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SAT0003

PIM1 REGULATES CD4+ T CELL EFFECTOR FUNCTION IN EARLY RHEUMATOID ARTHRITIS AND HAS POTENTIAL FOR USE AS A NOVEL, MEASURABLE THERAPEUTIC TARGET

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Background: We previously showed PIM1, among other STAT3-target genes, to be strikingly upregulated in circulating CD4+ T cells of treatment-naïve 'early' rheumatoid arthritis (eRA) patients^{1,2}. PIM1 has a recognised role in T cell development, has been implicated in the pathogenesis of autoimmunity³, and is the target of specific inhibitors in clinical development in oncology; for example PIM1 was recently identified as a promising target in triple-negative breast tumours⁴.

Objectives: We sought to understand the relevance of dysregulated PIM1 gene transcription for T-cell pathobiology during disease initiation and to determine the effects of its inhibition on T cell function. We hypothesised that, amongst a readily-identifiable, high PIM1 expressing subgroup of eRA patients, PIM1 overexpression in CD4+ T cells is a targetable early event in pathogenesis that lies downstream of STAT3-mediated IL-6 signalling.

Methods: Peripheral blood was obtained from consenting treatment-naïve arthritis patients or healthy donors and highly pure CD4+ T cells were isolated. PIM1 knock-down (SMARTpool siRNA, Dharmacon) or protein inhibition (small molecule inhibitors; PIM1-selective TCS-PIM-1 1, Tocris and panPIM AZD1208) was undertaken. Flow cytometric analysis was then used to assess activation and proliferation following CD3/CD28-mediated stimulation.

Results: In eRA, *ex vivo* CD4+ T cells exhibited an activated, hyper-proliferative phenotype compared with those isolated from healthy donors. Significantly higher PIM1 mRNA expression in CD4+ T cells was seen using PrimeFlow (flow cytometry) compared to that of other inflammatory arthritides. Both PIM1 knock-down, PIM1-specific and panPIM inhibition decreased the activation and proliferation of stimulated eRA (Figure 1A) and healthy donor CD4+ T cells. Moreover, in eRA, PIM1 and panPIM inhibition increased FoxP3 expression (Figure 1B) and increased the proportion of regulatory T cells (Figure 1C).

Figure 1. Early RA CD4+ T cell (A) proliferation is significantly decreased by either a PIM1-selective or a panPIM small molecule inhibitor, whereas (B) FoxP3 expression and (C) the proportion of regulatory T cells is increased.

Conclusion: Taken together, these data implicate PIM1 as prominent among genes whose induction may "pre-programme" CD4+ T cells to function aberrantly in disease. Conceivably, targeting PIM1 may prove an attractive approach to regulating aberrant CD4+ T cell effector function in an identifiable sub-population of early RA patients that exhibit high CD4+ T cell PIM1 expression in their peripheral blood.

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SAT0004

EVALUATION OF AUTOPHAGY IN INFILTRATING AND CIRCULATING LYMPHOCYTES FROM PATIENTS WITH SJOGREN'S SYNDROME

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Background: Autophagy is a lysosome mediated catabolic process that promotes cell survival during stress conditions. Aberrant regulation of autophagy is involved in the pathogenesis of a widening spectrum of autoimmune disorders: in particular, activation of autophagy pathway is implicated in aberrant survival of activated T cells. We recently demonstrated that autophagy is up-regulated in CD4+ T lymphocytes from patients with Sjogren's Syndrome (SS) both in peripheral blood and tissue. Moreover, aberrant autophagy correlates with systemic disease activity. Little is known on the role of autophagy in B cells homeostasis in SS.

Objectives: To investigate the activation of the autophagy pathway in lymphocytes infiltrating salivary gland in SS and peripheral blood from patients with SS.

Methods: Frozen SS minor salivary glands (MSG) from 20 patients were sectioned and evaluated with immunohistochemistry (IHC) by staining for CD3+, CD20+, and CD21+. MSG sections, sequential to IHC, were stained by Cresyl Violet and microdissected (Laser Capture Microdissection) in order to isolate and collect small infiltrates, large CD21- infiltrates, and large CD21+ infiltrates (GC-like structures). Microdissected GC from frozen human tonsils were used as control. The expression of autophagy genes Atg5 and MAP1LC3II (expressed as 2^{ΔΔCT} normalized to GAPDH) was determined by qPCR on microdissected tissue.

For studies on peripheral blood, 19 SS patients and 11 healthy controls were enrolled, and autophagy, expressed as the ratio between Mean Fluorescence Intensity and the isotopic control (rMFI), was evaluated by Cyto-ID[®] Autophagy detection kit in T lymphocytes (CD4+ and CD8+) and B lymphocytes (CD19+) using a FACSCalibur cytometer.

Results: Expression of the autophagy genes Atg5 and MAP1LC3 was significantly higher in the GCs from SS salivary glands compared to control tonsils (p<0.0001 and p=0.0013, respectively). In the salivary glands of SS patients, progressively higher expression levels of both Atg5 and MAP1LC3 mirrored the organization and severity of the inflammatory infiltrates (small vs. large CD21+ infiltrates p=0.0005 and p<0.0001 respectively).

Immunophenotyping studies revealed activation of the autophagy pathway in both circulating CD19+ and CD4+ lymphocytes from SS (p=0.04 and p=0.03 compared to HC); conversely, no pathway activation was detected in CD8+ cells. Of note, in patients with SS activation of autophagy was most marked in circulating CD19+ B cells compared the other subsets [(CD19+ vs CD4+ (p<0.0001); CD19+ vs CD8+ (p= 0.001)].

Conclusion: Our findings revealed that the autophagy pathway is aberrantly activated in lymphocytes infiltrating salivary glands of SS patients. Of note, progressively higher expression levels of autophagy genes mirrored the severity and extension of inflammatory infiltrates. Immunophenotyping studies revealed that activation of autophagy is preminent in B cells. Thus, activation of autophagy emerges as a feature of both infiltrating and circulating SS lymphocytes, and as mechanism possibly implicated in the activation and survival of autoreactive cells.

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