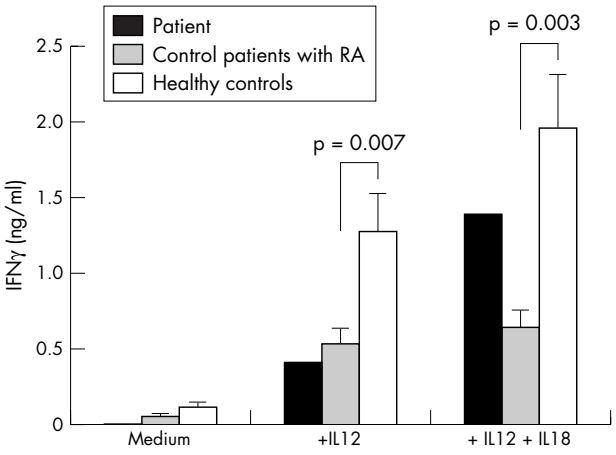

Figure 2 Th1 immune defect in this and other patients with RA. PBMC from the patient, control patients with RA (n = 15), and healthy controls (n = 14) were stimulated with or without 1 ng/ml of IL12 or a combination of IL12 (1 ng/ml) and IL18 (5 ng/ml). IFNγ concentrations in the culture supernatants were measured by an enzyme linked immunosorbent assay (ELISA). p Values compare controls and patients with RA.

The simultaneous occurrence of TB suggests that this patient had a cell mediated immune defect because she developed TB quickly after the initiation of infliximab treatment as for the other anti-TNF related cases.[2] To explore the systemic immune function, a bioassay based on the induction of IFNγ production by interleukin (IL) 12 and/or IL18 used peripheral blood mononuclear cells (PBMC) from this patient, 15 patients with RA, and 14 healthy controls.[6] PBMC from this patient were collected during treatment for TB and CML (September 2002) and were stimulated or not with IL12 (1 ng/ml) and IL18 (5 ng/ml) for 7 days. In comparison with the controls, the patient showed a reduced production of IFNγ in response to IL12 and IL18, which are key factors for a Th1 response (fig 2). This in vitro finding suggests the presence of a systemic immune defect as reflected by an acute TB reactivation. In this particular patient the additional contribution of CML and its treatment has to be considered.

DISCUSSION

Screening for TB, as would be done today in this particular case, should have prevented the severe reactivation seen here. Although such a procedure has been particularly effective, the long term immunosuppressive effects of infliximab are unknown. Infliximab treatment is still too recent for a full assessment of its long term safety to be made, and post-marketing follow up is required to define its long term effect on malignancies, particularly on lymphomas and leukaemias.

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Muscarinic acetylcholine receptor autoantibodies in patients with Sjögren's syndrome

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Sjögren's syndrome (SS) is an autoimmune disease characterised by lymphocytic infiltration into the lachrymal and salivary glands, leading to dry eyes and mouth. Infiltration is also found in the kidneys, lungs, thyroid, and liver. Immunohistochemical studies have shown that most infiltrating lymphocytes around the labial salivary and lachrymal glands, and kidneys are CD4 positive αβ T cells. Previous studies with polymerase chain reaction provide evidence about the T cell receptor Vβ and Vα genes on these T cells, and sequence analysis of the CDR3 region indicates some conserved amino acid motifs, supporting the notion that infiltrating T cells recognise relatively few epitopes on autoantigens.[1]

Candidate autoantigens recognised by T cells infiltrating the labial salivary glands of patients with SS have been analysed, and Ro/SSA 52 kDa, α-amylase, heat shock protein, and T cell receptor BV6[2] have been identified. However, there is no direct evidence that these reactive T cells really attack and destroy the salivary glands. In contrast, the presence of autoantibodies (Abs) against M3 muscarinic acetylcholine receptor (M3R) has been reported, and it is suggested that an immune reaction to M3R plays a crucial part in the generation of SS.[3–5] Robinson, *et al* demonstrated that human anti-M3R Abs reduce the secretory function in NOD.Igμnull mice.[3] Moreover, Bacman *et al* clearly showed that human Abs against the second extracellular loop of M3R could

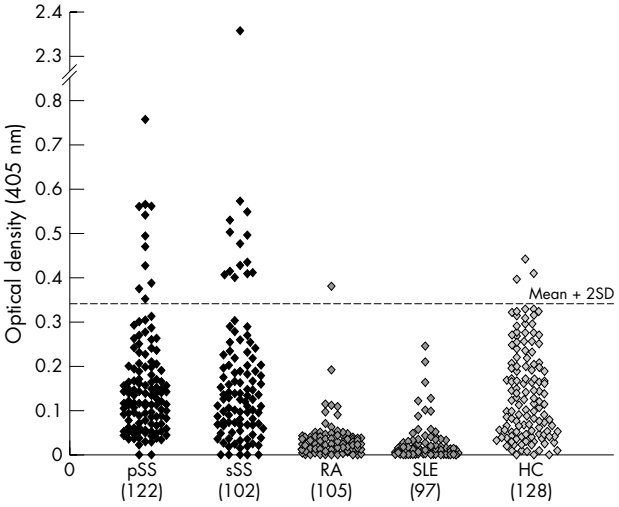

Figure 1 Optical density. pSS, primary Sjögren's syndrome; sSS, secondary Sjögren's syndrome, RA, rheumatoid arthritis, SLE, systemic lupus erythematosus, HC, healthy controls. Numbers in parentheses represent the number of patients in each group.

activate nitric oxide synthase coupled to the lachrymal gland M3R, suggesting that anti-M3R Abs are a new marker of dry eye SS.[4] The M3Rs are expressed on salivary and lachrymal glands, and thus they should be key receptors involved in the production of saliva and tears after stimulation of acetylcholine. Thence, autoantibodies against M3R could interfere with the production of saliva and tears. To test this hypothesis we analysed the prevalence of anti-M3R Abs in patients with SS.

Approval for this study was obtained from the local ethics committee and written informed consent was obtained from all patients and volunteers who participated in this study.

METHODS

Serum samples were collected from 122 Japanese patients with primary SS and 102 Japanese patients with secondary SS followed up at the Department of Internal Medicine, University of Tsukuba Hospital, Japanese Red Cross Mito Hospital, and Shimosizu National Hospital. All patients with SS satisfied the Japanese Ministry of Health criteria for the classification of SS. We also recruited 105 patients with rheumatoid arthritis, 97 with systemic lupus erythematosus, and 128 healthy subjects from our University.

A 25mer peptide (KRTVPPGECFIQFLSEPTITFGTAI) corresponding to the sequence of the second extracellular loop domain of the human M3R was synthesised (Kurabo Industries, Osaka, Japan). As a negative peptide, a 25mer peptide (SGSGSGSGSGSGSGSGSGSGSGS) was also synthesised (Kurabo Industries). Peptide solution (100 µl/well at 10 µg/ml) in 0.1 M Na_2CO_3 buffer, pH 9.6, was adsorbed to a Nunc-Immuno plate (Nalge Nunc International, Rochester, NY) at 4℃ overnight, and blocked with 5% bovine serum albumin (Wako Pure Chemical Industries, Osaka) in phosphate buffered saline (PBS) for 1 hour at 37℃. Serum at 1:50 dilution in blocking buffer was incubated for 2 hours at 37℃. The plates were then washed three times with 0.05% Tween 20 in PBS, and 1 µl of alkaline phosphatase conjugated goat antihuman IgG (Fc; American Qualex, San Clemente, CA) diluted 1:1000 in PBS was added for 1 hour at room temperature. After extensive washing, 100 µl of *p*-nitrophenyl phosphate (Sigma, St Louis, MO) solution (final concentration 1 mg/ml) was added as alkaline phosphatase substrate. Plates were incubated for 1 hour at room temperature and the optical density at 405 nm was measured by plate spectrophotometry (Bio-Rad Laboratories, Hercules, CA; fig 1). Determinations were performed in triplicate and standardised between experiments.

RESULTS AND DISCUSSION

The 25mer synthetic amino acid encoding the second extracellular domain of M3R was used as the antigen, because this portion has an important role in intracellular signalling.[6] The binding activity of Abs to the second extracellular domain of M3R is dependent on the concentration of Abs using serial-diluted quantitative assay (data not shown). Figure 1 shows that Abs against M3R were more commonly detected in the serum of patients with primary (11/122 (9%), $p<0.05$) and secondary SS (14/102 (14%), $p<0.05$) than in those with other autoimmune diseases such as rheumatoid arthritis (1/105 (1%)) and systemic lupus erythematosus (0/97 (0%)), or healthy subjects (3/128 (2%)). These results clearly showed that autoantibodies against M3R are specifically present in SS, suggesting that anti-M3R Abs could be used as a diagnostic marker in a subgroup of patients with SS (9–14%). The proportions of patients positive for anti-M3R Ab and anti-SSA Ab, anti-SSB Ab, rheumatoid factor, and antinuclear factor were 68%, 29%, 57%, and 83%. In contrast, the proportions of patients negative for anti-M3R Ab with these autoantibodies were 65%, 6%, 59%, and 76%, respectively. Thus, anti-SSB Ab is strongly associated with anti-M3R Ab ($p<0.05$), although the homology between SSB and the M3R molecule is very low and the detailed mechanism remain unclear. The clinical feature is not significantly different between in patients with SS positive for anti-M3R Ab and negative patients.

In conclusion, we detected autoantibodies against M3R in a subgroup of patients with SS, suggesting that anti-M3R Ab could be used as a new diagnostic marker for SS. Further experiments on the functional analysis of anti-M3R Abs in SS using M3R transfectant cell lines should shed light on the relationship between the presence of anti-M3R autoantibodies and the pathogenesis of SS.

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