Interleukin-6/STAT pathway is responsible for the induction of gene expression of REG Iα, a new auto-antigen in Sjögren’s syndrome patients, in salivary duct epithelial cells

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ABSTRACT

The regenerating gene, Reg, was originally isolated from a rat regenerating islet cDNA library, and its human homolog was named REG Iα. Recently, we reported that REG Iα mRNA as well as its product were overexpressed in ductal epithelial cells in the minor salivary glands of Sjögren’s syndrome (SS) patients. This study was undertaken to elucidate the role of cytokines and the subsequent intracellular mechanism for induction of REG Iα in the salivary glands of SS patients. We prepared a reporter plasmid containing REG Iα promoter (-1190) + 26 upstream of a luciferase reporter gene. The promoter plasmid was introduced by lipofection into human NS-SV-DC and rat AS salivary ductal cells. The cells were treated with interleukin (IL)-6, IL-8, and a combination of the two. Thereafter transcriptional activity of REG Iα was measured by luciferase assay. We found that IL-6 stimulation, but not IL-8, significantly enhanced the REG Iα promoter activity in salivary ductal cells. Deletion analysis revealed that the region of -141 to -117 of the REG Iα gene was responsible for the promoter activation by IL-6, which contains a consensus sequence for signal transduction and activation of transcription (STAT). The introduction of siRNA on human STAT3 abolished IL-6-induced REG Iα transcription. These results showed that IL-6 stimulation induced REG Iα transcription through STAT3 activation and binding to the consensus sequence of REG Iα promoter in salivary ductal cells. This IL-6/STAT-dependent REG Iα induction might play a role in the pathogenesis of SS.

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1. Introduction

The regenerating gene, Reg, was originally isolated from a rat regenerating islet cDNA library [1,2]. The Reg and Reg-related genes were isolated and revealed to constitute a multigene family, the Reg family, which consists of four subtypes (types I, II, III, and IV) based on the primary structures of the encoded proteins of the genes [2,3]. In humans, five functional REG family genes (REG Iα, REG Iα, REG II, REG III, HIP/PAP, and REG IV) have been isolated [2,3]. REG family gene products act as growth factors and promote cell proliferation and regeneration; therefore, they are considered to be important in various inflammatory diseases [2,3].

It has been reported that REG family gene expression was regulated by several cytokines or chemokines, such as interleukin (IL)-6, IL-8, IL-11, IL-22, interferon (IFN)γ, and cytokine-induced neutrophil chemoattractant-2p [4–10]. IL-6, a pleiotropic proinflammatory cytokine, fulfills its functions by activating Janus kinase (JAK) and subsequent signal transducer and activator of transcription (STAT) [11,12]. STAT plays a key role in transmitting cytokine signals as a transcription factor and in promoting cell proliferation and anti-apoptosis [13–16]. The involvement of STAT signaling in the REG gene family expression in gastrointestinal epithelial cells has also been reported [8,9,17].

Sjögren’s syndrome (SS) is a chronic autoimmune disease characterized by inflammation of the salivary and lacrimal glands; local or systemic overexpression of proinflammatory cytokines is involved in its pathogenesis [11,18–21]. It has been reported that proinflammatory cytokines such as IFNα, IFNγ, tumor necrosis factor (TNF)α, IL-12 and IL-18, along with other cytokines important in T and B cell activation and autoantibody production, such
as IL-6 and B cell activating factor (BAFF), are overexpressed in exocrine glands (such as salivary and lacrimal glands). Recently, we reported that REG1α mRNA and its product (REG1α protein) were overexpressed in ductal epithelial cells in the minor salivary glands (MSGs) of SS patients [22]. Furthermore, auto-antibodies against REG1α were found in SS patients and the anti-REG1α auto-antibody positive patients exhibited significantly lower saliva secretion than the auto-antibody negative patients [22]. We also showed that the mRNA levels of IL-6 and IL-8 were significantly higher in the SS MSGs than those in normal MSGs [22], suggesting that those cytokines might be involved in the overexpression of REG1α mRNA in the SS MSGs. However, the precise mechanism by which REG1α gene activation occurs has been elusive. This study was undertaken to elucidate the role of cytokines and the subsequent intracellular mechanism of the induction of REG1α mRNA in the salivary ductal cells of SS patients.

2. Materials and methods

2.1. Cell culture

To investigate why/how REG1α expression is induced in the salivary duct epithelial cells of SS patients [7], we searched salivary duct epithelial cells and found two available cell lines: NS-SV-DC and A5 cells. The NS-SV-DC cells, simian virus 40-immortalized cells derived from human salivary ducts, were kindly provided by Dr. M. Azuma (Department of Oral Medicine, Tokushima University School of Dentistry, Tokushima, Japan) and maintained in Keratinocyte SFM (Life Technologies, Carlsbad, CA) [23]. The A5 cells, derived from the salivary ducts of male Fischer 344 weanling rats by treating explanted tissue clumps with 3-methylcholanthrene, were kindly provided by Dr. B.J. Baum (National Institute of Dental and Craniofacial Research, NIH, Bethesda, MD) and maintained in DMEM supplemented with 10% FCS [24,25]. For the stimulation experiments, the cells were treated with 20 ng/mL human IL-6 (Roche, Mannheim, Germany), 200 ng/mL rat IL-6 (Wako Pure Chemical, Osaka, Japan), 100 nM IL-8 (Wako Pure Chemical, Osaka, Japan), 100 nM dexamethasone (Dx; MP Biomedicals, Santa Ana, CA) or combinations thereof.

2.2. Real-time reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA was isolated from the NS-SV-DC and A5 cells with an RNAProtect Cell Mini Kit (Qiagen, Hilden, Germany) as described previously [22,26]. The isolated RNA was reverse transcribed to the cDNA using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) for the real-time PCR template, as described previously [26,27]. The cDNA was subjected to PCR with the following primers: β-actin (NM_001101) sense primer, 5′-GCCAGAAGATGCCAAG-3′ and anti-sense primer, 5′-CAGAGCGGTACAGGATA-3′; REG1α (NM_002909) sense primer 5′-AGGAGAGTGCCACTGACTT-3′ and anti-sense primer 5′-TAGGAGCAGGGGCCACACTG-3′; STAT3 (NM_213662) sense primer, 5′-CAGGATGGCCCAATGAATGAC-3′ and anti-sense primer 5′-CCAGAGGATTATGGAACAC-3′. All the PCR primers were synthesized by NGRl (Sendai, Japan). Real-time PCR was performed using KAPA SYBR Fast qPCR Master Mix (Kapa Biosystems, Boston, MA) and a Thermal Cycler Dice Real Time System (Takara, Otsu, Japan), as described previously [22,26]. PCR was performed with an initial step of 3 min at 95 °C followed by 40 cycles of 3 s at 95 °C and 20 s at 60 °C for β-actin, and 40 cycles of 3 s at 95 °C and 20 s at 64 °C for REG1α. The level of REG1α mRNA level was normalized to the mRNA level of β-actin as an internal standard.

2.3. Construction of reporter plasmid and luciferase assay

Reporter plasmids were prepared by inserting fragments of human REG1α promoter gene (−1190/+26, −508/+26, −508/+26, −468/+26, −402/+26, −204/+26, −141/+26, and −117/+26) into pGL3-Basic vector (Promega, Madison, WI). NS-SV-DC and A5 cells were seeded in a 24-well plate at 1 × 10^5 cells per well, and promoter plasmids were transfected into those cells using Lipofectamine™ 2000 (Life Technologies) [26,28]. Six hours after transfection, the medium of each well was replaced with fresh medium containing various stimulants, such as IL-6 or IL-8, and incubated for an additional 24 h. The cells were harvested and extracts were prepared in Extraction Buffer (0.1 M potassium phosphate, pH 8.8/0.2% Triton X-100; Life Technologies). To monitor transfection efficiency, PCMV-SPORT-β-gal (Life Technologies) was co-transfected in all experiments at a 1:10 dilution. Luciferase activity was measured using a PicaGene luciferase assay system (Toyo-ink, Tokyo, Japan) and was normalized by the β-galactosidase activity as described previously [4,29,30].

2.4. RNA interference (RNAi)

Small interfering RNA (siRNA) directed against human STAT3 was synthesized by NGRl. The sense sequence of siRNA for human STAT3 was 5′-GGACCUUCCUGCUAGAUUt-3′. The Silencer® Select human scrambled siRNA was purchased from Ambion® and used as a control. The transfection of siRNA into NS-SV-DC cells was carried out using Lipofectamine® RNAiMAX Transfection Reagent (Life Technologies). The cells were transfected with 5 pmol each of siRNA in a 24-well culture dish as described previously [31].

2.5. Data analysis

All values were expressed as the mean ± SEM. The data were analyzed by unpaired two-tailed t-test using GraphPad Prism (GraphPad Software, La Jolla, CA). P value of < 0.05 was considered to be statistically significant.

3. Results

3.1. Induction of REG1α mRNA by IL-6

We reported previously that the mRNA levels of REG1α, IL-6 and IL-8 in the MSGs of SS patients increased significantly [22]. To investigate whether IL-6 or IL-8 up-regulate REG1α, we by real-time RT-PCR to analyze the REG1α mRNA expression in human NS-SV-DC salivary ductal cells. Treatment with IL-6 but not IL-8 or Dx,
induced REG la mRNA expression (Fig. 1). The combination of IL-6+Dx or IL-6+IL-8 had no additional effect compared to IL-6 alone. These results indicate that salivary ductal cells express REG la mRNA in response to IL-6 stimulation.

3.2. Activation of REG la gene promoter by IL-6

To determine whether the increase in REG la mRNA was caused by the activation of transcription, a 1216-bp fragment containing 1190-bp of the promoter region of the human REG la gene was fused to the luciferase gene and transfected into human NS-SV-DC and A5 rat salivary ductal cells. We found that IL-6 stimulation significantly enhanced REG la promoter activity in both the NS-SV-DC and A5 cells. However, treatment with IL-8 did not change the transcriptional activity of REG la (Fig. 2). These results suggest that REG la mRNA was induced by IL-6 in salivary ductal cells at the transcriptional level.

3.3. Localization of IL-6-responsive region in the REG la gene promoter

In order to identify the region essential for the transcription of the REG la gene by IL-6, progressive deletions of the REG la promoter were performed. Down to position −141, the deletions did not attenuate IL-6-induced REG la promoter activity; however, an additional deletion to −117 caused a remarkable decrease in IL-6-induced promoter activity of REG la (Fig. 3). These results indicated that the −141 to −117 promoter region of the REG la gene is responsible for REG la promoter activation by IL-6.

3.4. STAT3 is a key factor for REG1a gene transcription

A computer-aided search for sequences similar to known cis-acting elements revealed that the −141 to −117 region of the REG la gene contains consensus binding sequences for STAT. In order to verify the role of STAT3 in IL-6-induced REG la induction, siRNA for human STAT3 mRNA was introduced into NS-SV-DC cells, and IL-6-induced REG la mRNA expression was analyzed by real-time RT-PCR. As shown in Fig. 4, the introduction of siRNA for human STAT3 abolished both IL-6-induced STAT3 up-regulation and IL-6-induced REG la up-regulation.

4. Discussion

In our previous study, we reported that REG la protein was overexpressed in the ductal epithelial cells of MSGs of SS patients, and that the saliva secretion was attenuated with auto-antibodies to REG la [22]. We also showed that mRNA levels of IL-6 and IL-8 were significantly higher in the SS MSGs than in normal MSGs [22,32]. In the present study we showed that REG la overexpression in salivary ductal cells was induced by IL-6 but not by IL-8 at the transcriptional level.

IL-6, a potent proinflammatory cytokine, is involved in acute phase response, B cell proliferation and plasma cell formation, and T cell stimulation and recruitment [11]. High serum concentrations of IL-6 were found in primary SS (pSS) patients, and the levels of IL-6 concentration correlated with the degree of lymphocyte infiltration.
infiltration in the salivary gland [19,33,34]. Binding of IL-6 to the receptor leads to homodimerization of IL-6 receptor component gp130, which results in the activation of JAK and the subsequent phosphorylation of STAT3 [12]. STAT3 plays a central role in transmitting cytokine signals to the nucleus and promoting cell proliferation and anti-apoptosis [13–16]. Thus, the JAK/STAT pathway has been shown to be involved in carcinogenesis under a background of inflammation. Furthermore, accumulating evidence indicates that the JAK/STAT pathway might be involved in multiple immune functions: STAT1 and STAT4 mainly induce IFNγ expression in Th1 cells, STAT6 induces IL-4 expression in Th2 cells, and STAT3 induces IL-17 expression in Th17 cells [35].

Our results revealed that IL-6 stimulation enhanced REG lα gene expression through STAT3 activation in salivary ductal cells. The involvement of STAT signaling in REG family gene expression has been reported by other groups in different cell systems. Lee et al. reported that the IL-11/STAT3 signaling pathway was important in Helicobacter pylori CagA-directed REG 3γ (HIP/PAP) expression in gastric epithelial cells [8]. Sekikawa et al. showed that REG lα gene expression was regulated by the IL-22/STAT3 pathway in colon cancer cells and by the IL-6/STAT3 pathway in gastric cancer cells [9,17]. Most recently, we showed that REG lα gene expression was activated in human pancreatic β-cells by the combined stimulation of IL-6+Dx via JAK/STAT3 signaling [36]. These studies were implemented in gastrointestinal cell lines and pancreatic β-cells, and to the best of our knowledge, this is the first study to report an association between the IL-6/STAT3 pathway and REG lα expression in salivary ductal epithelial cells.

IL-8, also known as CXCL8, is a proinflammatory chemokine associated with the promotion of neutrophil chemotaxis and degranulation [37]. Several reports have indicated the involvement of IL-8 in the pathogenesis of SS. Cuello et al. reported that the MSG ductal epithelial cells of SS patients highly expressed IL-8 [38]. As mentioned above, IL-8 levels were also reported to be correlated with REG gene expression [7]. In our previous study, we showed that IL-8 mRNA was highly expressed in SS MSGs [22]. In the present study, however, we found that IL-8 did not induce REG lα expression in salivary ductal cells, suggesting that high levels of IL-8 in SS MSGs are not involved in REG lα up-regulation in the MSGs.

The REG l1 gene was originally found to be expressed in rat regenerating islets, but not in normal pancreatic islets [1]. RINm5F, a rat insulinoma-derived cell line, displayed a significant increase in cell numbers in the presence of REG l1/REG lα protein [2,4,26,39]. Human REG lα protein administration ameliorated diabetes in NOD mice, with an increase in the β-cell mass [40]. In addition, anti-REG lα auto-antibodies, found in the sera of diabetic patients, retarded the proliferation of pancreatic β-cells in vitro [39]. These results suggest that REG l1/REG lα protein stimulates the proliferation of pancreatic β-cells. We previously reported that pSS patients with anti-REG antibodies exhibited significantly lower salivary secretion [22], suggesting that IL-6-induced REG lα protein in salivary ductal cells is associated with the regeneration of damaged MSG ductal epithelial cells and that anti-REG auto-antibodies attenuate the proliferation/regeneration.

In the present study, we showed that the REG lα gene was activated by IL-6/STAT3 signaling in salivary duct epithelial cells. The STAT binding element was reported not only in the REG lα promoter but also in other REG family promoters, such as REG lγ, HIP/PAP, and REG lIII promoters [36,41], suggesting possible activation of the genes in the salivary ducts of SS patients through the IL-6/STAT3 axis. In our previous study, however, no REG lγ mRNA was detected either in either the control or the SS salivary glands. The mRNA levels of REG lIII and HIP/PAP were not different between the control and SS salivary glands. In contrast, REG lα mRNA levels were significantly higher in the salivary glands of SS patients than in those of controls [22]. In SS, the involvement of several cytokines other than IL-6, such as BAFF, IL-12/IL-23, and IFNγ, has also been reported [11]. Combination of these cytokines with IL-6 might contribute to REG lα-specific overexpression in the salivary duct epithelial cells of SS patients. As a result, anti-REG lα auto-antibody levels are elevated in SS patients [22] and salivary functions might be affected in SS patients.

Xerostomia, which is the chief manifestation of SS, is caused by salivary gland dysfunction resulting from immune-mediated inflammation. To alleviate xerostomia, treatment with conventional systemic immunosuppressive drugs has been tried as with other immune-mediated connective tissue diseases; however, the therapeutic effects were doubtful [42–45]. Recently, treatments using with monoclonal antibodies that target inflammatory cytokines or cell surface antigens were developed to treat several autoimmune diseases [46]; a monoclonal antibody against the IL-6 receptor exhibited its efficacy in rheumatoid arthritis [47]. As IL-6 has been shown to be highly expressed in the salivary glands of SS patients, blocking IL-6 and/or its receptor might have beneficial effects [19]. Our results, however, suggested that IL-6 was associated with regeneration of ductal epithelial cells via REG lα protein expression. Therefore, blocking IL-6 and/or its receptor might induce unwanted effects. Several trials have shown that rituximab, a chimeric anti-CD20 monoclonal antibody that binds to the B-cell surface antigen CD20, has beneficial effects in treating xerostomia in SS patients [48,49]. Rituximab therapy has also been shown to be effective in rheumatoid arthritis, reducing disease activity and decreasing auto-antibody production [50–52]. It is possible that the beneficial effects of rituximab in the SS patients were associated with B-cell depletion and the subsequent...
decrease in the production of pathogenic auto-antibodies, including anti-REG Iα auto-antibodies.

In conclusion, the present study showed that REG Iα transcription in salivary ductal cells was stimulated by IL-6. Our study also demonstrated that STAT3 bound the consensus sequence of REG Iα promoter and regulated transcription in ductal epithelial cells in response to IL-6 stimulation. It is suggested that overexpression of REG Iα protein in salivary ductal cells is dependent on the IL-6/STAT pathway and might play a role in the pathogenesis of SS.

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Appendix A. Supporting information

Supplemental data associated with this article can be found in the online version at [http://dx.doi.org/10.1016/j.bbrep.2015.05.006](http://dx.doi.org/10.1016/j.bbrep.2015.05.006).

References

[1] K. Terazomo, H. Yamamoto, S. Takasawa, K. Shiga, Y. Yonemura, Y. Tochino, H. Okamoto, A novel gene activated in regenerating islets, J. Biol. Chem. 263 (1988) 2111–2114.

[2] H. Okamoto, S. Takasawa, Recent advances in the Okamoto model: the CD38-cyclic ADP-ribose signal system and the regenerating gene protein (Reg)-Reg receptor system in β-cells, Diabetes 51 (2002) 5462–5473.

[3] Y.W. Zhang, L.S. Ding, M.D. Lai, Reg gene family and human disease, World J. Gastroenterol. 9 (2003) 2653–2661.

[4] T. Akiyama, S. Takasawa, K. Nata, S. Takasawa, K. Nata, S. Kobayashi, M. Abe, N.J. Shervani, T. Ikeda, K. Nata, M. Hatakeyama, L.M. Judd, A.S. Giraud, T.R. Menheniott, H. Seno, T. Fujimori, T. Chiba, Y. Kinoshita, Neutrophil chemoattractant 2 (REG3β) is up-regulated upon TNFα treatment in human intestinal cancer cell lines, Biochem. Biophys. Res. Commun. 397 (2010) 140–145.

[5] A. Yamauchi, I. Takahashi, S. Takasawa, K. Nata, N. Noguchi, T. Ikeda, T. Yoshikawa, N. Jinnai, H. Okamoto, A novel pancreatic protein receptor expressed in pancreatic islets by alternative splicing from type 2 ryanodine receptor gene, Biochem. Biophys. Res. Commun. 370 (2009) 743–748.

[6] H. Tsujinaka, S. Takasawa, H. Okamoto, Prevention of Reg Iα-induced β-cell proliferation by IL-6, Biochem. Biophys. Res. Commun. 404 (2010) 2888–2995.

[7] H. Okamoto, A. Itaya-Hironaka, A. Yamauchi, S. Sakuramoto-Tsuchida, T. Takashashi, T. Yoshikawa, T. Chiba, H. Sato, K. Adachi, R. Fukuda, M. Watanabe, S. Takasawa, H. Okamoto, Regulation of Reg3γ expression in islets from non-obese diabetic mice with β-cell regeneration, J. Molecular Med. 11 (2013) 2387–2397.

[8] K. Nakagawa, S. Takasawa, A. Yamauchi, I. Takahashi, T. Yoshikawa, H. Okamoto, Cyclin D1 activation through ATF-2 in Reg-induced pancreatic β-cell regeneration, FEBS Lett. 580 (2006) 585–591.

[9] K. Nakagawa, S. Takasawa, K. Nata, A. Yamauchi, A. Itaya-Hironaka, H. Okota, K. Yoshimoto, S. Sakuramoto-Tsuchida, T. Takashashi, A. Yamauchi, A. Itaya-Hironaka, H. Okota, H. Sato, K. Adachi, R. Fukuda, M. Watanabe, S. Takasawa, H. Okamoto, Prevention of Reg Iα-induced β-cell proliferation by IL-6/dexamethasone via activation of HGF gene regulation, Biochem. Biophys. Acta 1734 (2005) 295–299.

[10] S. Takasawa, A. Yamauchi, A. Itaya-Hironaka, A. Yamauchi, S. Sakuramoto-Tsuchida, T. Takashashi, T. Yoshikawa, H. Sato, K. Adachi, R. Fukuda, M. Watanabe, S. Takasawa, H. Okamoto, Prevention of Reg Iα-induced β-cell proliferation by IL-6, Biochem. Biophys. Res. Commun. 370 (2009) 749–754.

[11] S. Takasawa, A. Yamauchi, A. Itaya-Hironaka, A. Yamauchi, S. Sakuramoto-Tsuchida, T. Takashashi, T. Yoshikawa, H. Sato, K. Adachi, R. Fukuda, M. Watanabe, S. Takasawa, H. Okamoto, Prevention of Reg Iα-induced β-cell proliferation by IL-6, Biochem. Biophys. Res. Commun. 370 (2009) 749–754.

[12] A. Sekikawa, H. Fukui, K. Suzuki, T. Karibe, S. Fujii, K. Ichikawa, S. Tomita, T. Chiba, T. Fujimori, Involvement of the IL-22/REG Iα axis in ulcerative colitis, Lab. Investig. 90 (2010) 496–505.

[13] A. Sekikawa, H. Fukui, S. Fuji, A. Nanakai, N. Kanda, Y. Uenoyma, T. Sawabu, H. Hisatsune, T. Kusaka, S. Ueno, H. Nakake, H. Seno, T. Fujimori, T. Chiba, Possible role of REG Iα gene in ulcerative colitis and colitis cancer, Gut 54 (2005) 1437–1444.

[14] T. Hiranoe, K. Ishihara, M. Hibi, Roles of STAT3 in mediating the cell growth, differentiation and survival signals relayed through the IL-6 family of cytokine receptors, Oncogene 19 (2000) 2548–2556.

[15] J.I. Song, J.R. Grandis, STAT signaling in head and neck cancer, Oncogene 19 (2000) 2489–2495.

[16] S.O. Rahaman, P.C. Harbor, O. Chernova, G.H. Barnett, M.A. Vogelbaum, S.J. Haque, Inhibition of constitutively active STAT3 suppresses proliferation and induces apoptosis in glioblastoma multiforme cells, Oncogene 21 (2002) 8404–8413.

[17] A. Sekikawa, H. Fukui, S. Fujii, K. Ichikawa, S. Tomita, J. Mitoma, T. Chiba, T. Fujimori, REG Iα protein mediates an anti-apoptotic effect of STAT3 signaling in gastric cancer cells, Carcinogenesis 29 (2008) 76–83.

[18] T. Hirano, K. Ishihara, M. Hibi, Roles of STAT3 in mediating the cell growth, differentiation and survival signals relayed through the IL-6 family of cytokine receptors, Oncogene 19 (2000) 2548–2556.

[19] J. Bromberg, Stat proteins and oncogenesis, J. Clin. Investig. 109 (2002) 1139–1142.

[20] J.I. Song, J.R. Grandis, STAT signaling in head and neck cancer, Oncogene 19 (2000) 2489–2495.
Autoantibodies to REG, a beta-cell regeneration factor, in diabetic patients, Eur. J. Clin. Investig. 34 (2004) 752–758.

[40] D.J. Gross, L. Weiss, I. Reibstein, J. van den Brand, H. Okamoto, A. Clark, S. Slavin, Amelioration of diabetes in nonobese diabetic mice with advanced disease by linomide-induced immunoregulation combined with Reg protein treatment, Endocrinology 139 (1998) 2369–2374.

[41] K. Nata, Y. Liu, L. Xu, T. Ikeda, T. Akiyama, N. Noguchi, S. Kawaguchi, A. Yamauchi, I. Takahashi, N.J. Shervani, T. Onogawa, S. Takasawa, H. Okamoto, Molecular cloning, expression and chromosomal localization of a novel human REG family gene, REG III, Gene 340 (2004) 161–170.

[42] T.R. Reksten, K.A. Brokstad, R. Jonsson, J.G. Brun, M.V. Jonsson, Implications of long-term medication of oral steroids and antimalarial drugs in primary Sjögren’s syndrome, Int. Immunopharmacol. 11 (2011) 2125–2129.

[43] E.J. Price, S.P. Rigby, U. Clancy, P.J. Venables, A double blind placebo controlled trial of azathioprine in the treatment of primary Sjögren’s syndrome, J. Rheumatol. 25 (1996) 896–899.

[44] P.N. Skopouli, P. Jagiello, N. Tsifetaki, H.M. Moutsopoulos, Methotrexate in primary Sjögren’s syndrome, Clin. Exp. Rheumatol. 14 (1996) 555–558.

[45] S. Nakayamada, K. Saito, H. Umehara, N. Ogawa, T. Sumida, S. Ito, S. Minota, H. Nara, H. Kondo, J. Okada, T. Minoni, H. Yoshifui, H. Sano, N. Hashimoto, S. Sugai, Y. Tanaka, Efficacy and safety of mizoribine for the treatment of Sjögren’s syndrome: a multicenter open-label clinical trial, Mod. Rheumatol. 17 (2007) 464–469.

[46] A. Mócsai, L. Kovács, P. Gergely, What is the future of targeted therapy in rheumatology: biologics or small molecules, BMC Med. 12 (2014) 43–51.

[47] J.S. Smolen, A. Beaulieu, A. Rubbert-Roth, C. Ramos-Remus, J. Rovensky, E. Alecock, T. Woodworth, R. Alten, OPTION investigators, Effects of interleukin-6 receptor inhibition with tocilizumab in patients with rheumatoid arthritis (OPTION study): a double-blind, placebo-controlled, randomized trial, Lancet 371 (2008) 987–997.

[48] J.M. Meijer, P.M. Meiners, A. Vissink, F.K. Spijkervet, W. Abdulahad, N. Kamminga, E. Brouwer, C.G. Kallenberg, H. Bootsma, Effectiveness of rituximab treatment in primary Sjögren’s syndrome: a randomized, double-blind, placebo-controlled trial, Arthritis Rheum. 62 (2010) 960–968.

[49] F. Carubbi, P. Cipriani, A. Marrelli, P. Benedetto, P. Ruscitti, O. Berardi, L. Pantano, V. Liakoudi, S. Alvaro, A. Alunno, A. Manzo, F. Cicca, R. Geri, G. Triolo, R. Giacomelli, Efficacy and safety of rituximab treatment in early primary Sjögren’s syndrome: a prospective, multi-center, follow-up study, Arthritis Res. Ther. 15 (2013) R172.

[50] U.B. Cohen, P. Emery, M.W. Greenwald, M. Flodgard, R.A. Furie, M.C. Genovese, E.C. Keystone, E. Loveless, G.R. Burmester, M.W. Cravets, E.W. Hesse, T. Shaw, M.C. Totoritis, REFLEX Trial Group, Rituximab for rheumatoid arthritis refractory to anti-tumor necrosis factor therapy: results of a multicenter, randomized, double-blind, placebo-controlled, phase III trial evaluating primary efficacy and safety at twenty-four weeks, Arthritis Rheum. 54 (2006) 2793–2806.

[51] P.J. Mease, D.A. Revicki, J. Szczechinski, M. Greenwald, A. Kivitz, L. Barile-Fabris, J. Kalji, J. Eames, M. Leirisalo-Repo, Improved health-related quality of life for patients with active rheumatoid arthritis receiving rituximab: results of the dose-ranging assessment: international clinical evaluation of Rituximab in rheumatoid arthritis (DANCER) trial, J. Rheumatol. 35 (2008) 20–30.

[52] A. Vánsza, Z. Szabó, S. Szamosi, N. Bodnár, E. Végh, L. Gergely, G. Szucs, S. Szántó, Z. Sekecz, Longterm effects of rituximab on B cell counts and autoantibody production in rheumatoid arthritis: use of high-sensitivity flow cytometry for more sensitive assessment of B cell depletion, J. Rheumatol. 40 (2013) 565–571.