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### Costimulatory Molecules in Rheumatic Diseases Revisited with an Emphasis on Their Roles in Autoimmune Sjögren's Syndrome

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#### 1. Introduction

Sjögren's syndrome (SjS) is a chronic, inflammatory autoimmune disorder characterized by dry mouth and dry eyes, in which lymphocytic infiltration (primarily CD4 T-cells) of the salivary and lacrimal glands destroy their secretion abilities. Since abnormal activation of T-cells are a key feature of SjS, as well as for other autoimmune conditions, defining the processes for T-cell activation and inhibition are important for understanding SjS autoimmune pathogenesis.

The molecules CD80 (B7.1) and CD86 (B7.2), here forth collectively referred to as B7, are critical costimulatory ligands during the process of antigen presentation because of their abilities to support T-cell receptor (TCR)-mediated responses through their binding to and activation of CD28 receptors on T-cells. Binding of either of these B7 ligands to cytotoxic T-Lymphocyte Antigen 4 (CTLA-4, CD152) counter receptor are crucial for attenuating T-cell responses. Additionally, these processes of activation and inhibition have been shown to be modulated in part by regulatory T (Treg) cells, which are a naturally occurring cell population capable of directly suppressing effector T (Teff) cells activation and proliferation, especially to self-antigens.

In autoimmune diseases, dysfunction of Treg cells is a potential contributor to disease development, where costimulatory requirements for Treg cell proliferation and suppression capabilities may not be met. It is possible that the relative contribution of CD86/CD80 and CD28/ CTLA-4 signals to Treg and Teff cells could dictate the potency of suppression and phenotypes of cells. This is important in understanding autoimmune diseases where abnormal ex-



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pression of CD86 and CD80 is seen in the target tissue sites and dysregulation of specific Treg populations may contribute to development of autoimmune disorders.

Elucidating biological function and mechanisms of action of the CD86/CD80:CD28/CTLA-4 molecules have been the focus of much research over the past several decades since their seminal discoveries in the early 1990s. However, there are still many questions as to how these molecules interact to form specific immune responses in vivo, especially in the field of autoimmunity. In this book chapter, we will describe potential processes for T-cell activation and inhibition and investigate the contribution of abnormal costimulatory/inhibitory signals from CD80/CD86 to the establishment of autoimmune disorders, such as SjS.

## 2. Overview of costimulatory molecules for T-cell activation and inhibition

Much of the difficulties in achieving a cohesive understanding of the CD86/CD80:CD28/ CTLA-4 system stem from various complexities, such as, cell type-specific expression (including varying protein levels and kinetics of expression) and assorted ligand:receptor interactions that contribute to the overall immune phenotype. In this section, we will focus on the expression, interactions, and overall function of these receptors and ligands, especially related to Treg cell activation and suppression function.

#### 2.1. Expression of CD86/CD80:CD28/CTLA-4

The CD28 and CTLA-4 receptors are members of the CD28 family of immunoglobulin-like glycoproteins that are genetically linked on human chromosome 2 and mouse chromosome 1, although they each have distinct patterns of cell surface expression. CD28 is well known to be constitutively present at the surface of all T-cells (CD4 and CD8), where its surface levels are further increased to maximal by 24 hours following TCR activation. CTLA-4 on the other hand is found predominantly located in intracellular vesicles. CTLA-4 is well known to be rapidly removed from the cell surface by clatherin adaptor complex AP-2-mediated endocytosis, such that only a small fraction of CTLA-4 is present on the cell surface under steady-state conditions [1-5]. Following TCR activation CTLA-4 still retains its endocytosis capabilities [6] and yet, its surface concentration is quickly increased by 48 hours after T-cell activation. Delivery of CTLA-4 receptor to the immune synapse has also been shown to be associated with the strength of TCR signaling [7]; indicating that T-cells with higher affinity TCRs are potentially more likely to be attenuated by CTLA-4 receptor activation.

The two known CD28 and CTLA-4 ligands, CD86 and CD80, are members of the B7 family of immunoglobulin-like proteins expressed on a variety of antigen presenting cell (APC) types including dendritic cells (DCs), macrophages, and B-cells [8, 9]. Similar to the CD28 family, the B7 family of ligands appears to derive from gene duplication events, where they are genetically linked on human chromosome 3 and mouse chromosome 16, although, they too have very distinct patterns of expression from each other. CD86 is most commonly con-

stitutively expressed on the surface of professional antigen presenting cells (APCs) or cells such as monocytes [8-10]. On these cell types CD86 is generally more abundantly expressed than CD80. CD86 is rapidly upregulated and maximally expressed by 48 hours in response to its interaction with CD28 receptor or other inflammatory stimuli [9, 11, 12]. Typically, CD80 is not constitutively present on unactivated cells. Following initiation of CD28 or inflammatory stimulation the expression of CD80 is produced at a much slower rate [9]. Interestingly, immature DCs (and Langerhans cells) show evidence that CD80 is the predominantly expressed ligand compared to CD86 [13-15]. These findings are indicative of a predominant role for CD80 in immature or regulatory cells [16-18].

#### 2.2. Relative interaction properties of CD86/CD80:CD28/CTLA-4

The CD28/CTLA-4 receptors and the CD86/CD80 ligands function as multi-subunit proteins comprising of identical protein subunits that provide unique ligand:receptor interactions. CD28 is well known as a homodimer. However, this homodimer configuration only possesses a single ligand binding site, in stark contrast to CTLA-4 homodimer, which is shown to possess two ligand binding sites [19, 20]. Regarding the ligands themselves, CD80 exists as a weak noncovalently bound homodimer based on analytical ultracentrifugation and crystal structure of CD80 [21]. This same dimeric conformation was also observed following crystallization of CD80 complexed with CTLA-4 [20, 21] and photo-bleaching FRET confirmed that CD80 exists predominantly as a dimer on the surface of APCs [22]. On the other hand, CD86 primarily exists as a monomer as detected by analytical ultracentrifugation and gel filtration studies [23]. The crystal structure analysis of CD86 also revealed poor dimer interface [21], but interestingly, crystallization of CD86 complex with CTLA-4 indicates that it can potentially form a dimer as well as crosslink CTLA-4 receptors [24]. Again, crystallization of CD86 without CTLA-4 or in solution indicates that CD86 is unlikely to form stable dimers [19, 23]. Findings based on photo-bleaching FRET also confirmed that CD86 exists as a monomer on the cell surface [22], where CD86 tends to have a faster association and dissociation rate than CD80 to either receptors [25]. The issue for potential receptor mediated dimerization of CD86 is currently unresolved. These findings altogether suggest that CD28 is restricted to having one ligand binding partner, whereas, CTLA-4 is potentially capable of crosslinking linking a single ligand, probably CD80, and forming complex network of interactions receptor:ligand interactions.

Along these similar lines, actual affinity of CTLA-4 for the B7 ligands is much higher than with CD28. However, the differences between CD80 and CD86 binding to T-cell receptors are less remarkable. Relative interaction properties of these molecules indicate preferential interaction between CD80 and CTLA-4, whereas CD86 more biased towards CD28 [19, 20]. CD80 binds CTLA-4 with a modest 10-fold higher affinity than does CD86 ligand [19]. As such, all other things being equal, when CD86 is expressed on APCs, then CD28 is more favored interaction than CTLA-4 at the T-cell:APC interface. Thus, the activation signals elicited by CD86:CD28 are less likely to be attenuated by coincident CTLA-4 ligation and enhancing the costimulatory effects [19]. Actual preferences of each B7 molecule with CD28 and CTLA-4 were tested in APCs deficient in either CD86 or CD80, where relative seques-

tration of CD28 and CTLA-4 in the immunological synapse was evaluated. Results from this study do indicate that CD80 does preferentially bind to CTLA-4 and CD86 shows better interaction with CD28 on the cell surface [26].

#### 2.3. Functions of CD86/CD80:CD28/CTLA-4

As mentioned previously, the receptors CD28 and CTLA-4 are members of the CD28 family of immunoglobulin-like glycoproteins expressed on both CD4+ and CD8+ T-cells. These receptors share the same B7 ligands; however, they have opposing functions on T-cells. CD28 receptor activation supplies additional signals to support TCR-mediated responses, such as, T-cell proliferation, cytokine production, cell survival, and promotes T-cell help to B cells [1, 27-29]. As expected, CD28 deficient mice have impaired T-cell activation in response to antigen [30], as well as, defective B cell responses [29, 31]. These additional costimulatory signals provided by CD28 have been suggested to function to decrease the threshold for T-cell activation, thereby, reducing the contact time with APC required for T-cell activation [32, 33]. CTLA-4 receptor activation provides signals to effectively attenuate T-cell responses. The inhibitory function of CTLA-4 was first described from CTLA-4 deficient mice that exhibit a fatal CD4+ T-cell hyperproliferation and multi-organ infiltration [34-36]. These abnormally activated T-cells are potentially reactive to multiple self-antigens despite apparently normal thymic selection [37]. These T-cells are primarily mediated through activation of CD28, since mice deficient in both CTLA-4 and B7 do not develop disease [38, 39] and where a specific mutation in CD28 prevents disease induction [40]. CTLA-4 has now been better established in its role in the suppressive function of Treg cells [41-44]. The mechanisms by which CTLA-4 in Treg cells directly suppresses immune responses includes the delivery of negative signal towards inhibition of Teff cell proliferation and activation [45, 46], as well as, through the removal of B7 molecules from the surface of the APCs [47]. However, there is also the potential for CTLA-4 competition with CD28 for ligands that could contribute to suppression functions as well. Altogether, CTLA-4 has a major role in modulating CD28mediated activation.

The differences in CD80 and CD86 function in regards to T-cell activation and inhibition are not so well appreciated. There is still a general perception that CD80 and CD86 are interchangeable costimulators with differences only in kinetics of expression and cell type distribution. Despite the obvious overlapping functions, these B7 molecules do show evidence of distinct biological effects, predominantly with CD80 having more inhibitory roles and effects on Treg cells. A classic example of this phenomenon is in the non-obese diabetic (NOD) mouse model. In this model, blockade of CD80 by monoclonal antibodies (MAbs) exacerbates disease, while blockade of CD86 prevents disease [48]. It is possible that with blocking of CD86, the available CD80 could have enhanced interactions and would thus be free to interact with CTLA-4, thus attenuating T-cell activation. Blocking of CD80 would potentially free CD86 to interact with CD28 making it more likely to provide help activate self-reactive T-cells. Since NOD Tregs appear more dependent on CD80 for their maintenance, these selfreactive T-cells may not be attenuated as well [49]. A more recent regulatory function has also been found for CD80, where CD80 acts as an alternate ligand for programmed death ligand 1 (PD-L1, B7H1, CD274) [50]. PD-L1 is well known to induce inhibitory signals in T-cells and to inhibit T cell proliferation. The affinity of CD80 for PD-L1 is intermediate between its affinity for CD28 and CTLA-4, yet still threefold less than the affinity of PD-L1 for PD-1 [50]. The function of this CD80:PD-L1 interaction in immune responses was shown in vivo where this pathway was shown to prevent alloimmune responses and where these tolerogenic effects were mediated by the interactions of PD-L1 on APCs eliciting an inhibitory signal through CD80 expressed on T-cells [51]. Specific blockade of this CD80/PD-L1 interaction indicates that the inhibitory signal from CD80 but not PD-L1 is responsible for attenuation of T-cell expansion and enhancement of T-cell anergy. Blocking of this interaction specifically led to enhanced expansion and restored antigen responsiveness in previously anergized T-cells [52].

#### 2.3.1. Regulatory T cells

There are several lines of evidence suggesting that the expression of CD80 and CD86 ligands by APCs are important in maintaining Treg cell homeostasis and suppressive function in the periphery. B7 blockade experiments have indicated that continual expression of B7 in the periphery is necessary to maintain the Treg compartment as indicated by the reduced CD25 expression following blockade [53]. Additionally, blocking antibodies to B7 also was capable of inhibiting the natural turnover of adoptively transferred Treg cells in vivo [54].

The relative contribution of CD86 and CD80 to Treg cell responses is more difficult to describe because of their overlapping functions, however, definite differences have been indicated. The relative expression levels of CD86 and CD80 on DCs are well known to be modulated during the progression from immature to mature state. For example, DCs expressing high levels of CD86, makes them particularly proficient at driving Treg cell proliferation [55], where CD86 was shown to be more important than CD80 in this regard [56]. These findings are all consistent with CD86 being a better ligand for CD28 in the face of CTLA-4 expression and potentially better at stimulating Treg proliferation in these settings. CD80 appears to contribute more to the inhibitory functions of Treg cells through its involvement with CTLA-4. For example, in the presence of antibodies to either CD80 or CTLA-4, Treg suppression abilities were impaired when the CD25- responder T cells are not exposed to the blocking antibodies [56]. Evidence also suggests that Teff cells from B7-deficient mice are resistant to suppression by wild type Tregs, where CD80 on Teff cells was largely shown to be the dominant ligand for mediating suppression capabilities of Treg cells [57]. This effect is potentially mediated by a CD80 cell-intrinsic negative signal into Teff cells that helps facilitate suppression. The receptor for B7 that mediated this effect was not identified, but due to the requirement for Treg cells it appears to be mediated by CTLA-4 [57]. The transfection of either CD80 or CD86 into Chinese hamster ovary cells (CHO), indicate CD80 could direct CTLA-4-mediated inhibition of resting human T cells through the activities of CD25+CTLA-4+ Treg cells [43]. Additional to the effects of CD80 through CTLA-4, suppressive functions of CD80 have shown that the PD-L1/CD80 pathway also leads to promotion of the in vivo expansion of donor natural Tregs in allogeneic recipients [58]. The proposed mechanism of action is that IFN-gamma upregulates tissue expression of the PD-L1 on APCs and parenchymal cells. Expression of PD-L1 on those cells in normal tissues is thought to be capable of interacting with CD80 expressed on Treg cells, promoting development and maintenance of Treg cells [58].

Therefore, it is possible that under a steady state condition, low CD80 and PD-L1 expressed by immature DCs preferentially interacts with CTLA-4 and CD80 predominantly expressed by Treg cells, thus, promoting Treg function and maintenance. However, specific mechanisms of controlling Treg cell maintenance and suppression functions through these signaling molecules are still areas requiring exploring. Following inflammatory stimuli and DC maturation, where there is a relative upregulation of CD86 compared to CD80 along with higher levels of antigenic stimulation, Treg suppressive capacity should become reduced. This is supported by the finding that CD28 and TCR stimulation could antagonize suppressive function of Treg cells [43, 59]. Therefore, CD80 and CD86 may have somewhat opposing roles in aiding suppressive capacity through CTLA-4 vs. activation of T-cells cells through CD28, where their relative expression could influence this balance (Figure 1).



**Figure 1.** Mechanism by which Treg cells balance responses to B7 ligand stimulation. (A) Relatively high expression of CD80 on APC favours enhancing suppressive function of Treg cell and inhibits T-cell proliferation. (B) Increase in CD86 following activation of APC favours inhibition of Treg cells and enhances T-cell proliferation. Specific signalling requirements of Treg activation and inactivation are still to be determined.

#### 3. Impact of CD86/CD80 molecules in autoimmune disease

The relative contribution of CD86 and CD80 to the development of autoimmunity is difficult to evaluate because of the diverse cells and interactions involved in disease pathogenesis. However, interesting trends have appeared where alterations in relative expression of CD86 and CD80 in the target tissues and alterations in Treg numbers and function may contribute

to the onset of autoimmune diseases. In this section we will review some information on the rheumatic autoimmune diseases systemic lupus erythematosus and rheumatoid arthritis as well as cover important findings regarding autoimmune diabetes. The major focus of this section will be on elucidating the pathogenesis of Sjögren's syndrome as it is related to target tissue expression of B7 molecules and role of Treg cells in these tissues.

#### 3.1. Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a chronic multi-organ autoimmune disease distinguish by imbalanced T-cell homeostasis towards activated Teff cell subsets Th1 and Th2 and can affect multiple organs and systems of the body. It is well characterized by the number and variety of autoantibodies produced and is well marked by anti-nuclear antibody production. Many of the symptoms are caused by impaired antigen-antibody complex clearance and thus triggering inflammatory responses in multiple sites. Because of the difficulty in evaluating patient disease progression several mouse models have been used to evaluate how abnormal costimulation and Treg cells contribute to disease. The autoimmune BXD2 mice indicates that type I IFN can promote autoimmune responses through the upregulation of CD86high expression on marginal zone precursor B-cells, which were shown to be located at the T-B-cell border of the spleen germinal centers [60]. In regards to Treg maintenance, the (NZB x NZW)F1 and (SWR/NZB)F1 lupusprone mice had reduced numbers of CD4+CD25+ cells [61]. There was no intrinsic defect in the suppressive function of the (NZB x NZW) F1 mice [62]. Additionally, only marginal defects in Treg suppression were observed in MRL/Mp mice [63]. MRL/lpr mice with strong lupus disease were found to have normal percentages of peripheral CD4+CD25high T-cells and that Foxp3 expression was unaltered. However, they do display a reduced capacity to suppress and effector CD4+CD25- T-cells were significantly less susceptible to suppression. Importantly they also found that CD80/CD86 were underexpressed on Teff, APCs, and on Treg cells, suggesting that the reduction in these molecules could be contributing to the reduced abilities of Treg cells to suppress [64].

#### 3.2. Rheumatoid arthritis

Rheumatoid Arthritis (RA) is a chronic inflammatory disease characterized by inflammation of the joints and surrounding tissue including mononuclear cell infiltration of synovial tissue. Predominant lymphocytes present in rheumatoid synovium focal infiltrates are lymphocytes (predominantly CD4+ T-cells with relatively few positive for CD25), macrophages, and plasma B cells. The expression of CD86 was readily detectable in the synovium compared to osteoarthritis synovial [65]. In contrast, CD80 expression was not significantly expressed in the synovium [65]. Overall, the expression of CD80 on APCs in the synovium is generally low, while expression of CD86 is relatively high and is expressed on several APCs including DCs, B-cells, and macrophages [66-70]. Blocking of CD28 signaling pathway has also been shown to prevent or even treat autoimmune diseases. For instance, CD28 deficient mice are resistant to collagen induced arthritis [72] and inhibition of both CD80 and CD86

during the induction phase of collagen induced arthritis prevents the development of disease [71, 73, 74]. Arthritis is also abolished and autoantibody production is suppressed in MLR/lpr mice lacking CD28, however, the accumulation of abnormal T cells is almost unchanged [75]. Indicating CD28 may have a better role in aiding B cell responses in this model. Most importantly, treatment with blocking CTLA-4-Ig also significantly improved signs and symptoms and Treg function of RA patients in clinical trials [76]. In general, Treg cells with diminished suppressive capacity were obtained from synovial fluid of patients with RA [77, 78]. Functional studies of Treg cell defects in mice related to RA indicate that defects in CD4+Foxp3+ Treg cells in K/BxNsf mice exhibited earlier onset and more aggressive progression of arthritis than K/BxN littermates [79]. This was accompanied by plasmacytoid dendritic cells expressing high levels of CD86 and CD40, but not CD80, in synovia and increase memory CD4+ T-cells in the spleen and draining lymph nodes [79]. These mice also exhibit an abnormal accumulation of mature plasma cells in spleen and associated loss of bone marrow plasma cells. These plasma cells were also less susceptible to cell death [80]. Overall, it appears the function of CD28 to the pathogenesis of RA, potentially mediated through over expression of CD86 appears to have a major function in disease pathogenesis.

#### 3.3. Autoimmune diabetes

B7 molecules have potential influence on autoreactive T cells in animals genetically predisposed to autoimmune disease. Adoptive transfer of NOD B-cells previously blocked with B7 MAbs along with diabetogenic T-cells into NOD.scid mice protected associated type 1 diabetes [81]. Again, as mentioned previously NOD mice treated with blocking CD86 MAbs prevents the spontaneous development of diabetes, whereas blocking CD80 accelerates and worsened disease [48]. Treatment of NOD mice older than 10 weeks of age with blocking CD86 MAbs did not alter the course of disease [48]. These results indicate that in the NOD background, the influence of CD86 to promotion and CD80 to suppression of disease occurs early on in disease pathogenesis. Breeding of CD86 deficiency onto NOD background prevented the development of diabetes, as expected from previous studies. However, aging mice around age 20 weeks would develop a peripheral neuropathy characterized by demyelization and defective nerve function due to mononuclear cell infiltration of peripheral nerves [82]. This was accompanied by a high level of CD80 expression on the APCs in the spleen as well as on the nerves of affected animals [82]. This is similar to reports seen in Experimental Autoimmune Epithelitis (EAE) and Multiple Sclerosis (MS) patients [83, 84], where downregulation of CD86 and upregulation of CD80 is observed on CNS-infiltrating cells and splenocytes. These somewhat conflicting findings for autoimmune diseases can potentially be explained by differences in interplay of local cellular interactions, cytokines and chemokines that may alter B7 expression. In this case, high expression of CD80 could potentially allow activation of CD28 signaling and autoimmune activation rather than suppression.

There is still some debate on whether Treg cells contribute to autoimmunity with regards to their deficits and functionality in autoimmune diabetes. Treg depletion or B7-deficiency (significant loss in CD4+CD25+ cell population) in NOD mice leads to accelerated disease onset

[53, 54]. The importance of Treg number to quality of suppression was shown with polyclonal Treg adoptive transfer to NOD.scid at a 2:1 ratio (Treg:Th1) was unable to suppress, while 5:1 ratio was able to provide protection in approximately half of the recipients. Therefore, with sufficiently large populations of Tregs, there may be adequate numbers of antigen-specific Tregs capable of suppressing diabetes [85]. It is interesting to note that Treg cells developed in vitro from induced GAD-IgG transduced splenocytes were capable of suppressing diabetes in NOD mice. This was accompanied by a higher ratio of CD80 compared to CD86 expression in splenocytes, which was sufficient to allow development of functional Treg cells (Foxp3+) in this model [86-88]. Depletion of CD4+CD25+ cells from transduced splenocytes transferred into NOD mice showed increased ratio of CD86 to CD80 in splenocytes. Blocking of CD80, but not CD86, reduced the relative quantity of Foxp3 [87]. Along these lines, a progressive decrease was observed in the Treg cell:Teff cell ratio in inflamed islets but not in pancreatic lymph nodes in NOD mice. Intra-islet Treg cells expressed reduced amounts of CD25 and Bcl-2, where the administration of low-dose interleukin-2 (IL-2) promoted Treg cell survival and protected mice from developing diabetes. Together, these results suggest intra-islet Treg cell dysfunction is a root cause of the progressive breakdown of self-tolerance and the development of diabetes in nonobese diabetic mice [89]. However, there is still some debate on whether NOD mouse Treg cells are sufficient in regulating Teff, since Tregs from NOD and B6g7 mice were equally effective but NOD T conventional cells were hyper-responsive to stimulation [90].

#### 3.4. Sjögren's syndrome

Recently in SjS, as with some of the other autoimmune diseases previously mentioned, the emphasis of pathogenicity has been placed on CD86 expression in the salivary gland environment. Expression of CD86 and CD80 in SjS patient salivary gland tissue compared to patients with nonspecific sialadenitis was first described in 1999, where expression of both ligands were found in ductal and acinar regions of immunohistochemically stained tissue sections [91]. It was recently shown that the functional expression of CD86 on salivary gland epithelial cells derived from SjS patients are capable of interacting with CD28, but its binding to CTLA-4 was reduced [92]. Therefore, salivary epithelial cells are possibly functioning to promote production of IL-2 and T-cell proliferation. This paper also indicates that expression of CD80, contrary to other immunohistochemistry results suggesting that CD80 is expressed [93],[94], could not be established in their salivary gland epithelial cells. It is difficult to establish the relative expression of these molecules in vivo, since several other studies were conducted on cell lines derived from patient biopsies under cytokine stimulating conditions or from immunohistochemistry of tissue sections using BB1 antibody, which potentially shares reactivity with non-B7 proteins such as MHC class II invariant chain [92, 95]. Further studies may be required to verify the production of CD80 as well as the relative expression of each of these ligands in the salivary glands of patients and healthy controls.

However, in C57BL/6 (B6) mice the overall expression of B7 is relatively low (CD86 greater than CD80) in normal mouse submandibular salivary glands compared to lymphoid tissues and is located predominantly along the ducts and among acini as previously indicated in

human patients (data unpublished, [96]). Presumably, these cells are salivary gland epithelial cells, where the expression of low level costimulatory ligands is presumed normal and possibly protective. As for mouse models for SjS, retinoblastoma-associated protein 48 (RbAp48) transgenic mice, using a salivary gland specific promoter [97], resulted in the development of SjS-like symptoms. In salivary gland tissues of these mice the protein expression of MHC class II, CD86, CD80, and ICAM-1 were all upregulated in affected mice salivary glands. Where the expression levels of CD86 is higher than CD80 in the SMX, indicating a potential pathogenic role of CD86 to disease initiation [96]. Another mouse model of SjS, the C57BL/6.NOD-Aec1Aec2 is a double congenic mouse model of primary SjS syndrome that contains two genetic regions (Idd3 and Idd5) derived from the NOD mouse model [98]. These genetic regions confer spontaneous development of SjS-like syndrome on the B6 background. A recent study using this model highlighted the importance of local B7 molecules and signaling through CD28 to disease progression [99]. In this report AAV transduced expression of the CTLA-4-Ig (blocks B7 molecules interactions) in the salivary glands of SjS-prone mouse model. Delivery of this gene construct via AAV prior to disease onset prevented glandular damage and lymphocytic cell infiltration commonly associated with disease, as well as, prevented loss of saliva secretion in the treated mice [99]. This was also accompanied by a significant increase in transforming growth factor beta-1 (TGF-B1) in the salivary glands and draining lymph nodes of these mice [99]. These results suggest a potential regulatory role (either Treg or epithelial cells) involved with treatment of CTLA-4-Ig. Authors of this paper did not explore the mechanisms of action of this molecule. Along similar lines, another study involving treatment of blocking CD86 MAbs to another mouse model of primary SjS, the NFS/sld 3-day thymectomized mutant, was shown to prevent the autoimmune lesions and autoantibody production to a-fodrin [100]. Along with previous lines of research indicating a negative regulatory role of CD80, no significant effects were seen in mice treated with anti-CD80 MAbs [100]. These results together outline the importance of costimulation to disease onset and severity and highlight the influence of CD86-CD28 as a potential mediator of disease initiation in salivary and lacrimal glands.

Results regarding Treg involvement in the pathogenesis of SjS have been contradictory. However, increasing evidence details defects in Treg contributing to disease. Recently it has also been shown that in situ patrolling Tregs are essential for protection against autoimmune exocrinopathy. In this system, CCR7 deficient mice were unable to allow Treg egress from lymph nodes into peripheral tissues such as salivary and lacrimal glands, and that this resulted in disease resembling SjS [101]. Interestingly, wild-type C57BL/6 mice evaluation showed approximately 30% lacrimal and 23% salivary expression of Foxp3+ cells under steady-state conditions, while CCR7-deficient mice had approximately 7% and 9% respectively [101]. The Foxp3-deficient scurfy mouse model (essentially deficient in Treg cells) adds more complexity to the issue of roles of Tregs, since these mice do not develop inflammation or inflammatory cell infiltration into their salivary or lacrimal glands, however, adoptive transfer to Rag-deficient mice induces multi-organ inflammation in salivary, lacrimal glands, stomach, small intestine, pancreas, colon, and even skeletal muscle. This included inflammation in the lacrimal and salivary glands as well as inhibited salivary function, where infiltration was located primarily in the acini and granular convoluted tubules [102].

SJS patients have also been shown to have decreased number of Tregs (CCR7+Foxp3+) in salivary glands compared to controls [101]. In line with this direct tissue evidence, there are also several other reports of fewer salivary Tregs present in patients with SJS [103, 104] and reports of Tregs with decreased suppression capabilities [105]. Reports of increased number of Foxp3+ Treg cells in the salivary glands of patients with SJS [105, 106], was positively correlated with the grade of infiltration as evaluated by Chisholm score [106]. The authors do not address potential mechanisms for this observation. The observed number of Foxp3+ cells in the peripheral blood was unchanging between SJS, RA, and healthy controls [106]. It is possible that the observations of increased numbers of Treg cells in the salivary glands of patients with SJS could be a result of proliferative enrichment due to increased costimulatory signaling (CD28 and CD86), and may have altered suppressive function as a result.

#### 4. Conclusions

In autoimmune diseases, dysfunction of Treg cells is a potential contributor to disease development, where costimulatory requirements for Treg cell proliferation and suppression capabilities may not be met. Based on reviewed research on autoimmune diseases, it appears that contribution of CD86 to Teff cells via CD28 signaling are potentially one of the initiating factors of disease, at least in the cases RA, autoimmune diabetes, and SjS. The lack of CD80 regulatory effects on these diseases appears in general to be due to a lack of expression on certain cell types, such as resident epithelium as in RA and SjS. In the previously mentioned model where CD80 helps suppressive Treg function, this relative lack of CD80 earlier in disease onset could be contributing to the allowance of autoreactive T-cells to respond towards self-antigens. However, regulatory mechanisms of B7 ligand expression as well as the differential signaling mechanisms that control Treg maintenance and function with regards to CD80 and CTLA-4 signaling pathways have yet to be elucidated. Some of the previously mentioned contradictory findings as to abnormal expression of B7 molecules and their function in autoimmune diseases could be explained by multiple factors such as genetic background, relative expression of B7 ligands, immune microenvironment, and timing of critical immunological events. Better understanding costimulatory and inhibitory requirements of CD80 and CD86 are worth further looking into since clinical trials involving CTLA-4-Ig (Abatacept, Orencia) have shown promise in clinical improvement for RA [107] but not so well in controlling flares in patients with non-life threatening SLE [108]. Clinical trials are also underway for MS and Type I diabetes, where findings do show efficacy in controlling disease activity in MS [109] and delaying beta cell loss in diabetes patients [110]. There is no data available for use of CTLA-4-Ig in SjS patients. However, the previously mentioned findings with CTLA-4-Ig in SjS-prone mice do show promise for the use of costimulatory blockade in prevention of disease [99]. Mechanisms involving these observed protective effects of blocking B7 with regards to Treg maintenance and function should also be explored, since regulation of specific Treg populations may contribute to development of autoimmunity. Overall, there are still many questions as to how the CD86/CD80:CD28/CTLA-4 family interacts to form specific immune responses in vivo, especially in the areas of Treg development, homeostasis, and suppression function. It is well established that Treg cells are important to the prevention of autoimmunity. Whether targeting costimulatory molecules to drive desirable Treg development and function to a complete reversal of disease phenotype needs to be further clarified in autoimmune diseases, especially in SjS.

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