

Th1/Th2 Immune Balance and Other T Helper Subsets in IgG4-Related Disease

Masafumi Moriyama and Seiji Nakamura

Abstract IgG4-related disease (IgG4-RD) is a systemic disease characterized by elevated serum IgG4 levels and a strong infiltration of IgG4-positive plasma cells in various organs. IgG4-RD patients also frequently suffer from allergic diseases, including asthma and atopic dermatitis. It is well known that T helper type 2 (Th2) cells have an important role in the initiation of allergic diseases, and Th2 cytokines such as interleukin (IL)-4 and IL-13 promote class switching to IgG4. Therefore, IgG4-RD is considered to be a Th2-predominant disease. However, other Th subsets, including regulatory T cells and T follicular helper cells, have recently received increasing attention with regard to the pathogenesis of IgG4-RD. Exploring the interconnected network of Th subsets in IgG4-RD is a highly promising field of investigation. In this review, we focus on the localization and functions of individual Th subsets to clarify the involvement of these cells in the pathogenesis of IgG4-RD.

Contents

1	Introduction.....	1
2	Th1/Th2 Balance	2
3	Other CD4 ⁺ T Helper Subsets	3
3.1	Th17 Cells	3
3.2	Treg Cells	3

Competing interests: The authors declare no competing interests.

M. Moriyama

OCT Research Center, Faculty of Dental Science, Kyushu University, Fukuoka, Japan

S. Nakamura (✉)

Division of Maxillofacial Diagnostic and Surgical Sciences, Section of Oral and Maxillofacial Oncology, Faculty of Dental Science, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

e-mail: seiji@dent.kyushu-u.ac.jp

© Springer International Publishing Switzerland 2016

Current Topics in Microbiology and Immunology

DOI 10.1007/82_2016_40



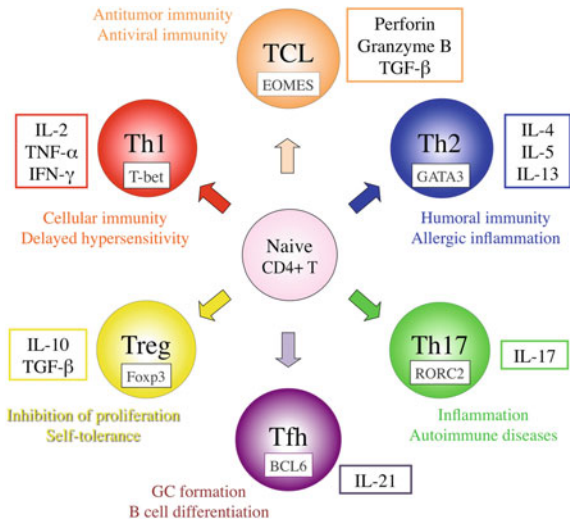
3.3 Tfh Cells
3.4 CD4⁺ CTL Cells.....
4 Conclusions.....
References

1 Introduction

IgG4-related disease (IgG4-RD) is a novel clinical entity characterized by severe fibrosis and marked infiltration of IgG4-positive plasma cells with hyperplastic ectopic germinal centers (GCs) in multiple target organs, including the pancreas (Hamano et al. 2001), bile duct (Zen et al. 2004), kidney (Takeda et al. 2004), lung (Zen et al. 2009), lymph nodes (Sato et al. 2009), thyroid (Dahlgren et al. 2010), retroperitoneum (Hamano et al. 2002), and lacrimal and salivary glands (Yamamoto et al. 2005). IgG4-RD has a wide range of presentations, such as autoimmune pancreatitis (AIP), cholangitis, interstitial nephritis, interstitial pneumonitis, lymphadenopathy, Riedel thyroiditis, retroperitoneal fibrosis, and Mikulicz’s disease.

Notably, IgG4 is a T helper type 2 (Th2)-dependent immunoglobulin and has low affinity for its target antigen. Th2 cytokines such as interleukin (IL)-4 and IL-10 direct naïve human B cell immunoglobulin isotype switching to IgG4 and IgE production (Meiler et al. 2008). As shown in Fig. 1, at least six subsets of CD4⁺ Th cells have been identified to date: Th0, Th1, Th2, Th17, regulatory T (Treg), and follicular helper T (Tfh) cells, which are generally considered to maintain homeostasis of the immune system. Interestingly, various diseases may be induced by the impaired regulation of these cells (King et al. 2008). Recent studies suggested that

Fig. 1 CD4⁺ T helper subsets lineage. *Abbreviations* Th, T helper; Treg, regulatory T; Tfh, follicular helper T; CTL, cytotoxic T cell



particular Th subsets might be involved in the initiation of IgG4-RD (van der Neut et al. 2007; Rispens et al. 2009). This review will summarize the results of recent studies seeking to understand the role of Th cell subsets in IgG4-RD.

2 Th1/Th2 Balance

Th1 cells produce IL-2, interferon (IFN)- γ , and tumor necrosis factor (TNF)- α , which induce the inflammatory responses responsible for cellular immunity. In contrast, Th2 cells produce IL-4, IL-5, and IL-13, which provide help for humoral immunity. Th2 responses can also counteract Th1-mediated microbicidal action. Many allergic and autoimmune disorders are associated with polarization of the Th1/Th2 balance (Kennedy et al. 1992; Rapoport et al. 1993). IL-4 causes immunoglobulin isotype switching to IgG4 and IgE production (Punnonen et al. 1993). IgG4-RD patients also frequently develop allergic disease, including bronchial asthma, allergic rhinitis, and atopic dermatitis, with severe eosinophilia and elevation of serum IgE levels (Zen et al. 2007). Thus, many studies of this disease suggest that Th2 polarization contributes to the pathogenesis of IgG4-RD (Zen et al. 2007; Tanaka et al. 2012; Nakashima et al. 2010; Maehara et al. 2012b; Miyake et al. 2008). We also previously reported that peripheral CD4⁺ Th cells from patients with IgG4-related dacryoadenitis and sialadenitis (IgG4-DS), so-called Mikulicz's disease, revealed deviation of the Th1/Th2 balance toward Th2 (Miyake et al. 2008). Furthermore, the expression profile of cytokines in salivary glands from patients with IgG4-DS suggested that Th2 immune reactions might play a key role in IgG4 production (Tanaka et al. 2012).

However, the mechanism of Th2 immune activation in IgG4-RD remains to be clarified. In a recent study, we focused on IL-33 as a recently identified cytokine that directly stimulates ST2, the IL-33 receptor, expressed by Th2 cells, eosinophils, and mast cells, to induce production of IL-4, IL-5, and IL-13 (Schmitz et al. 2005). IL-33 is mainly expressed by epithelial cells but also by a wide variety of cell types, including dendritic cells, macrophages, fibroblasts, mast cells, and osteoblasts. Our current data demonstrate that the expression of IL-33 in salivary glands from IgG4-DS patients is significantly higher than that in patients with chronic sialadenitis and healthy controls. Interestingly, the expression of IL-33 in salivary glands was detected around ectopic GCs only in IgG4-DS patients. To confirm the identity of IL-33-producing cells around ectopic GCs, we analyzed the coexpression of immune cell markers and IL-33. The localization of IL-33 was consistent with that of CD163-positive M2 macrophages. In addition, mRNA expression of IL-33 was positively correlated with that of Th2 cytokines only in IgG4-DS patients (manuscript in preparation). These results suggest that the main IL-33 producing cells in IgG4-DS might be M2 macrophages and that they are deeply involved in activation of Th2 immune responses.

In contrast, Okazaki et al. (2000) examined the Th1/Th2 balance in peripheral blood mononuclear cells (PBMCs) in patients with AIP. The number of CD4⁺ Th

cells producing IFN- γ and the amount of IFN- γ secreted per cell were significantly increased compared with controls. However, IL-4 was not increased, suggesting that AIP might be mediated by a Th1-predominant immune reaction. With regard to the other organs, several studies reported that IgG4-DS showed a predominance of Th1 cells similar to IgG4-related sclerosing sialadenitis (Ohta et al. 2012). Moreover, Mattoo et al. (2014) analyzed circulating Th2 memory cells and reported that only IgG4-RD patients who had a history of atopic dermatitis showed an increase in circulating Th2 memory cells. They concluded that IgG4-RD promotes Th2 responses resulting from concomitant atopic manifestations. This discrepancy between studies might be explained by the aberrant activation of Th2 responses caused by differences in the severity of the disease or the presence of allergic disease.

3 Other CD4⁺ T Helper Subsets

3.1 *Th17 Cells*

The Th1/Th2 paradigm was expanded by the identification of Th17 cells, a subset of CD4⁺ Th cells characterized by their ability to produce IL-17 and expression of the transcription factor ROR γ t, and which play a crucial role in the induction of autoimmunity and allergic inflammation by mediating the recruitment of neutrophils and macrophages to affected tissues (Infante-Duarte et al. 2000). Our previous data demonstrated that both Th1 and Th17 cells that infiltrated around ductal epithelial cells might be deeply involved in the initiation of Sjögren's syndrome, a chronic autoimmune disease targeting the exocrine glands, specifically the salivary and lacrimal glands (Moriyama et al. 2012; Maehara et al. 2012b). In contrast, IgG4-DS showed non-periductal lymphocytic infiltration and mild destruction of epithelial cells. However, Th-17-related molecules were rarely seen in the salivary glands (Tanaka et al. 2012; Maehara et al. 2012a). From these findings, we speculate that IgG4-DS might not be a Th17-mediated autoimmune disease.

3.2 *Treg Cells*

Treg cells are essential for the maintenance of immunological self-tolerance and immune homeostasis by direct contact with effector immune cells or by the secretion of anti-inflammatory cytokines, such as IL-10 and transforming growth factor (TGF)- β . Treg cells exert their effects through the modulation of both T and B cell responses, and two subsets of Treg cells, CD4⁺ CD25⁺ Foxp3⁺ Treg cells and

IL-10-producing Tr1 cells, play a key role in regulating effector T cell functions (Shevach et al. 2006).

Allergic immune responses develop in response to the Th2 cytokines IL-4 and IL-13, which are responsible for IgG4 and IgE production by B cells. Interestingly, it was shown that the class switching to IgG4 is caused by co-stimulation with IL-4 and IL-10 and that IL-10 decreased the switching of IL-4-induced IgE but elevated the switching of IL-4-induced IgG4. This specific isotype switching is regarded as a 'modified Th2 response' (Maizels and Yazdanbakhsh 2003). Zen et al. (2007) reported that CD4⁺ CD25⁺ Foxp3⁺ Treg cells strongly infiltrated into the affected tissues and produced IL-10 and TGF- β in cases of IgG4-related sclerosing pancreatitis and cholangitis. In addition, we found that mRNA expression of IL-4, IL-10, and Foxp3 in salivary glands from patients with IgG4-DS was positively correlated with the IgG4/IgG ratio obtained immunohistochemically (Tanaka et al. 2012). These results suggest that Th2 and regulatory immune reactions might promote B cells to produce IgG4.

3.3 *Tfh Cells*

Tfh cells were identified as a unique Th phenotype, contributing to isotype switching, somatic hypermutation, and GC formation. Several studies reported that Tfh cells control the functional activity of effector Th cells and promote ectopic GC formation via IL-21, which impairs B cell differentiation (Vinuesa et al. 2010). IL-4 and IL-21 receptor knockout mice have significantly reduced IgG responses, indicating that IL-21 cooperates with IL-4 to regulate humoral immune responses (Ozaki et al. 2002). Furthermore, IL-21 directly inhibits IL-4-induced IgE production and induces the switching to IgG4 by co-stimulation with IL-4 and IL-21 (Kitayama et al. 2008). We previously found that expression of IL-21 correlated with the number of GCs formed in salivary glands from patients with IgG4-DS, further suggesting that aberrant IL-21 production might induce multiple GC formation and the switching of IgG4.

Recent studies demonstrated that the Tfh population comprises functionally distinct subsets characterized by specific patterns of surface antigens and that at least three subsets exist: Tfh1, Tfh2, and Tfh17 cells (Morita et al. 2011). Tfh1 cells secrete IFN- γ upon activation and have limited class switching activity. Tfh2 and Tfh17 cells induce the switching of various immunoglobulin isotypes (IgM, IgA, IgG, and IgE). Akiyama et al. (2015) reported that the number of circulating Tfh2 cells was increased in IgG4-RD and correlated with the number of plasmablasts and serum IgG4 and IL-4 levels. Moreover, the number of plasmablasts and levels of serum IgG4 and IL-4 decreased after glucocorticoid treatment, while that of Tfh2 cells remained unchanged. They concluded that Tfh2 cells are the underlying pathogenic T cell population in IgG4-RD.

3.4 CD4⁺ CTL Cells

Cytotoxic CD4⁺ T cells (CD4⁺ CTLs) are characterized by the production of IFN- γ , granzyme B, and perforin, and are distinct from any conventional CD4⁺ Th subset (Van de Berg et al. 2008). Mattoo et al. (2016) reported that circulating CD4⁺ CTLs were clonally expanded in IgG4-RD and the dominant T cells in disease tissues. The circulating CD4⁺ SLAMF7⁺ CTLs in IgG4-RD patients declines concomitant with a clinical response to rituximab therapy. In addition, the ratio of CD4⁺ GrB⁺ CTLs in IgG4-DS lesions was positively correlated with the serum IgG4 levels, as well as the number of affected organs. This suggests that the dysregulation of CD4⁺ CTLs might play a key role in the pathogenesis of IgG4-RD.

4 Conclusions

The physiological role of IgG4 antibody in IgG4-RD is poorly understood. IgG4 antibodies are generally considered to have a poor ability to activate complement and immune complex formation, which leads to anti-inflammatory activity. Moreover, specific antigens recognized by IgG4 have yet to be identified. From these findings, we speculate that the aberrant IgG4 production in IgG4-RD does not behave as a tissue-destructive immunoglobulin, but as a secondary reactive response induced by a unique cytokine milieu (Fig. 2).

Innate immunity has recently received much attention regarding the initiation of IgG4-RD. Several studies indicated that abnormal innate immune responses via toll-like receptors (TLRs) expressed by monocytes/macrophage enhanced Th2 immune responses and the immunopathogenesis of IgG4-RDs (Watanabe et al. 2012; Fukui et al. 2015). Macrophages are known to play an important role in the immune response to foreign substances or microbes by presenting pathogen antigens to antigen-specific Th cells. Notably, M2 macrophages scavenge debris and contribute to angiogenesis, wound healing, and fibrosis by producing IL-10 and CCL18 (Van Gorp et al. 2010). Thus, we have analyzed the relationships between M2 macrophages and the initiation of IgG4-RD and demonstrated the predominant infiltration of M2 macrophages in affected lesions from patients with IgG4-RD (Furukawa et al. 2015). In our latest study, we performed microarray analysis of gene expression in salivary gland samples to identify innate immune molecules and found that macrophage receptor with collagenous structure (MARCO) was identified as a disease-associated molecule in IgG4-RD. Moreover, the expression pattern of MARCO was consistent with that of the M2 macrophage marker CD163 (Ohta et al. 2016).

Thus, research accumulated in recent years has enhanced our understanding of the immunological background of IgG4-RD. However, additional research is still required to further elucidate the pathogenesis of IgG4-DS, especially the connection between innate and adaptive immunity.

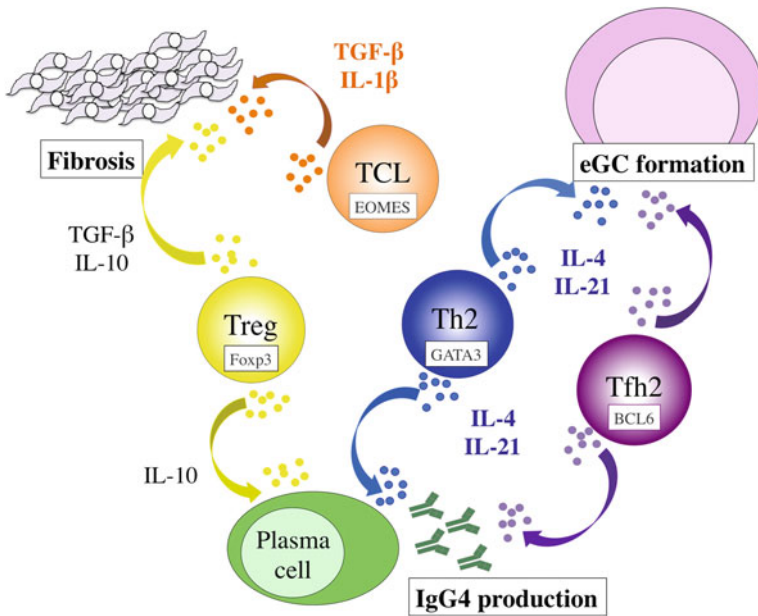


Fig. 2 Schematic model of Th subsets in IgG4-RD. Th2, Treg, and Tfh2 cells play key roles in GC formation and IgG4 production. Treg cells and CD4⁺ CTLs promote local fibrosis by IL-1β and TGF-β

Funding This work was supported in part by grants from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (26293430, 26670869), the ‘Research on Measures for Intractable Diseases’ project, a matching fund subsidy from the Ministry of Health Labour and Welfare, Japan (H26-026, H26-064), the ‘Practical Research Project for Rare/Intractable Diseases,’ a matching fund subsidy from the Japan Agency for Medical Research and Development (H26-275, H26-313).

References

- Akiyama M, Suzuki K, Yamaoka K et al (2015) Number of circulating follicular helper 2 T Cells correlates with IgG4 and Interleukin-4 levels and plasmablast numbers in IgG4-related disease. *Arthritis Rheumatol* 67(9):2476–2481
- Dahlgren M, Khosroshahi A, Nielsen GP et al (2010) Riedel’s thyroiditis and multifocal fibrosclerosis are part of the IgG4-related systemic disease spectrum. *Arthritis Care Res* 62 (9):1312–1318
- Fukui Y, Uchida K, Sakaguchi Y et al (2015) Possible involvement of Toll-like receptor 7 in the development of type 1 autoimmune pancreatitis. *J Gastroenterol* 50(4):435–444
- Furukawa S, Moriyama M, Tanaka A et al (2015) Preferential M2 macrophages contribute to fibrosis in IgG4-related dacryoadenitis and sialoadenitis, so-called Mikulicz’s disease. *Clin Immunol* 156(1):9–18

- Hamano H, Kawa S, Horiuchi A et al (2001) High serum IgG4 concentrations in patients with sclerosing pancreatitis. *N Engl J Med* 344(10):732–738
- Hamano H, Kawa S, Ochi Y et al (2002) Hydronephrosis associated with retroperitoneal fibrosis and sclerosing pancreatitis. *Lancet* 359(9315):1403–1404
- Infante-Duarte C, Horton HF, Byrne MC et al (2000) Microbial lipopeptides induce the production of IL-17 in Th cells. *J Immunol* 165(11):6107–6115
- Kennedy MK, Torrance DS, Picha KS et al (1992) Analysis of cytokine mRNA expression in the central nervous system of mice with experimental autoimmune encephalomyelitis reveals that IL-10 mRNA expression correlates with recovery. *J Immunol* 149(7):2496–2505
- King C, Tangye SG, Mackay CR (2008) T follicular helper (TFH) cells in normal and dysregulated immune responses. *Annu Rev Immunol* 26:741–766
- Kitayama D, Sakamoto A, Arima M et al (2008) A role for Bcl6 in sequential class switch recombination to IgE in B cells stimulated with IL-4 and IL-21. *Mol Immunol* 45(5):1337–1345
- Maehara T, Moriyama M, Nakashima H et al (2012a) Interleukin-21 contributes to germinal centre formation and immunoglobulin G4 production in IgG4-related dacryoadenitis and sialoadenitis, so-called Mikulicz's disease. *Ann Rheum Dis* 71(12):2011–2020
- Maehara T, Moriyama M, Hayashida JN et al (2012b) Selective localization of T helper subsets in labial salivary glands from primary Sjogren's syndrome patients. *Clin Exp Immunol* 169:89–99
- Maizels RM, Yazdanbakhsh M (2003) Immune regulation by helminth parasites: cellular and molecular mechanisms. *Nat Rev Immunol* 3(9):733–744
- Mattoo H, Mahajan VS, Della-Torre E et al (2014) De novo oligoclonal expansions of circulating plasmablasts in active and relapsing IgG4-related disease. *J Allergy Clin Immunol* 134(3):679–687
- Mattoo H, Mahajan VS, Maehara T et al (2016) Clonal expansion of CD4⁺ cytotoxic T lymphocytes in IgG4-related disease. *J Allergy Clin Immunol*
- Meiler F, Klunker S, Zimmermann M et al (2008) Distinct regulation of IgE, IgG4 and IgA by T regulatory cells and toll-like receptors. *Allergy* 63(11):1455–1463
- Miyake K, Moriyama M, Aizawa K et al (2008) Peripheral CD4⁺ T cells showing a Th2 phenotype in a patient with Mikulicz's disease associated with lymphadenopathy and pleural effusion. *Mod Rheumatol* 18(1):86–90
- Morita R, Schmitt N, Bentebibel SE et al (2011) Human blood CXCR5(+)/CD4(+) T cells are counterparts of T follicular cells and contain specific subsets that differentially support antibody secretion. *Immunity* 34(1):108–121
- Moriyama M, Hayashida JN, Toyoshima T et al (2012) Cytokine/chemokine profiles contribute to understanding the pathogenesis and diagnosis of primary Sjogren's syndrome. *Clin Exp Immunol* 169:17–26
- Nakashima H, Miyake K, Moriyama M et al (2010) An amplification of IL-10 and TGF-beta in patients with IgG4-related tubulointerstitial nephritis. *Clin Nephrol* 73(5):385–391
- Ohta N, Makiyama S, Okano M et al (2012) Roles of IL-17, Th1, and Tc1 cells in patients with IgG4-related sclerosing sialadenitis. *Laryngoscope* 122(10):2169–2174
- Ohta M, Moriyama M, Maehara T et al (2016) DNA microarray analysis of submandibular glands in IgG4-related disease indicates a role for MARCO and other innate immune related proteins. *Medicine* 95(7):e2853
- Okazaki K, Uchida K, Ohana M et al (2000) Autoimmune-related pancreatitis is associated with autoantibodies and a Th1/Th2-type cellular immune response. *Gastroenterology* 118(3):573–581
- Ozaki K, Spolski R, Feng CG et al (2002) A critical role for IL-21 in regulating immunoglobulin production. *Science* 298(5598):1630–1634
- Punnonen J, Aversa G, Cocks BG et al (1993) Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. *Proc Natl Acad Sci USA* 90(8):3730–3734

- Rapoport MJ, Jaramillo A, Zipris D et al (1993) Interleukin 4 reverses T cell proliferative unresponsiveness and prevents the onset of diabetes in nonobese diabetic mice. *J Exp Med* 178 (1):87–99
- Rispens T, Ooievaar-De Heer P, Vermeulen E et al (2009) Human IgG4 binds to IgG4 and conformationally altered IgG1 via Fc-Fc interactions. *J Immunol* 182(7):4275–4281
- Sato Y, Kojima M, Takata K et al (2009) Systemic IgG4-related lymphadenopathy: a clinical and pathologic comparison to multicentric Castleman’s disease. *Mod Pathol* 22(4):589–599
- Schmitz J, Owyang A, Oldham E et al (2005) IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* 23:479–490
- Shevach EM, DiPaolo RA, Andersson J et al (2006) The lifestyle of naturally occurring CD4⁺ CD25⁺ Foxp3⁺ regulatory T cells. *Immunol Rev* 212:60–73
- Takeda S, Haratake J, Kasai T et al (2004) IgG4-associated idiopathic tubulointerstitial nephritis complicating autoimmune pancreatitis. *Nephrol Dial Transplant* 19(2):474–476
- Tanaka A, Moriyama M, Nakashima H et al (2012) Th2 and regulatory immune reactions contribute to IgG4 production and the initiation of Mikulicz disease. *Arthritis Rheum* 64 (1):254–263
- Van de Berg PJ, van Leeuwen E, ten Berge IJ et al (2008) Cytotoxic human CD4⁺ T cells. *Curr Opin Immunol* 20:339–343
- van der Neut Kolfschoten M, Schuurman J, Losen M et al (2007) Anti-inflammatory activity of human IgG4 antibodies by dynamic Fab arm exchange. *Science* 317(5844):1554–1557
- Van Gorp H, Delputte PL, Nauwynck HJ (2010) Scavenger receptor CD163, a Jack-of-all-trades and potential target for cell-directed therapy. *Mol Immunol* 47:1650–1660
- Vinuesa CG, Linterman MA, Goodnow CC et al (2010) T cells and follicular dendritic cells in germinal center B-cell formation and selection. *Immunol Rev* 237(1):72–89
- Watanabe T, Yamashita K, Fujikawa S et al (2012) Involvement of activation of toll-like receptors and nucleotide-binding oligomerization domain-like receptors in enhanced IgG4 responses in autoimmune pancreatitis. *Arthritis Rheumatol* 64(3):914–924
- Yamamoto M, Harada S, Ohara M et al (2005) Clinical and pathological differences between Mikulicz’s disease and Sjogren’s syndrome. *Rheumatology* 44(2):227–234
- Zen Y, Harada K, Sasaki M et al (2004) IgG4-related sclerosing cholangitis with and without hepatic inflammatory pseudotumor, and sclerosing pancreatitis-associated sclerosing cholangitis: do they belong to a spectrum of sclerosing pancreatitis? *Am J Surg Pathol* 28(9): 1193–1203
- Zen Y, Fujii T, Harada K et al (2007) Th2 and regulatory immune reactions are increased in immunoglobulin G4-related sclerosing pancreatitis and cholangitis. *Hepatology* 45(6): 1538–1546
- Zen Y, Inoue D, Kitao A et al (2009) IgG4-related lung and pleural disease: a clinicopathologic study of 21 cases. *Am J Surg Pathol* 33(12):1886–1893