

## **Reovirus-1 (a dsRNA Virus) and Poly (I:C) Induce High Expression of BAFF mRNA and Protein by Salivary Epithelial Cells Through TLR3 / Type I-interferon - Dependant and - Independent Pathways**

Marc Ittah<sup>1</sup>, Corinne Miceli-Richard<sup>1</sup>, Jacques-Eric Gottenberg<sup>1</sup>, Jérémie Sellam<sup>1</sup>, Pierre Eid<sup>2</sup>, Christine Lepajolec<sup>1</sup>, Pierre Lebon<sup>3</sup>, Xavier Mariette<sup>1</sup>. <sup>1</sup>Bicetre Hospital, Le Kremlin Bicetre, France; <sup>2</sup>CNRS-UPR 9045, Villejuif, France; <sup>3</sup>Saint-Vincent de Paul Hospital, Paris, France  
Presentation 1360

**Background:** B-cell activating factor (BAFF) plays a key role for promoting B-lymphocyte activation and survival in primary Sjögren's syndrome (pSS). We have previously reported that salivary gland epithelial cells (SGEC) have the property to express and secrete BAFF after stimulation by type I interferon (IFN), suggesting the pivotal role of SGEC and IFN in the pathophysiology of pSS.

**Objective:** To investigate if viral infection could induce BAFF expression in SGEC. If so, define if BAFF induction pathways were Toll-like receptors (TLRs) and/or type I IFN dependant.

**Methods:** Cultures of SGEC were established from minor salivary glands obtained from 7 patients with sicca symptoms . Expression of TLRs was investigated by RT-PCR. BAFF mRNA and protein expression was analyzed by Q-PCR and ELISA after stimulation of the different TLRs by agonists or viruses. The endosomal pathway was inhibited by chloroquine. Type I IFN induction pathway was inhibited by an anti-IFNAR1 antibody. The proinflammatory cytokines IL-6, IL-8, and RANTES were analyzed by ELISA.

**Results:** We detected expression of TLR-2, -3, and -7, but not TLR-9 mRNA. Chemical agonists of TLR-2 (PGN), TLR-7 (R837) and TLR-9 (CpG) did not induce BAFF expression. HSV1 (a dsDNA virus) induced BAFF mRNA but no protein. Reovirus (a dsRNA virus) and Poly (I:C) (a TLR-3 agonist) induced high expression of BAFF mRNA (ratio BAFF/actin:  $81.37 \pm 15.89$  (SEM) and  $100.45 \pm 19$  respectively). Likewise BAFF protein was detected in the supernatants of SGEC after reovirus infection and poly (I:C) stimulation:  $76.32 \text{ pg/mL} \pm 7.27$  and  $62.09 \pm 5.83$  respectively. After reovirus infection, the inhibitions of endosomal pathway by chloroquine and of IFN pathway by anti-IFNAR antibody partially down-regulated BAFF mRNA by 37 % and 40 %, respectively ( $p=0.029$  and  $p=0.03$ ) and BAFF protein by 31 % and 24 % ( $p= 0.03$  and  $p=0.08$ ). After poly (I:C) stimulation, the inhibition of endosomal pathway was weaker than with reovirus-1 and IFNAR1 blockade had no effect on BAFF mRNA and protein. IL-6, IL-8 and RANTES were significantly up-regulated after reovirus-1 infection and poly (I:C) stimulation ( $p<0.01$ ). Chloroquine inhibited reovirus-induced IL-6 and IL-8 secretion ( $p<0.01$ ).

**Conclusions:** dsRNA virus infection and poly (I:C) stimulation directly induce high expression of BAFF mRNA and protein by SGEC probably through two different mechanisms. The first one implicates TLR-3 and type I IFN dependent pathways, the second one is TLR-3 and type I IFN independent and could involve NF-kB through the RIG-I/MDA5 pathway, two intracellular receptors for dsRNA. This high induction of BAFF by SGEC after viral infection sheds some new lights on the mechanisms of induction of autoimmunity by innate immunity.