Overview

Patients with Psoriatic Arthritis (PsA) often present a complex of clinical features, including: Arthritis in the peripheral joints, inflammation of the entheses, (at the junction of tendon to bone), bone erosion and new bone formation leading to dactylitis.

Serum concentration of IL-23 is reported to be elevated in patients with ankylosing spondylitis. Recently, overexpression of IL-23 in mice has been shown to lead to axial and peripheral enthesitis, as well as new bone formation by acting on resident T cells. Here we apply and characterize a preclinical model of PsA induced by systemic IL-23 expression in mice. Follow up and quantification of inflammation and bone lesions were demonstrated by means of dual fluorescence and X-Ray imaging.

Materials & Methods

A quantity of 3 µg mcIL-23 enhanced Episomal Expression Vector (IL-23 EEV, SBI) was injected into 8 week-old B10. RIII male mice (Charles River, France) by hydrodynamic i.v. injection. Control animals received Ringer solution.

Circulating levels of IL-23 were measured at 5 days post injection (Mouse IL-23 Quantikine ELISA Kit, R&D systems).

Inflammation of the paw and fingers were scored according to Sherlock et al. Inflammation and new bone formation were assessed with in vivo non-invasive molecular imaging, using ProSense680 and OsteoSense750EX probes (Perkin Elmer, France), respectively. Images were captured using the Bruker In-Vivo Xtreme imaging system. Bone damage and dactylitis were confirmed using high magnification X-ray imaging system. At the terminal endpoint, hind limbs were collected for histology analysis.

In vivo Fluorescence and X-ray Imaging

At 24 hours before imaging, probes were administered to mice (0.8 nmol/10 g, i.p.) according to the workflow shown in Table 1.

<table>
<thead>
<tr>
<th>Day</th>
<th>Group 1 (control)</th>
<th>Group 2 (mIL23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Ringer hydrodynamic delivery</td>
<td>IL 23 EEV hydrodynamic delivery</td>
</tr>
<tr>
<td>5</td>
<td>Blood sampling for IL 23 assay</td>
<td>ProSenseTM in vivo imaging</td>
</tr>
<tr>
<td>7</td>
<td>ProSenseTM in vivo imaging</td>
<td>ProSenseTM / OsteoSenseTM / X-ray in vivo imaging</td>
</tr>
<tr>
<td>21</td>
<td>ProSenseTM in vivo imaging</td>
<td>ProSenseTM / OsteoSenseTM / X-ray in vivo imaging</td>
</tr>
<tr>
<td>35</td>
<td>X-ray in vivo imaging with magnification stage</td>
<td>ProSenseTM / OsteoSenseTM / X-ray in vivo imaging</td>
</tr>
<tr>
<td>43</td>
<td>ProSenseTM / OsteoSenseTM / X-ray in vivo imaging</td>
<td>ProSenseTM / OsteoSenseTM / X-ray in vivo imaging</td>
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Table 1: Experimental workflow
Granulocyte infiltration and bone remodeling were measured using a Bruker In-Vivo X-Limitor the imaging system, equipped with a deeply cooled 4 MP CCD camera. Fluorescent images were acquired with the following camera settings: 5 sec acquisition time, f-stop 2.8, binning 1 x 1, X-ray filter 0.2 mm and an X-ray energy of 45 kVp. All images were taken with a 190 mm x 190 mm field of view (FOV).

For the analysis of bone damage and dactylitis, mice were assessed using X-ray imaging with magnification stage of the Bruker In-Vivo Xlimitor at 10 sec acquisition time, f-stop 2.1 x 1 binning, 0.4 mm X-ray filter, X-ray energy 45 kVp.

Image Analysis and Presentation

Signal intensity analysis was performed using Molecular Imaging Software version 7.1 (Bruker BioSpin, Billerica, MA, USA). A free form Region of interest (ROI) was drawn around forelimbs and hind limbs. Based on the control mouse images of Group 1, a fluorescence signal dynamic range threshold of 400 AU was selected. ROI data were exported and saved to Microsoft Excel® files, while images were exported as JPEG files.

Histology:

At sacrifice, the hind paws were fixed in buffered 10% formalin, decalcified, paraffin embedded and sectioned. For each paw, one µm thick section every 10 sections was collected and stained with hematoxylin-eosin (HE) and one adjacent section stained with anti-MPO for neutrophil detection.

Statistical Analysis

For each ROI, the signal mean and standard error to the mean (s.e.m.) were calculated. A statistically significant difference between the study groups was evaluated with Prism® software using a one-way ANOVA followed by a Dunnett’s multiple comparisons post-hoc test. In Figures presented here, the statistical significance level observed was symbolized as follows: * = p<0.05, ** = p<0.01, *** = p<0.001.

Results/Discussion

From day 8 onwards an IL23-induced swelling of paw and fingers could be observed and which is in line with findings from Sherlock et al.1 (Figure 1, upper panel). Follow-up of the inflammatory process was assessed also using non-invasive molecular imaging (Figure 1 lower panel). Good correlation was observed between PsA clinical scores of mice and granulocyte infiltration (Figure 1). In addition, localization of the fluorescence around the heel was compatible with inflammatory pathology focused on the entheses as reported previously1 and thus represent the molecular footprint of IL23-induced swelling (Figure 2).

In the later phase of the disease, bone lesion and dactylitis, both hallmarks of the disease, were clearly visible before sacrifice. Planar 2D X-ray imaging has demonstrated micro-calculifications at these sites displaying highest bone formation and remodeling (Figure 3).

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In vivo fluorescence imaging in combination with high resolution, low dose X-ray together provided a sensitive, non-invasive approach for the detection of inflammation and bone damage processes in a preclinical model of PsA, induced by systemic IL-23 expression in mice. These data were supported by a clinical gold-standard as well as histopathology analysis. The non-invasive model characterisation of PsA processes introduced here, provides future investigations a new, high throughput experimental platform for conducting rapid, statistically robust evaluations of therapy/new therapy combinations in the field of PsA.

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References
