matsumoto

University of Tsukuba, Department of Internal Medicine, Ibaraki, Japan

Correspondence: Takayuki Sumida, university of Tsukuba, Department of Internal Medicine, 1-1-1 Tennodai, Tsukuba City, Ibaraki 305-8575, Japan. tsumida@md.tsukuba.ac.jp

Summary

M3 muscarinic acetylcholine receptor (M3R) is expressed in exocrine glands (e.g., salivary glands [SGs] and lachrymal glands), and plays a crucial role in exocrine secretion. M3R reactive T cells have been detected in circulating mononuclear cells of 40% of patients with Sjögren’s syndrome (SS), and the major T cell epitopes of M3R in those patients with HLA-DR B1 × 0901 are located in the second loop of M3R. Moreover, autoantibodies (autoAbs) against M3R are also present in sera of around 50% of patients with SS, and several B cell epitopes, such as N-region, 1st, 2nd, and 3rd loop of M3R, have been identified. Functional analysis using human SG cell lines showed that autoAbs against the 2nd loop of M3R suppressed intracellular Ca^{2+} influx, suggesting inhibition of saliva secretion. To clarify whether the M3R reactive immune response induces autoimmune sialadenitis (AIS), M3R^{−/−} mice were immunized with M3R synthetic peptides and their splenocytes transferred into Rag1^{−/−} mice. The recipients developed severe sialadenitis, and cell transfer studies indicated that T cells are key factors in the pathogenesis of AIS. These results indicate that the M3R immune reaction plays a key pathogenic role in AIS, suggesting that M3R molecule acts as an autoantigen in the pathogenesis of SS.

T cells and autoantigens (autoAgs) in various organs of patients with Sjögren’s syndrome (SS)

SS is an autoimmune disease characterized by infiltration of lymphocytes into the lachrymal glands (LGs) and the salivary glands (SGs) leading to dry eyes and mouth. Infiltration of the same cells is also found in the kidneys, lungs, thyroid, and liver. Immunohistochemical studies have shown that most infiltrating lymphocytes are CD4^{+}CD8^{−} T cells [1]. The Ag specificity of T cells is governed by the T cell Ag receptor (TCR) expressed on T cells. Thus, the usage of TCR α and TCR β genes have been examined by immunological and molecular biological methods [1–9]. Employing polymerase chain

In this issue

Latest update on the primary Sjögren’s syndrome
P. Youinou and J.D. Pers, Brest, France

Do we need new diagnostic criteria for Sjögren’s syndrome?
T. E. Daniels, San Francisco, USA

Autoantibodies in Sjögren’s syndrome: Clinical Presentation and Regulatory Mechanisms
A.G. Tzioufas et al. Athens, Greece

Pathogenic role of anti-M3 muscarinic acetylcholine receptor immune response in Sjögren’s syndrome
T. Sumida et al. Ibaraki, Japan

Pathophysiological cytokine network in primary Sjögren’s syndrome
J.D. Pers et al. Brest, France

B cell populations and sub-populations in Sjögren’s syndrome
N. Hamza et al. Groningen, The Netherlands

Central and peripheral neurological complications of primary Sjögren syndrome
A.I. Fauchais et al. Limoges, France

Predictors for the development of non-Hodgkin lymphoma in primary Sjögren’s syndrome
M.V. Jonsson et al. Bergen, Norway

Biologic treatments in Sjögren’s syndrome
S. Bowman and F. Barone, Birmingham, United Kingdom
reaction of clones ad lines derived from SG- and LG- eluted T lymphocytes, immunofluorescence staining of tissue sections, or fluorescence-activated cell-sorter (FACS) analysis, several studies have demonstrated the presence of TCR VIβ 2.13 [1,2] and Vα genes on T cells present in the SGs, LGs, kidneys and peripheral blood (PB) of patients with SS, suggesting a preferential selection of TCR genes. Moreover, sequence analysis of the complementary-determining region 3 has unveiled certain conserved amino acid motifs. These observations support the notion that infiltrating T cells recognize relatively few epitopes on the autoAg [1–4]. AutoAgs recognized by T cells that have infiltrated the SGs have been analyzed. Several studies [10–16] have identified various p autoAg candidates, such as Ro/SSA 53 kDa [10,16], α-amylase, heat shock protein, and TCR V β6 [17]. In 2006, we provided evidence for the presence of M3 muscarinic acetylcholine receptor (M3R) reactive T cells in PB of half of the patients with SS [18]. Since M3R is expressed in exocrine glands, such as SGs and LGs, and plays a crucial role in exocrine secretion (figure 1), we focused on M3R as an autoAg recognized by T cells in SS patients.

**M3R-reactive T cells in PB mononuclear cells (PBMCs) from patients with Sjögren’s syndrome**

The second extracellular domain of M3R is an interesting molecule, because it plays an important role in intracellular signaling. The 25-mer synthetic amino acids encoding the second extracellular domain of M3R (KRTVPPGECFIQFLSEPTITFGTAI, AA213-237) were used as the antigen for T cells, and the number of interferon (INF)-γ-producing T cells was counted by FACS using a magnetic activated cell sorting secretion assay. The proportion of INF-γ-producing T cells among PBMCs was high in 40% of SS patients with HLA-DR B1 × 0901 allele [5,6]. This finding indicates that M3R (2nd position) reactive INF-γ-producing autoreactive T cells are definitely present among PBMCs of SS patients, suggesting that M3R reactive T cells are involved in the development of SS.

The 25 mer amino acids (KRTVPPGECFIQFLSEPTITFGTAI, AA213-237) contain anchored motifs that bind to HLA-DR B1 × 0901, indicating that this protein should be one of T-cell epitopes on the M3R molecule [18]. Moreover, we demonstrated previously that VPPGECFIQFLSEPT (M3R 223 I→K) and VPPGECFIAFLESEPT (M3R 224 Q→A) are candidate altered peptide ligands of the second extracellular domain of M3R.

**Auto-M3 muscarinic acetylcholine receptor antibodies (Abs) in Sjögren’s syndrome patients**

Previous studies confirmed the presence of autoAbs against M3R, and suggested the potential pathogenic role of immune reaction to M3R in the development of SS [18]. Robinson et al. [19] demonstrated that human anti-M3R Abs reduce the
secretory function in NOD immunoglobulin-null mice. Moreover, Bacman et al. [20] reported that human anti-M3R Abs against the second extracellular loop of M3R could activate nitric oxide synthetase coupled to the LGs M3R, suggesting that anti-M3R Abs are potential marker of dry eye SS. The M3Rs are expressed in the SGs and the LGs, and are thus potential key receptors involved in the production of saliva and tears after stimulation of acetylcholine. Hence, autoAbs against M3R could interfere with the production of saliva and tears [21]. We reported previously that the prevalence of anti-M3R Abs in adult patients with SS and child-onset SS was 42 to 54% [22,23] (figure 2). Analysis of B cell epitopes recognized by anti-M3R Abs identified several B cell conventional and newly created epitopes including N-terminal, first, second and third loops of M3R molecule, suggestive of epitope spreading [24].

For functional analysis, human SG cell lines were pre-incubated with IgG separated from sera of anti-M3R Ab-positive and -negative SS, and intracellular Ca\(^{2+}\) concentrations \([Ca^{2+}]_i\) measured after loading with Fluor-3 and stimulation with cevimeline hydrochloride. Abs against the 2nd loop positive SS-IgG inhibited the increase in \([Ca^{2+}]_i\) induced by cevimeline, while Abs to the N-terminal positive and to 1st loop increased in \([Ca^{2+}]_i\), and Abs to the 3rd loop had no effects (figure 3). We established [25] two monoclonal anti-M3R Abs (mAbs) against the 2nd loop of M3R in B6 mice immunized by synthetic amino acids encoding 2nd position of M3R [25]. These two anti-M3R2nd mAbs also reduced \([Ca^{2+}]_i\), as demonstrated by in vitro assays. These findings support the notion that the function of anti-M3R Abs is different among B cell epitopes and Abs against 2nd portion of M3R could be involved in the suppression of saliva secretion in SS patients.

**M3 muscarinic acetylcholine receptor-induced autoimmune sialadenitis (AIS)**

To clarify the role of the immune response to M3R in the pathogenesis of SS, M3R\(^{-/-}\) mice were immunized with murine M3R peptides and their splenocytes were inoculated into Rag1\(^{-/-}\) (M3R\(^{-/-}\)→Rag1\(^{-/-}\)) mice [26]. High serum levels of anti-M3R Abs and low saliva volume were detected in M3R\(^{-/-}\)→Rag1\(^{-/-}\) mice. Histological examination showed marked infiltration of mononuclear cells in the salivary glands, and immunohistochemical analysis demonstrated that the majority of these cells were CD4+ T cells with a few B cells and several IFN-\(\gamma\) and interleukin (IL)-17-producing cells (figure 4). Furthermore, apoptotic cells were also present in the SGs. The incidence of AIS was significantly lower in M3R\(^{-/-}\)→xIFN-\(\gamma\)→Rag1\(^{-/-}\) mice than in the controls, suggesting that IFN-\(\gamma\) acts as an effector cytokine in the development of AIS (Iizuka et al., submitted). These histological and immunohistochemical findings in M3R\(^{-/-}\)→Rag1\(^{-/-}\) mice are similar to those in the SGs of patients with SS. We call these mice with SS-like sialadenitis the M3R-induced AIS model.

In another series of experiments, the transfer of CD3+ T cells alone from M3R\(^{-/-}\) mice immunized with M3R peptides into
Figure 3
B cell epitopes on M3 muscarinic acetylcholine receptor (M3R) and function of anti-M3R Abs

Figure 4
Histological and immunohistochemical analyses of salivary glands of M3 muscarinic acetylcholine receptor (M3R)$^{-/-}$→Rag1$^{-/-}$ mice
**Box 1**

**Take-home messages**

- M3R reactive T cells were detected in 40% of patients with SS, suggesting that the M3R immune response might function as an autoantigen recognized by autoreactive T cells in SS.
- AutoAbs against M3R were identified in around 50% of SS patients and Abs to the 2nd loop of M3R lowered [Ca^{2+}], suggesting that certain anti-M3R Abs act as pathogenic Abs.
- In M3R-induced sialadenitis (MIS) mice, CD4+ T cells were essential for the development of sialadenitis, suggesting that M3R reactive T cells play a crucial role in the pathogenesis of SS-like AIS.
- Extrapolation of these studies to human suggests that the M3R immune response could be important in the development of SS.

Rag1−/− mice resulted in cell infiltration and destruction of epithelial cells in the SGs (figure 5). Our preliminary data suggest that the major T cell epitope on M3R might be the 1st domain of M3R, because T cells can recognize the 1st domain of M3R and produce both IFN-γ and IL-17. M3R−/− mice immunized with only the 1st domain of M3R-induced sialadenitis in M3R−/−→Rag2−/− mice (Asashima et al. in preparation), suggesting that M3R reactive T cells play a pathogenic role in the development of AIS like SS.

We conclude that the immune response to M3R plays a crucial role in the pathogenesis of SS-like AIS. Take-home messages are available in box 1.

**Disclosure of interest:** the authors declare that they have no conflicts of interest concerning this article.

**References**


[3] Dwyer E, Itescu S, Winchester R. Characterization of the primary structure of T cell receptor β chains in cells infiltrating the...


