Paradoxical role of IL-1β in osteoblast may generate a different signal that regulates osteoblast markers expressed in the heterogeneous subchondral bone changes in OA. Understanding these mechanisms could pave the way towards targeted therapeutic interventions.

Acknowledgement: This study was supported by the Academy of Medical Sciences and the Material Science Institute Lancaster University.

Disclosure of Interests: None declared


THU0024 INHIBITION OF CELL PROLIFERATION AND PROMOTION OF INTERLEUKIN-8 PRODUCTION BY T-614 IN CULTURED HUMAN AORTIC ADVENTITIAL FIBROBLASTS

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Background: Takayasu's arteritis (TA) is an inflammatory fibrosing arteritis that affects predominantly the aorta and its main branches. Recently, growing evidence suggests that adventitial fibroblasts play an essential role in vascular inflammation [1]. Tumor necrosis factor-α (TNF-α) can reportedly induce inflammation of vascular adventitial fibroblasts[2]. T-614 (iguatimod), a novel disease-modifying antirheumatic drug, has been widely used in rheumatoid arthritis in China and Japan [3]. However, the effect and mechanism of T-614 on TA have received little attention.

Objectives: We report the effects of T-614 on cell proliferation and interleukin-8 (IL-8) production in cultured human aortic adventitial fibroblasts (HAAF), and explored its possible effect on the treatment of TA.

Methods: HAAF were cultured with 0, 5, 50, 100, or 250 μg/ml T-614 in the absence or presence of 10 ng/ml TNF-α in vitro. Cell viability of HAAF was determined by a modified MTT assay. Supernatant IL-8 level was measured by enzyme linked immunosorbert assay.

Results: (1) After subculture, HAAF were polygonal or spindle-shaped under the microscope (Figure 1A, B). (2) In the presence of TNF-α, compared with the contrast group, cell viability of HAAF significantly decreased in 50, 100, and 250 μg/ml T-614 treatment groups (OD value: P<0.01, P<0.001, respectively; survival fraction (SF): P<0.05, P<0.001, P<0.001, respectively) (Table I, Figure 2). However, there was no significant difference in cell viability between TNF-α stimulated and unstimulated groups at the same concentration of T-614. In the absence and presence of TNF-α, T-614 suppressed HAAF cell viability dose-dependently (OD value: r = -0.915, P<0.001 and r = -0.926, P<0.001, respectively; SF: r = -0.897, P<0.001; r = -0.885, P<0.001, respectively). (3) In the absence of TNF-α, compared with the contrast group, IL-8 level in 5 and 100 μg/ml T-614 treated groups were significantly higher (P<0.05); in the presence of TNF-α, IL-8 level in 5, 50, and 100 μg/ml T-614 treated groups were significantly higher (P<0.001, P<0.001, P<0.001, respectively) (Table I, Figure 3). There was a negative correlation between supernatant IL-8 level and the concentration of T-614 in groups stimulated with TNF-α (r = -0.670, P<0.001). TNF-α increased IL-8 level in the control group and various concentrations of T-614 treated groups (all P<0.001).

Table I. Effects of T-614 on TNF-α stimulated cell viability of HAAF (OD value)

<table>
<thead>
<tr>
<th>T-614(μg/ml)</th>
<th>OD value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ng/ml TNF-α</td>
<td>0.24±0.038</td>
</tr>
<tr>
<td>5</td>
<td>0.04±0.050</td>
</tr>
<tr>
<td>50</td>
<td>0.34±0.061</td>
</tr>
<tr>
<td>100</td>
<td>0.27±0.034</td>
</tr>
<tr>
<td>250</td>
<td>0.09±0.019</td>
</tr>
</tbody>
</table>

TNF-α, tumor necrosis factor-α; HAAF, human aortic adventitial fibroblasts. Data are shown as mean ± SD of 3 independent experiments, each in triplicate. *P < 0.001, †P < 0.01 vs. control group (0 μg/ml T-614) within each group alone.

Conclusion: T-614 can inhibit the proliferation of HAAF and promote IL-8 production; therefore, it may provide a new immunotherapeutic intervention for TA.

REFERENCES:
Background: Psoriatic arthritis (PsA) is frequently associated with enthesitis. It has been proposed that inflammatory processes at the synovio-enthesal complex are involved in the pathogenesis of inflammatory arthritides such as RA, tenocytes are a major component of tendons and entheses and play a central role in tendon inflammation observed in PsA.

Objectives: To investigate whether PsASF and tenocytes show significant interactions while being stimulated with the above cytokines alone as well as in combination with the aim to find out whether these may contribute to the pathogenesis of PsA.

Methods: SF were isolated from patients with PsA undergoing joint surgery. Human tenocytes were acquired commercially and isolated from hamstring tendon tissue of patients undergoing hamstring tendon ACL reconstruction. PsASF and tenocytes were stimulated with IL-1β, TNF-α, IFN-γ, IL-15 and IL-23 alone and in combination. Direct cell co-culture experiments were performed at a 1:1 ratio of both cell types in parallel to experiments with single cell type cultures. IL-6 levels were measured by ELISA to quantify the immunological activation of the cells.

Results: PsASF as well as tenocytes showed strong responses to IL-1β (tenocytes [1173-fold, n=3]; PsASF [56-fold, n=3]) and TNF-α (tenocytes [110-fold, n=3]; PsASF [9-fold, n=3]) stimulation regarding IL-6 secretion. IFN-γ alone had only minimal effects on both cell types but acted synergistically when applied together with IL-1β (tenocytes [218-fold, n=3]; PsASF [129-fold, n=3]) and TNF-α (tenocytes [24-fold, n=3]; PsASF [19-fold, n=3]). IL-15 and IL-23 alone showed no effect but the data suggest a small antagonistic effect against IL-1β (tenocytes IL-15 [211-]/IL-23 [27%], n=3; PsASF IL-15 [19%], n=3) and TNF-α induced IL-6 secretion. Overall, PsASF and tenocytes showed similar responses in the single cell type stimulation experiments. Co-culturing PsASF and tenocytes did not reveal any synergistic or antagonistic interactions in regards to any of the cytokines used.

Conclusion: Our data suggest that tenocytes and PsASF do not interact in a way that would promote inflammation within the synovio-enthesal complex. Also, as far as the induction of IL-6 is concerned, PsASF and tenocytes are not major target cells of IL-15 and IL-23. IFN-γ, however, may be able to promote inflammation in combination with other cytokines in both cell types.

Disclosure of Interests: Felix Dechant: None declared, Klaus Frommer: None declared, Neal M. Millar: None declared, Iain McInnes: Grant/research support from: Projekt supported by an unrestricted educational grant from Celgene GmbH., Elena Neumann: None declared, Ulf Müller-Ladner: None declared, Stefan Rehart: None declared, Klaus Frommer: None declared, Iain McInnes: Consultant for: AbbVie, Celgene, Galvani, Lilly, Novartis, Pfizer, UCB Pharma, Stefan Rehart: None declared, Ulf Müller-Ladner: Grant/research support from: Projekt supported by an unrestricted educational grant from Celgene GmbH., Elena Neumann: None declared