Looking Into the Future—Emerging Therapies based on pathogenesis

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ABSTRACT

Previously in this textbook, improvement in the knowledge on the pathogenesis of primary Sjögren's syndrome (pSS) has already been emphasized, as well as the absence of any specific drug for treatment of this disease. In this section, we will try to reconcile pathogenesis and treatment by focusing on the crucial pathogenic steps that could be targeted by emerging therapies. The main new insights into the pathogenesis of the disease are represented by the accumulating data on the involvement of type-I interferon and the triggers of B lymphocyte activation in pSS. First, we will summarize the pathogenic involvement of type-I interferon (IFN) and B-cell-activating-factor of the TNF family (BAFF) in B-cell activation. Interestingly, these recent genetic and pathogenic studies evidenced number of similarities between pSS and lupus, and pSS could be considered as a sort of lupus of mucosa. We will subsequently discuss the different therapeutics that could target such an IFN-BAFF-B lymphocyte axis.

Keywords : interferon, BAFF, TLRs, rituximab, anti-CD22

OVERVIEW ON THE PATHOGENESIS OF pSS

Initial steps

The initial steps in the pathogenesis of pSS involve the interaction between environmental/genetic factors and epithelial cells. Infectious triggering factors or other "danger signals" may have different consequences:

Breach of self-tolerance

Environmental factors, like viruses might contribute to the triggering of the disease by enhancing autoantigen presentation through molecular mimicry or surface exposure of endogenous ribonucleoproteins like SS-A, SS-B, normally hidden from the immune system, in apoptotic blebs, or exosomes. The breach of tolerance might be of central or peripheral origin. Regarding the peripheral origin of the breach of tolerance, some studies have investigated the role of blood or salivary regulatory T-cells, without any consistent evidence of decrease or functional defect in pSS (1,2). Thus, to date, there is no rationale for potential cell-therapy based on the induction of regulatory T-cells in pSS.

Activation of innate immunity and interferon pathways

Recently, the presence of interferon-producing cells and of IFN-α has been shown exclusively in salivary glands of patients with pSS. Likewise, 3 different gene expression studies (2 studies in salivary glands (3,4), 1 in PBMCs (5) demonstrated the activation of interferon pathways in pSS. The type I interferon response is, at least partly, genetically determined. Gene polymorphisms of IRF5, a pivotal transcription in IFN pathways are associated with pS (6). In addition to the role of

genetic factors, immune complexes are the key drivers of the persistent activation of IFN pathways.

One of the main pathogenic consequences of the activation of IFN pathways is the induction of BAFF (B-cell-activating factor of the TNF family, also termed BLyS). BAFF promotes B-cell survival and antibody secretion. Autoreactive B-cells are more dependent on BAFF for their survival than alloreactive B-cells. The BAFF/APRIL "system" has 5 members : BAFF and APRIL(a proliferation-inducing ligand), present on monocytes, dendritic cells and activated T cells, and their receptors. BAFF, which has membrane-bound and soluble forms, is recognized by 3 receptors: BAFF-receptor (BR3), TACI on B and T cells, BCMA on B cells (Figure 1). BAFF-transgenic mice develop polyarthritis and clinical features of lupus and SS (7). In patients with pSS, serum BAFF levels correlate with levels of autoantibodies (anti-SSA/SSB, rheumatoid factor) (8).

BAFF expression is also increased in salivary glands of patients with pSS compared with controls. Interestingly, Sellam et al recently found a decrease of BAFF-R expression on B cells of patients with pSS and with SLE (9). This decrease of BAFF-R was correlated with disease activity in both diseases. There was a negative correlation between BAFF-R expression on B cells and serum BAFF level, suggesting that high serum BAFF level negatively regulates BAFF-R expression on B cells, either by internalisation of the receptor or by shedding out of the membrane.

The role of APRIL in autoimmunity is less clear than that of BAFF. Indeed, APRIL transgenic mice do not develop either B cell abnormalities, serological, nor clinical signs of autoimmunity (10).

Interestingly, through TACI targeting, APRIL could have a dual effect:

- a B-cell stimulatory effect mainly on immunoglobulin isotype switch. Indeed, TACI signalling is involved in immunoglobulin switch. TACI mutations are found in 10% of patients with common variable immunodeficiency (11).
- a negative signal on B cells explaining hyperactivation of B cells in TACI -/- mice (12). Accordingly, APRIL could serve as a homeostatic down-modulator of B-cell hyperactivation induced by BAFF.

* Multiple cellular origins of BAFF in pSS

In pSS, BAFF is not only expressed by the usual "professional" secreting cells (monocytes and dendritic cells), but also by :

- *resident cells of target organs of autoimmunity.* Salivary epithelial cells may express and secrete BAFF, both in patients with SS and healthy subjects (13). Interestingly, this expression is largely increased by stimulation with type 1 or type 2 interferon (IFN). Patients with SS seem to be more sensible to the effect of type 1 interferon for inducing BAFF expression and secretion by salivary epithelial cells. Thus, resident cells of target organs of autoimmunity are not only passive victims, but also play an active role by secreting BAFF after innate immune stimulation, resulting in activation of autoreactive B lymphocytes. The active contribution of epithelial cells to the pathogenesis of pSS, for which the term of "autoimmune epithelitis" (14) has been proposed, is also illustrated by their expression of HLA class II molecules, costimulation

molecules (CD80 or B7-1, CD86 or B7-2, CD40), adhesion molecules (ICAM-1) (14) and of some innate immune receptors, like Toll-like-receptors (TLRs).

- *T cells.* The first report by from Groom et al in 2002 showed the presence of BAFF within the salivary lymphoid infiltrate characteristic of this disease. Later, Lavie et al demonstrated that both T cells of the infiltrate and epithelial cells could express BAFF (15). In autoimmune conditions, BAFF can also be expressed and secreted by circulating blood T cells by monocytes. In some pathologic conditions, T cells could also express BAFF (16, 17).

- *B cells.* Recently, Deridon et al suggested that the B cells of the infiltrate, which are the target of BAFF and express the different receptors of BAFF, could also express the ligand (BAFF itself), leading to an autocrine pathway for BAFF secretion and activation of B cells (18)

**Regulation of BAFF secretion*

IFNS are the main cytokines which stimulate BAFF secretion. Firstly, it was shown that type 2 g-IFN was able to induce BAFF in monocytes and dendritic cells. Then, Litinskiy et al demonstrated than type 1 IFN could induce BAFF secretion by monocytes (19). Type 1 IFN is also able to induce BAFF secretion by salivary epithelial cells (14). Viruses, or *double-stranded RNAs*, are capable to induce directly and strongly, BAFF secretion by salivary epithelial cells or bronchial epithelial cells, using pathways dependent or not on TLRs and IFN (20). Other cytoplasmic RNA sensors, such as PKR, could be involved (21). Thus, immune complexes might contribute to the persistence of BAFF overexpression in pSS. *BAFF* gene polymorphism is not associated with the disease, but might also regulate BAFF secretion (22).

BAFF overexpression results in polyclonal B-cell activation inside target organs and might contribute to the development of lymphoma

BAFF, along with other cytokines and chemokines, creates a microenvironment supportive of B-cell aggregation and differentiation, and production in target organs of the disease of anti-SS-A/SS-B antibodies, with structural features remarkably similar to germinal centres observed in lymph nodes (23). The co-expression of BAFF and BAFF-receptor (BR-3) in some B lymphocytes might contribute to BAFF-mediated autocrine loop of B-cell activation in salivary glands (18). Persistent polyclonal B-cell activation by BAFF overexpression and by immune complexes stimulating the RF-bearing activity of some B-cell receptors might result in the development of lymphomas in 5% of patients with pSS (16 to 18-fold increase compared with the general population (24, 25). Interestingly, increase in serum BAFF is associated with a poor survival in patients with lymphoma (26). BAFF might thus represent one of the bridges joining innate immunity and autoimmune B-cell activation, but also between autoimmunity and lymphoma in pSS.

Other cytokines, chemokines and adhesion molecules are involved in the pathogenesis of the disease

A predominant local Th1 response is observed in salivary glands of patients with pSS, with increased levels of IL-2, IFN-γ, but also of other pro-inflammatory cytokines, like IL-1 and TNF-α. However, Th2 cytokines are also secreted, with high peripheral blood levels of IL-6 and IL-10, cytokines which promote antibody

secretion. A decrease of TGF-β, though controversial, and IL-4 expression in salivary glands of pSS patients has been reported. T-cell-attracting chemokines, such as CXCL-9 (Mig) and CXCL-10 (IP-10) are involved in the migration of T cells in the salivary glands. B-cell-attracting chemokines (CXCL-12 or SDF-1, CXCL-13 or BCA-1) are expressed by epithelial cells, endothelial cells (CXCL-13), contributing to the recruitment of B cells and activated T cells (27,28). An increase of IL-17 was also reported (29).

Autoantibody secretion is pivotal for the persistence of autoimmunity

Anti-SSA/SSB might complex with double-stranded RNAs (Y-RNA) or single-stranded RNA and drive the continuous stimulation of interferon-producing cells through their Fc and Toll-like receptors. The persistent activation of the interferon pathways might thus be related to a vicious circle, in which the environment interacts with genetic factors (HLA-DRB1*15 and DRB1*03 associated with production of autoantibodies (30) and subsequent formation of immune complexes with self RNA; increased secretion of IFN in patients with predisposing IRF5 haplotypes (31, 6)) to drive the mutually co-stimulatory innate and adaptive immune response (Figure 2). Immune complexes might not only stimulate BAFF secretion indirectly, by promoting the activation of IFN but also directly, as shown by the induction of BAFF by epithelial expression after double-stranded RNA stimulation (20). Thus, immune complexes lead to the enhancement of IFN activation and of BAFF secretion, mediating the characteristic B-cell hyperactivation observed in pSS.

Glandular hypofunction rather than glandular destruction (Figure 3)

Recent data suggest that the glandular dysfunction in SS could result from immune mediated inhibition of secretory processes rather than from glandular destruction (32). First, apoptosis of the salivary gland epithelial cells has been shown to be a rare event (33). Secondly, many patients with pSS with disabling objective dryness retain large amounts (30-50%) of histological normal salivary glands. Last, this residual tissue is functional *in vitro* and can be stimulated *in vivo* in patients with pSS using systemic sialogogues.

Salivary and lachrymal secretion is controlled by the binding of acetylcholine on type-3 muscarinic acetylcholine (M3) receptors on the surface of the acinar cells (Figure 3). Many interactions between the immune system and the secretory process could inhibit this neuroexocrine junction:

- inhibition of neurotransmitter release by cytokines (IL-1, TNF- α) (34)

- enhanced breakdown of acetylcholine by increased levels of cholinesterase in pSS (35)

- blockade of M3 receptors by antimuscarinic autoantibodies (36)

- altered NO production and intracellular calcium mobilization

- altered fluid movement due to abnormal distribution of aquaporins (AQP). Salivary acinar cells express AQP5 on the apical cell membrane and AQP1 on the basolateral membrane. Some data indicated that the expression of AQP5 could be reduced at the luminal membrane of salivary acinar cells in pSS but a recent study showed that the distribution of AQP5 in SS was unaltered (37).

EMERGING THERAPIES

Prerequisite for the development of new drugs in pSS

Disease activity score

It is mandatory to validate a consensual disease activity score. After the very interesting preliminary works of Vitali et al (38) and Bowman et al (39), an international consensus Sjögren's activity score is being set up on behalf of EULAR (40).

Selection of patients

For each drug evaluated, the target population should be defined: patients with glandular symptoms only (which must have residual salivary flow, so that a possible improvement can be assessed) or with associated systemic features, patients with recent onset or longstanding disease. Depending on the mechanism of action of the drug evaluated, specific inclusion criteria might be necessary. Likewise, for B-cell inhibitors, controlled trials should first focus on patients with systemic involvement and/or increase in B-cell biomarkers.

New insights into the pathogenesis of pSS explain the inefficacy of TNF antagonists

Increase of BAFF could explain the lack of efficacy of anti-TNF in SS

Two randomized controlled trials, one with infliximab (41), one with etanercept (42) demonstrated absence of efficacy of TNF blockers in SS. A recent work showed the reason of the failure of anti-TNF. On etanercept and not on placebo, pSS patients experienced an increased in type 1 interferon and BAFF secretion which could explain the absence of improvement (43).

Blockade of the IFN-BAFF-B lymphocyte axis

Inhibition of the triggering factors of IFN activation

The main potential therapeutic targets leading to IFN activation are immune complexes, Fcγ receptors, and innate immune receptors including TLR and other RNA sensors. Indeed, immune complexes have to be internalized by dendritic cells and B lymphocytes through Fcγ receptors. Then immune complexes activate TLRs in late, acidified, endosomes, and might also stimulate other cytoplasmic RNA sensors, such as RIG-I, MDA-5 or PKR. Various strategies could be envisioned to inhibit subsequent IFN activation and BAFF secretion:

- Inhibition of immune complexes uptake by inhibitors of Fcγ receptors
- Increase in expression of “inhibitory” Fcγ receptors (Fcγ RII-B)
- Antagonists of TLRs involved in the recognition of immune complexes (TLR3, 7, 8 and 9)

- Inhibition of the activation of endosomes using hydroxychloroquine.

  This “old” drug has shown some interesting effects, notably on the decrease of gammaglobulin levels, in open studies, which might result from the inhibition of TLRs. Thus, the therapeutic interest of hydroxychloroquine should be reassessed in controlled trials (a French multicenter controlled trial is ongoing)

- Inhibition of other RNA sensors, such as PKR

IFN blockade

Recently it was presented the first phase 1 study with an anti-type 1 IFN monoclonal antibody in SLE with good safety and promising efficacy (44). Safety and efficacy have to be confirmed in controlled phase II-III studies.

However, inhibiting type 1 IFN could lead to adverse side effect. In diseases for which an interferon signature has been demonstrated, BAFF targeted therapy could be safer and maybe as efficient.

Antagonists of BAFF and APRIL

BAFF could be a possible bridge between innate and adaptive immunity, and between autoimmunity and lymphoma in pSS, which makes it a particularly interesting therapeutic target.

To date, three different drugs have been designed (Figure 4):

- Belimumab is a monoclonal anti-BAFF antibody which targets only BAFF (45),
- Atacicept is a TACI-Fc molecule which targets both BAFF and APRIL,
- BR3-Fc targets only BAFF

To date, two large phase 2 studies (400 to 500 patients each) have been presented with belimumab. In RA, the results are rather disappointing with around 30 % of ACR 20 response in all belimumab groups versus 15 % in the placebo group (46). This may be explained by the fact that B-cell activation in RA may not be driven only by BAFF. In SLE, the results are more encouraging. Although the primary end-point (decrease of SLEDAI of more than three points) was not achieved in the whole study including 449 patients, the analyse restricted to the 70 % of patients with antinuclear antibodies or anti-DNA antibodies showed a significant effect of belimumab on the decrease of disease activity measured by SLEDAI and anti-DNA antibody level. Phase 3 studies are ongoing in SLE to confirm these preliminary results and phase 2 studies in SS should begin. Phase 2 studies with atacicept and BR3-Fc are ongoing in RA.

Another possible interest of anti-BAFF therapy is its using just after rituximab treatment. Indeed, in all autoimmune diseases treated with rituximab, an increase in serum BAFF after rituximab therapy was observed (47-50). This increase is not exclusively related to the disappearance of BAFF-binding B cells in peripheral blood. Thus, two studies showed a true homeostatic feedback characterized by the increase in BAFF mRNA expression in monocytes after rituximab (48, 50). This increase in BAFF after rituximab could favour the stimulation of new autoimmune B cells. Using BAFF targeted therapy after rituximab to avoid this BAFF increase could therefore be of interest.

B-cell depletion

Rituximab in SS

Rituximab, a monoclonal anti-CD20 antibody has been approved in the treatment of anti-TNF refractory rheumatoid arthritis. Three randomized controlled studies demonstrated its efficacy in this pathology.

Targeting B cells seems also very promising in SS. To date, rituximab has been used in three open studies included 15 to 16 patients (51, 52) and in case reports of lymphomas complicating SS (53, 54) (Table 1). In two of these open studies (51, 52), efficacy on dryness was restricted to patients with early diseases. The third open study included patients with systemic complications since B cell hyperactivity is the higher in this category of patients (55). There was a clear effect of rituximab on systemic complications: parotidomegaly, synovitis, cryoglobulinemia-associated vasculitis. Individual cases with lung or renal infiltrate also improved. However, in this study, there was no change in subjective or objective dryness.

In a first randomized control trial of rituximab in SS (56), 17 patients without any systemic complications were included. Due to the low number of patients, there was no statistically significant difference on the primary end-point (fatigue assessed by visual analogic scale (VAS)) between the two groups, but the decrease of fatigue was statistically significant only in the rituximab group (decrease of around 50 % versus 20 % in the placebo group).

In a second controlled study (57), thirty patients – selected to have salivary flow rates above 0.15 ml/minute -- were randomized to rituximab treatment (n= 20) or placebo. There was a significant improvement in the visual analog scale score for oral and mouth dryness and in salivary secretion rates in the rituximab treated group.

Of note, approximately 10 % of treated patients of the different studies (case reports, open and controlled studies) presented serum sickness-like disease 3 to 7 days after rituximab infusion (fever, arthralgia and purpura). This complication, usually benign, must be differentiated from immediate infusion reactions which are probably due to cytokines release and which will not recur after the next infusions. Serum sickness disease may occur after treatment with chimeric antibodies. Curiously, it is exceptional after treatment of lymphoma with rituximab and has not been described in the randomized control trials of rituximab in RA. Cases of serum sickness diseases have also been described in open studies of rituximab in lupus. The higher frequency of serum sickness disease in SS may be due to hypergammaglobulinemia which is much more common in SS and SLE than in RA.

Other B cells targeted therapy : other anti-CD20, anti-CD22

With the development of humanized or human anti-CD20 monoclonal antibodies or small oral molecules against CD20, the interest of B-cell inhibition using other agents might be further evaluated in pSS.

An open study including 15 patients has been performed with epratuzumab, an anti-CD22 monoclonal antibody (58). This anti-B-cell antibody leads to only partial B-cell depletion (50% in blood). The results of this open study are interesting with improvement of dryness, fatigue and pain VAS. Moreover, salivary flow seems to be improved in patients with early disease. A controlled trial is now necessary to confirm these data.

Other therapeutic perspectives

Inhibition of other cytokines and chemokines

Inhibition of IL-6, which has a demonstrated efficacy in RA, or IL-21, other pivotal cytokines for B-cell activation, might be of interest in pSS. Inhibition of lymphotoxine-beta, which allows, along with BAFF, the formation of germinal centre-like structures in salivary glands, also deserves to be evaluated. Indeed, baminercept, a lymphotoxine-beta receptor-immunoglobulin fusion protein has a dramatic effect in reducing salivary B-cell infiltrates of NOD mice. Moreover, salivary flow was partially restored in the treated mice (59)

Inhibition of T-cell costimulation

Abatacept (CTLA-Ig), which has demonstrated its efficacy in RA, could be of therapeutic interest in pSS. Abatacept could also interact with the antigen-presenting cell properties of epithelial cells, which express CD80 and CD86.

Gene therapy

For patients with glandular symptoms only, it could be speculated that progress in designing harmless vectors might make gene therapy become a true possibility in pSS (60, 61). The good candidate genes for gene therapy remain to be determined.. Of interest is the ongoing evaluation of the possibility of aquaporin-1 gene local livery in the salivary glands, currently evaluated in the NIH for radiotherapy-related dryness (phase 1).

CONCLUSION

Looking into the future. for therapies of pSS, we can feel rather optimistic. pSS is a wonderful model of autoimmunity for translational research, with an easy access to target organs of autoimmunity. Interestingly, recent genetic and pathogenic studies evidenced number of similarities between pSS and lupus, and pSS could be considered as a sort of lupus of mucosa. In pSS like in lupus, new pathogenic pathways have been and will be unravelled, resulting in the definition of new targets. To convert our optimism into the improvement of patients in daily care, a consensual disease activity score must be validated and clinicians and pharmaceutical companies have to design new trials with adequate inclusion criteria. Indeed, pSS is not a lost battle for the development of efficient biotherapies !

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Table 1. Efficacy and indications of RTX in patients with pSS

Authors, years	Number of patients	Indications of RTX	Efficacy for lymphoma	Efficacy for systemic features	Efficacy for objective dryness	Efficacy for subjective dryness	Adverse events
Somer, 2003 [53]	1	Lymphoma	Yes	NR	Yes	Yes	No
Voulgarelis, 2004 [54]	4	Lymphoma (4/4)	4/4 (100%)	3/3 (100%)	NM	NM	2/4 (50%) 2 IRR
Harner, 2004 [62]	1	Lymphoma	Yes	NR	NM	Yes	NM
Ramos-Casals, 2004 [63]	2	Lymphoma (2/2)	Yes	NR	NM	NM	NM
Pijpe, 2005 [51]	1	Lymphoma	Yes	NR	Yes	Yes	No
Gottenberg, 2005 [64]	6	Lymphoma (2/6)	1/2 (50%)	NR	N (0/2)	3/6 (50%)	2/6 (33%)
		Systemic features (4/6)	NR	4/4 (100%)			1 SSR, 1IRR
Ahmadi-Simab, 2005 [65]	1	Scleritis	NR	Yes	NM	NM	NM

Pijpe, 2005 [51]	15	Lymphoma (7/15)	3/7 (43%)		28.6% (2/7)	Yes	6/14 (43%)
		Early pSS (8/15)		NM	100% (7/7)		3 SSR, 2 IRR
Ring, 2005 [66]	1	Renal tubular acidosis	NR	No	Yes	Yes	No
Seror, 2006 [55]	16	Lymphoma (5/16)	4/5 (80%)	NR	2/16 (18%)	5/16 (36%)	4/16 (25%)
		Systemic features (11/16)	NR	9/119 (82%)			2 SSR, 2 IRR
Meijer, J., 2008 [57]	30	Mainly glandular symptoms	NR	Yes	Yes	Yes	1 SSR, 2 IRR
Devauchelle V, [52]	16	Mainly glandular symptoms	NR	Yes	No	Yes	1 SSR, 2 IRR
Saint-Clair, 2007 [67]	12	Glandular symptoms	NR	NR	No	No	4 IRR
Dass, 2008 [56]	17	Glandular symptoms	NR	NR	No	No	1 SSR, 2IRR

IRR: infusion-related reaction; NR: not relevant; NM: not mentioned; SSR: serum sickness-like reaction

Figure 1. The BAFF(BLyS) / BAFF receptors system : a crucial role for B cell activation in autoimmune diseases, notably in pSS.

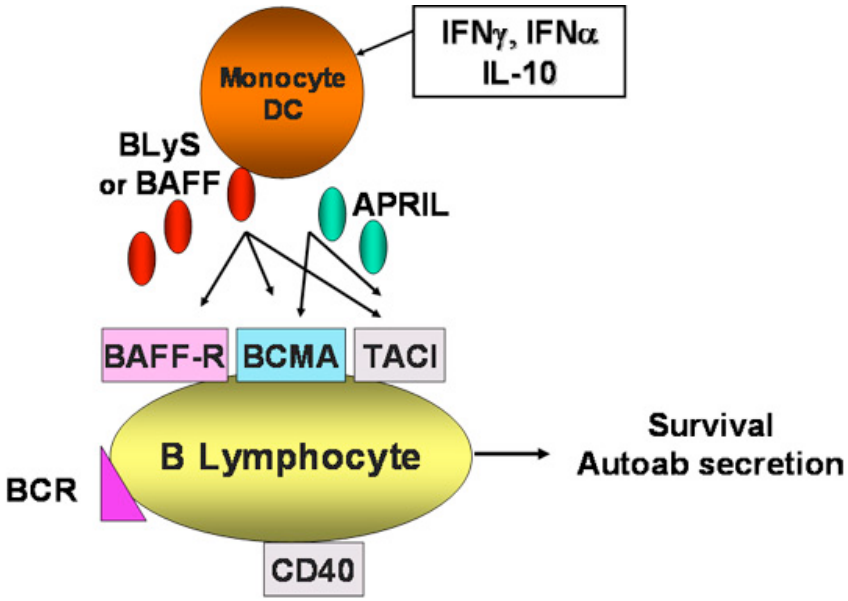

Figure 2. Contribution of genetic and environmental factors, innate and adaptive immunity to the pathogenesis of pSS. pDC: plasmacytoid dendritic cell, mDC: myeloid dendritic cell

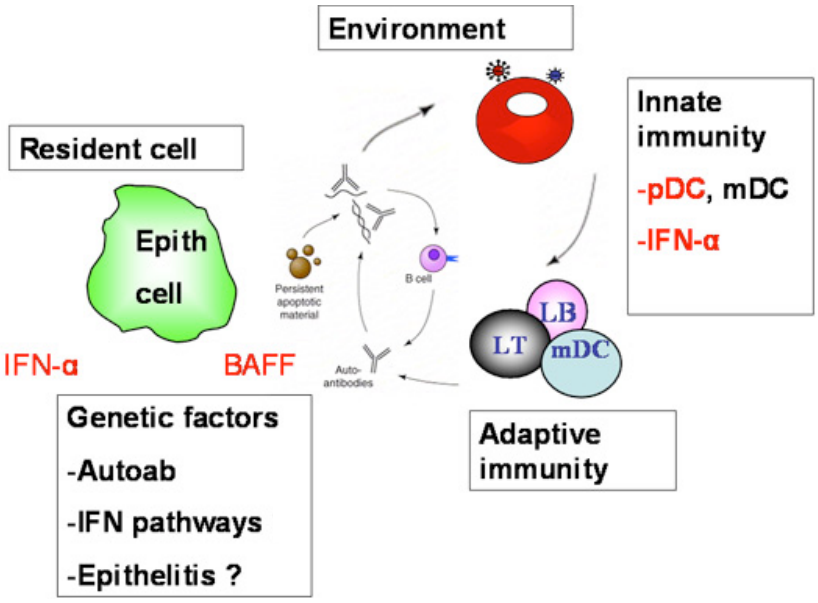

Figure 3 - Dysfunction of the neuro-exocrine synapse in pSS, due to metalloproteases, autoantibibodies, cytokines, and T-lymphocyte mediated cytotoxicity.

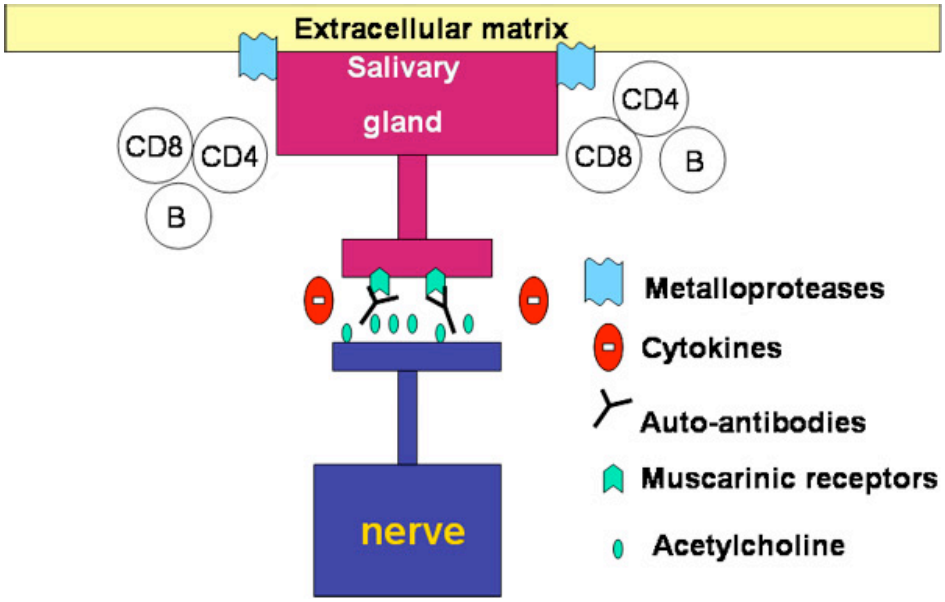

Figure 4: The 3 treatments on development inhibiting BAFF or BAFF + APRIL