The Role of Notch Signalling in the Pathogenesis of Psoriasis
ABSTRACT

**Background:** Angiogenesis plays a critical role in the pathogenesis of psoriatic arthritis (PsA) and psoriasis (Ps) and is one of the first histologically detectable features of inflammation in the synovial tissue and lesional skin. Notch signalling pathways are critical for angiogenesis and endothelial cell (EC) fate; however, the mechanisms regulating these processes in the inflamed joint remain to be elucidated. In this study, we examine the expression of Notch signalling components in PsA and Ps, and examine whether Notch signalling mediates the effect of TNFα inhibition.

**Materials and methods:** Immunohistochemical analysis for Notch-1 and Jagged-1 was performed in synovial tissue sections from PsA (n=14) compared to healthy control synovial tissue (n=5) and in Ps lesional skin (n=6) pre/post anti-TNFα therapy (Infliximab). To examine the direct effect of TNFα on Notch-1 signalling, synovial fibroblasts (K4IM) were cultured for 24 hrs with TNFα (10ng/ml) and protein and mRNA expression analysed (n=3). Notch-1 and Jagged-1 mRNA was quantified by Real-time PCR and Notch-1IC was assessed by Western blot analysis.

**Results:** Increased expression of Notch-1 and Jagged-1 was demonstrated in PsA synovial tissue compared to healthy control synovium (p<0.05). Expression was localised to perivascular/vascular, sub-lining and lining layer regions. Increased expression of Notch-1 and Jagged-1 was also demonstrated in Ps lesional skin compared to uninvolved skin (p<0.05), expression of which was significantly inhibited following TNFi therapy (p<0.05). TNFα significantly induced Notch-1 and Jagged-1 mRNA expression (p<0.05) and induced protein expression of Notch-1 IC, indicating active transcription.

**Conclusion:** Together these results suggest that Notch-1 signalling may in part mediate the pro-inflammatory effects of TNFα in Ps and PsA. The Notch-1 signalling pathway may represent a potential therapeutic target for Ps and PsA.

**Keywords:** Notch, Psoriasis, Psoriatic Arthritis, Angiogenesis, Inflammation
INTRODUCTION

The clinical course of inflammatory arthritis is variable ranging from a mild self-limiting arthritis to a rapidly progressive disease with disabling joint destruction, extra-articular manifestations and an increase in long-term morbidity and mortality. Psoriatic Arthritis (PsA) is one of the most common inflammatory arthritides accounting for 15% of referrals to early arthritis clinics and has considerable morbidity (Kane et al, 2003). PsA was originally defined by Wright as an erosive polyarthritis in the presence of psoriasis, usually seronegative for rheumatoid factor (RF) (Wright, 1956), and then expanded to include peripheral or axial involvement, in the presence of skin or nail psoriasis, usually seronegative for RF (Moll and Wright, 1973). A new classification by Veale et al 1994 included (1) an asymptomatic oligoarthritis with few erosions and good preservation of joint function; (2) a RF negative but frequently erosive symmetrical polyarthritis; (3) predominant spondylitis. CASPAR (Classification criteria for Psoriatic Arthritis) group proposed a revised criteria for the diagnosis of PsA in 2006, where a diagnosis of PsA could be made with a sensitivity of 98.7% and a sensitivity of 91.4%, which was superior to previously published classification (Taylor et al, 2006).

Initially, PsA was considered a benign disease, with one study suggesting only 11% of patients developed erosions over 7 years (Shbeeb et al, 2000). However, accumulating evidence has proven that the course of PsA, if undertreated, may lead to serious long term morbidity and increased mortality. Increased radiographic joint damage, elevated laboratory markers of inflammation, elevated swollen joint counts, female gender and baseline functional disability are all associated with a poor disease outcome in both PsA and RA (Roberts et al, 1976; Deighton et al, 1992; Gladman et al, 1998; Zangger et al, 1998; Dossaers-Bakker et al, 2002). Furthermore, PsA patients have associated psoriasis which is a chronic inflammatory dermatosis affecting the skin, hair, nails and joints. This characteristic lesion is a well-defined erythematous scaly plaque with a sharply delineated edge that appears at any site on the body but most commonly affects the elbows, knees, intergluteal cleft, nails and scalp.

Tumor necrosis factor alpha (TNFα) is a pro-inflammatory cytokine involved in the regulation of
innate immunity and inflammation. The use of TNF inhibitors (TNFi) has revolutionised the treatment of PsA and Ps. TNFi therapies have been shown to improve disease activity indices (clinical and laboratory) as well as inhibiting radiographic progression (Bathon et al, 2000; Lipsky et al, 2000; Weinblatt et al, 2003; Fransen et al, 2006; Gladman et al, 2007). Early initiation of therapy can reduce disability and future disease burden (Smolen et al, 2003; Breedveld et al, 2006; Emery et al, 2008). Furthermore, Saber et al 2011 demonstrated a higher percentage of PsA patients achieve DAS remission compared to RA, with rate of remission significantly greater in PsA. However, TNFi therapies are expensive and have potentially serious side-effects. An important goal for rheumatologists/dermatologists treating PsA and Ps patients is to identify markers that can (1) predict response to TNFi therapy, (2) predict remission rates and (3) predict those patients who can maintain remission following withdrawal of TNFi therapy (Hyrich et al, 2006; Nam et al, 2009).

The initiating trigger involved in the pathogenesis of PsA remains unknown; however angiogenesis is thought to be one of the primary events. Distinct macroscopic vascular morphology in the joints of early PsA has been demonstrated, characterised by elongated, dilated, tortuous vessels with no branching (Reece et al, 1996; Fearon et al, 2003), which is associated with differential expression of pro-angiogenic factors in synovial fluid and tissue such as VEGF, Ang 1/2, Placental growth factor (PIGF), platelet-derived growth factor (PDGF), Neural Cellular Adhesion Molecule (NCAM) or Transforming Growth Factor β1 (TGFβ1), which can promote either immature or mature stable vessels (Asahara et al, 1998; Fraser et al, 2001; Darland and D'Amore 2001; Lobov et al, 2002; Fearon et al, 2003; Schomber et al, 2007; Zhang et al, 2008; Koch et al, 2003; Kennedy et al, 2010). In Ps a similar blood vessel pattern has been demonstrated with microvessels of the papillary dermis characterised by elongated, tortuous and dilated vessels, with increased EC proliferation and activation (Braverman et al, 1974; Braverman et al, 1977; Brooks et al, 1994). Furthermore, increased expression of VEGF, Ang2, PECAM, PDGF, PIGF, NRP-2 and podoplanin have been demonstrated in psoriasis skin (Krane et al, 1991; Cañete et al, 2004; Markham et al, 2006; Henno et al, 2010).

Angiogenesis and persistent inflammation is highly dependent on specific signalling pathways that will preserve the integrity of the newly formed vessel and induce cell survival. One such
pathway is the Notch signalling pathway, which is highly conserved playing a pivotal role in vascular development by receptor-ligand interactions, cell-cell communication and cell fate decisions. Notch-ligand interactions are critically important for vessel tip cell selection (De Smet et al 2009), for EC-pericyte interactions and vascular network (Sullivan et al, 2003; Liu et al, 2003; Armulik et al, 2005; Gridley, 2007). Four Notch receptors (Notch 1-4) and five ligands (Jagged-1, -2, Delta-1, -3 and -4) have been identified in mammalian cells (Iso et al, 2003; Gridley, 2007). After binding, Notch is cleaved intracellularly (Notch IC) and translocates to the nucleus and transcriptionally activates Notch target genes (Hes and Hrt) which regulate cell fate decisions (Lai, 2002; Iso et al, 2003). Over-expression or knockdown studies have shown that Notch IC signalling determines proliferation, differentiation and apoptosis in several mammalian cell types (Iso et al, 2003) and can result in embryonic lethality due to defects in vasculogenesis, angiogenesis and EC migration (Xue et al, 1999; Huppert et al, 2000; Duarte et al, 2004; Gale et al, 2004).

Notch-1 expression has been demonstrated in RA synovial tissue and lesional skin, with levels of expression associated with disease severity (Gao et al 2012, Liu et al, 2010), however to date no studies have examined the localised distribution of the Notch ligands or downstream signalling components. While Notch-Delta-4 interactions are known to mediate growth factor regulation of EC, such as VEGF, studies have also shown that in response to pro-inflammatory cytokines Notch-1 may preferentially interact with Jagged-1 (Sainson et al, 2008). While the role of the Notch signalling pathway is well established in the vasculature, recent studies suggest it may also have a role in regulating inflammation. During development of the invasive synovium in the collagen induced arthritis mouse model, T cell responses are mediated by Jagged-1 (Kijima et al, 2009). TNFα has been shown to induce synoviocytes proliferation via Notch-1 activation (Ando et al, 2003). In addition, studies have demonstrated that Notch-1 is associated with keratinocyte maturity and cell differentiation (Thélu et al, 2002; Okuyama et al, 2007). Furthermore, Notch signalling can interact with key transcriptional regulators involved in the pathogenesis of inflammatory arthritis and psoriasis such as NF-κB, HIF1α, STAT and CREB (Nakajima et al, 2004; D’Altri et al, 2011; Gao et al, 2012) however, the precise mechanism involved have yet to be elucidated.
In the present study, we examined Notch-1 expression in PsA patients with moderate/severe psoriasis following TNFi therapy and examine if TNFα can directly induce Notch-1 signalling in vitro.
MATERIALS AND METHODS

Arthroscopy and Synovial tissue collection

Patients with active psoriatic arthritis were recruited from the Rheumatology clinics prior to commencing biologic therapy. PsA was classified according to previously defined criteria (Veale et al, 1994). Normal control tissue from patients undergoing interventional arthroscopy for ligament tears was also obtained. All research was performed in accordance with the Declaration of Helsinki and approved by the St. Vincent’s University Hospital (SVUH) medical research and ethics committee. All patients provided fully informed written consent. Clinical assessments included tender and swollen joint count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), disease activity score (DAS) and global health visual analogue scale (VAS). Arthroscopy of the inflamed knee was performed under local anaesthetic using a Wolf 2.7 mm needle arthroscopy and biopsies obtained from the site of inflammation under direct visualisation as previously described (Ng et al, 2010). Biopsies were embedded in OCT (Tissue Tek, Sakura, The Netherlands) for immunohistochemical analysis. Normal skin biopsies (n = 5) were obtained as controls.

Skin Biopsies from patients with Psoriasis pre post TNFi

Patients with moderate-severe psoriasis, who had failed systemic therapy, were recruited from Dermatology/Rheumatology clinics at St Vincent’s University Hospital. The study was approved by the Research and Ethics Committee. Following fully informed, written consent patients underwent clinical assessment and skin biopsy, prior to anti-TNFα therapy. Patients received Infliximab (Remicade, Centocor, Malvern, Pa) infusions (3-5mg/kg at weeks 0 and 6. Patients (n=10) were followed up through week 12. Punch biopsies (6 mm) were taken from clinically involved and uninvolved skin at week 0. Following 12 weeks TNFi therapy another biopsy was obtained from the original site of inflammation to assess changes in inflammatory cellular/molecular mechanisms. Clinical assessments including Disease Activity Score using the 28 joint count (DAS28) (Fransen J and van Riel PL, 2005; Gladman DD et al 2006; Saber et al, 2011) and the Health Assessment Questionnaire (HAQ) were also assessed at 0 and 12 weeks. Normal skin biopsies n=6 were obtained from healthy control subjects. Histological specimens were embedded in Tissue-Tek OCT compound (Sakura, Zoeterwoude, Netherlands), snap frozen in liquid nitrogen and stored at –80°C.
Immunohistochemistry

7 μm OCT sections were placed on glass slides coated with 2% 3-amino-propyl-triethoxy-silane (Sigma-Aldrich) and dried overnight at room temperature. Tissue sections were fixed in acetone for 10 minutes and air-dried. Non-specific binding and endogenous peroxidase activity was blocked using 10% casein and 0.3% H2O2 respectively. A routine three-stage immunoperoxidase labelling technique incorporating avidin-biotin-immunoperoxidase complex (DAKO, Glostrup, Denmark or Vectastain, Vector Laboratories, UK) was used. Separate sections were incubated with rabbit polyclonal anti-Notch-1 (Millipore, Temecula, CA, USA) or an appropriate isotype IgG control (Santa Cruz Biotechnology). Colour was developed in solution containing diaminobenzadine-tetrahydrochloride (Sigma-Aldrich), 0.5% H2O2 in PBS buffer (pH 7.6). Slides were counterstained with haematoxylin (BDH Laboratories, Poole, UK) and mounted.

Slides were analysed using a well established semi-quantitative scoring method ranging from 0-4 (0 = no staining, 1=<25%, 2=25-50%, 3=50-75%, 4=>75% staining) (Youssef et al, 1998; Ng et al, 2010). The synovial sections were scored separately for perivascular/vascular, lining layer and sub-lining layer regions and skin sections were scored separately for the dermal, epidermal and vascular regions.

Culture of K4 cell line fibroblasts

Commercial human K4 fibroblast cells immortalised with SV40 virus (K4s) (Kind gift from Evelyn Murphy) were incubated in RPMI media (Invitrogen) supplemented with 10% Foetal Bovine Serum (FBS), 2% HEPES Buffer, 1% Penicillin Streptomycin, and 0.8% Fungizone. Cells were grown to confluence in T75 flasks at 37oC in humidified air with 5% CO2 and used between passages 30-45. Cells were serum starved for 24hrs and then culture in the presence of TNFα (10ng/ml) for 24 hrs. Protein and total RNA were isolated from cultured cells as outlined below.

K4 cells mRNA Extraction

Total RNA from K4 cells was obtained using the Stratagene Absolutely RNA Miniprep Kit (Agilent technologies, Santa Clara, CA, USA). RNA quantity was assessed by spectrophotometer. The integrity of RNA samples was assessed using a bioanalyzer (Agilent). Samples with a 260:280nm ratio of 1.9-2.1 and an RNA integrity number between 7 and 10 were used in
subsequent experiments. Isolated RNA was stored at -80°C. Total RNA 400ng was reversed transcribed to cDNA. Gene expression was analysed by relative quantification with preoptimized conditions using Lightcycler PCR technology (Roche Diagnostics. Lewes, UK). Specific TaqMan primers for Notch-1 (hs00413187_ml), and Jagged-1 (hs01070032) and DLL-4 (hs01070032) were acquired from Applied Biosystems (Applied Biosystems, Cheshire, UK). β-actin (4333764T) was used as endogenous controls. All primers and sequences were obtained from Applied Biosystems.

**Reverse Transcription of K4 cells RNA**

Total RNA isolated from K4 cells was reverse transcribed into cDNA. SuperScript II RNase Reverse Transcriptase (Gibco BRL) was used to synthesize first-strand cDNA. 400 nanograms of total RNA was added to a 10 μl reaction volume containing 200 units of SuperScript II in reverse transcriptase buffer, 100 mM dithiothreitol (supplied with the reverse transcriptase enzyme), 40 units of RNasin Ribonuclease Inhibitor (Promega), 1.25 mM each dATP, dCTP, dGTP, and dTTP (Promega), RNase-free water, and 500 ng of oligo(dT) (Promega). Reverse transcription was performed at 42°C for 50 minutes. The reaction was terminated by incubation at 95°C for 15 minutes. Complementary DNA was stored at -20°C for future use.

**Polymerase chain reaction**

PCR primers and probes for Notch-1, Jagged-1 and DLL-4 were designed by Applied Biosystems (Assays on-Demand). β-actin was used as an endogenous control. Amplification reactions contained 1μl of cDNA, 10μl of TaqMan Fast Universal Master Mix (Applied Biosystems), and 1μl of primer and probe mix and were brought to a total volume of 20μl by the addition of RNasefree water. All reactions/negative controls were performed in duplicate using 96-well plates on a LightCycler 480 system (Roche Diagnostics). Thermal cycling conditions were as recommended by the manufacturer (Applied Biosystems). Relative changes in gene expression were determined using the ΔΔCt method.

**Preparation of Protein Lysates and Western Blot**

Following culture K4 cells were trypsinised and pellets were collected after centrifugation. The resulting cell pellets were lysed and protein was extracted in ice-cold RIPA
(radio-immunoprecipitation assay) buffer (Sigma-Aldrich) containing 10µg/ml phosphatase inhibitor cocktail and 10µg/ml protease inhibitor cocktail (Sigma-Aldrich). Protein concentration was measured using a BCA assay (Pierce Chemical Co, Rockford, IL, USA). Proteins from K4 protein lysates were resolved on SDS-PAGE (10% resolving, 5% stacking gels) prior to transfer onto nitrocellulose membrane (Amersham Biosciences, Buckinghamshire, UK). Membranes were blocked for 2 hours in wash buffer containing 5% non-fat dried milk at room temperature with gentle agitation. Following three 15 minute washes in wash buffer, membranes were incubated with rabbit polyclonal anti-Notch-1 (1:500; Millipore) (dilution in PBS containing 0.05% Tween 20 and 2.5% non-fat dried milk at 4°C overnight with gentle agitation. β-actin (1:5000; Sigma-Aldrich) was used as loading control. Membranes were incubated; following three 15 minute washes, in 1:1000 dilution of horseradish peroxidase conjugated anti-rabbit IgG or anti-mouse IgG (Amersham Biosciences) for 2 hours. Following three final 15 minute washes, the ECL TM detection reagent (Amersham Biosciences) was placed on the membranes for 5 minutes before they were exposed to Hyperfilm ECL.

**Statistical Analysis**

Data is expressed as the mean ± SEM. Statistical analysis was performed using SPSS 18 for Windows (SPSS). For non parametric data Wilcoxon signed rank test for related samples and Mann Whitney U for non paired samples were used. Student’s t-tests were used to analyse parametric data. P values less than 0.05 were considered significant.
RESULTS

Demographic characteristics of PsA patients

Fifteen patients, 7 males and 8 females between the ages of 35-79 (median 57) were included in the study. Disease activity was assessed and a DAS28 composite clinical score was calculated for each patient (Table 1).

<table>
<thead>
<tr>
<th>Psoriatic Arthritis (n=15)</th>
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<tbody>
<tr>
<td><strong>Age</strong></td>
</tr>
<tr>
<td>52 (35-79)</td>
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<tr>
<td><strong>Gender (f)</strong></td>
</tr>
<tr>
<td>8 (53%)</td>
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<tr>
<td><strong>DAS28</strong></td>
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<td>3.95 (1.96-4.93)</td>
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Table 1: PsA patient demographics and disease activity score.

Expression and localised distribution of Notch signalling components in inflamed synovial tissue.

To examine Notch-1 and Jagged-1 localised expression, immunohistochemical analysis was performed in synovial tissue sections from PsA (Notch n=14, Jagged-1 n=8) and healthy control synovial tissue (n=5). Representative images of Notch-1 (A) and Jagged-1 (D) expression in PsA showing localised expression in the perivascular/vascular sublining and lining layer regions are shown in Figure 1, with minimal expression observed in healthy control tissue (B, E). Negative staining was observed for IgG control (C, F). Semi-quantification demonstrated increased Notch-1 and Jagged-1 expression in PsA vs. healthy control tissue (p<0.05) (Figure 2).
Figure 1: Representative images of Notch-1 (A), Jagged-1 (D) and IgG control (C,F) staining in PsA synovial tissue with minimal Notch-1 (B) and Jagged-1 (E) expression observed in control synovial tissue. Magnification 10x.
Figure 2: Semi-quantification of Notch-1 and Jagged-1 expression in synovial tissue. Semi-quantification of Notch and jagged-1 expression in the lining layer, sub-lining and vascular regions of PsA synovial tissue (n=15) compared to control synovium (n=5). Data represented as the mean±SEM. *p<0.05 significantly different from Control. **p<0.005 significantly different from Control.
Demographic characteristics of Psoriasis patients
Ten patients, 7 males and 3 females between the ages of 22-67 (median 45), undergoing TNFi therapy were included in the study. All patients had previously undergone treatment with a variety of combinations of topical, photo and systemic therapies including methotrexate (MTX), cyclosporine (CSA), neotigason and ultraviolet light type A/B (UVA/B). Disease activity was assessed and a DAS28 composite clinical score was calculated for each patient pre and post therapy (Table 2). These treatments were unsuccessful in adequately resolving the patient symptoms. All patients completed the 12 week study and received the Infliximab infusions at weeks 0 and 12.

<table>
<thead>
<tr>
<th>Psoriasis Skin</th>
<th>Pre TNFi Treatment (n=10)</th>
<th>Post TNFi Treatment (n=10)</th>
</tr>
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<tbody>
<tr>
<td>Age</td>
<td>48.3 (22-67)</td>
<td>48.3 (22-67)</td>
</tr>
<tr>
<td>Gender (f)</td>
<td>3 (30%)</td>
<td>3 (30%)</td>
</tr>
<tr>
<td>DAS28</td>
<td>5 (3.39-6.31)</td>
<td>2.4 (0.88-4.2)</td>
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</table>

Table 2: Psoriasis patient demographics and disease activity score.

Expression and localised distribution of Notch-1 and Jagged-1 signalling components in inflamed skin biopsies.
Notch-1 receptor was strongly expressed in the dermal, vascular regions and basal layer of the epidermis of involved skin (A) compared to uninvolved skin (B). Jagged-1 ligand was strongly expressed in the dermal, vascular regions and basal layer of the epidermis of involved skin (C) compared to uninvolved skin (D). (Figure 3) Negative staining was also observed for IgG control in involved and uninvolved skin. Notch-1 epidermal and vascular expression was significantly higher in involved skin compared to uninvolved skin in the (p<0.05) (Figure 4).

Expression and localised distribution of Notch signalling components in inflamed skin biopsies pre and post 12 weeks anti-TNFα therapy.
Expression of Notch-1 and Jagged-1 was assessed in psoriasis skin biopsies at baseline and 12 weeks post anti-TNFα therapy. Representative images of Notch-1, at baseline (A) and 12 weeks post therapy (B) and Jagged-1, at baseline (C) and 12 weeks post therapy (D) are shown in Figure 5 which shows a reduction in expression at 12 weeks. Quantification demonstrated a significant decrease in Notch-1 in the epidermal regions at 12 weeks post therapy (Figure 6) (p<0.05).
Figure 3: Representative images of Notch-1 and Jagged-1 expression in involved skin compared to uninvolved skin.

Representative images of increased expression of Notch-1 (A), and Jagged-1 (C) in involved skin compared to Notch-1 (B) and Jagged-1 (D) in uninvolved skin. Original magnification 10x.
Figure 4: Comparison of Notch and Jagged-1 expression in involved skin compared to uninvolved skin.

Semi-quantification of Notch pathway components in involved compared to uninvolved skin. Quantified expression of Notch-1 (n=10) and Jagged-1 (n=6), in epidermal, dermal and vascular regions of involved compared to uninvolved skin. Data represented as the mean±SEM. *p<0.05 significantly different from Uninvolved skin.
Figure 5: Representative images of Notch-1 and Jagged-1 expression in skin pre/post TNFi therapy.

Representative images of Notch-1 and Jagged-1 at baseline compared to 12 weeks post TNFi therapy. Increased expression of Notch-1 (A) and Jagged-1 (C) at baseline compared to Notch-1 (B) and Jagged-1 (D) at 12 weeks post TNFi. Original magnification 10x. Negative staining was observed for IgG control.
Figure 6: Notch-1 and Jagged-1 expression in skin pre/post TNFi therapy.

Semi-quantification of Notch-1 and Jagged-1 expression at baseline and at 12 weeks post TNFi. Quantified expression of Notch-1 (n=10) and Jagged-1 (n=6) in epidermal dermal and vascular regions at baseline compared to 12 weeks post therapy. Data is represented as the mean±SEM. *p<0.05 significantly different from post therapy.
Figure 7: TNFα upregulation of Notch-1 expression in synoviocytes.

To examine the direct effect of TNFα on Notch-1 signalling, K4IM were cultured with TNFα (10ng/ml) and protein and mRNA expression was analysed (n=3). TNFα induced a two fold increase in Notch-1 mRNA expression (Figure 7A) and induced Notch-1 IC expression as demonstrated by Western blot (Figure 7B); indicating TNFα induced activation of the Notch pathway. Furthermore, TNFα induced a fourfold increase in Jagged-1 mRNA expression (p<0.05) (Figure 7C).
DISCUSSION

Angiogenesis plays a critical role in the pathogenesis of PsA/Ps and is one of the first histologically detectable features in inflammation. Under normal physiological conditions angiogenesis is tightly regulated by the balance of pro and anti-angiogenic factors. However, in inflammatory disorders this process is highly dysregulated and is critically dependent on blood vessel stability and maturation to propagate the disease state.

At a pathological level, PsA synovial and Ps lesional skin are characterised by dysregulated angiogenesis which is associated with a distinct morphology (Reece et al, 1996; Fearon et al, 2003; Canete et al, 2004; Braverman et al, 1974; Braverman et al, 1977). Elevated levels of pro-angiogenic molecules, such as VEGF, TGFβ-1, MMP-9, PDGF-B and Ang1/2 are associated with the distinct vascular features observed in these diseases (Asahara et al, 1998; Fraser et al, 2001; Darland and D’Amore 2001; Lobov et al, 2002; Fearon et al, 2003; Koch et al, 2003; Schomber et al, 2007; Zhang et al, 2008; Kennedy et al, 2010). However to date little is known about the downstream survival signalling pathways involved in mediating these processes in PsA and Ps.

One such pathway is the Notch signalling pathway which is critically involved in the mechanism of blood vessel formation, branching, stability and morphogenesis (Hellstrom et al, 2007; Suchtings et al, 2007; Dufraine et al, 2008). Notch signalling plays an important role in many cell fate decisions, including EC survival. Many studies have shown that Notch receptor-ligand interaction is over-expressed in many vascular diseases and cancers. (Allenspach et al, 2002; Nickoloff et al, 2003; Rizzo et al, 2008; Segarra et al, 2008). Studies have shown that the inhibition of Notch signalling elements results in embryonic lethality, defects in angiogenic vascular remodelling, abnormalities in vessel structure, branching and patterning and dysfunctional vascular tip versus stalk cell development (Park et al, 1998; Tallquist et al, 1999; Villa et al, 2001; Gustafsson et al, 2005; Hellstrom et al, 2007; Adams et al, 2007). Therefore, the dysregulation of the Notch pathway is strongly associated with endothelial and vascular dysfunction.

In this study, we demonstrate increased Notch-1 and Jagged-1 expression in PsA inflamed synovium. The expression of Notch-1 and Jagged-1 in the PsA synovium is consistent with previous studies showing expression localised to the vasculature and lining layer regions of the RA synovium (Gao et al, 2012; Yabe et al, 2005). Furthermore, studies have demonstrated expression of Notch and/or its receptors in RA synoviocytes, which are the invasive cells of the synovial lining layer (Ando et al, 2003; Ishii et al, 2001) and shown that Notch-1 can mediate TNFα-induced synoviocyte proliferation (Ando et al, 2003; Nakazawa et al, 2001). Additionally, Jagged-1 can
induce inflammation in a CIA model of arthritis through regulating T cell responses and hypoxia is associated with increased Notch-1-IC expression in RA SM and fibroblasts (Gao et al, 2012; Kijima et al, 2009).

Notch-1 expression has also been demonstrated in Ps skin biopsies (Abdou et al, 2012), however, this is the first study to demonstrate expression of Jagged-1 in Ps skin, and that Notch-1 and Jagged-1 expression are inhibited by TNFi. Furthermore, we demonstrated Notch signalling components in the epidermis suggesting a proliferative role which may be related to the hyperactivity component of the inflamed epidermis in the pathophysiology of psoriasis. This is supported by in vitro studies showing that Notch-1 is associated with keratinocyte maturity and cell differentiation (Okuyama et al, 2007; Thélu et al, 2002). Jagged-1 has been found to mediate the recruitment of immune cell infiltrates to the epidermal, dermal and vascular regions of the skin (Ambler and Watt, 2010).

The functions of the Notch-1 ligand, Jagged-1, involves the mediation of vessel branching as well as recruitment and activation of pericytes to the vessel wall, as deletion studies have illustrated vascular defects occur in their absence (Hofmann and Iruela-Arispe, 2007; High et al, 2008; Benedito et al, 2009). In Jagged-1 knock-out mouse models, retinal angiogenesis was impaired, manifesting in a lower density of blood vessels and fewer tip cells (Benedito et al, 2009). Additionally, when Jagged-1 was specifically over-expressed in retinal endothelial cells, increased angiogenesis and cell tips formation was observed (Benedito et al, 2009). Elevated levels of Jagged-1 are associated with poor prognosis in renal cancer patients (Wu et al, 2011), while human haploinsufficiency of Jagged-1 causes Alagille Syndrome, which results in cardiovascular, liver, eye and skeletal abnormalities (Li et al, 1997). It has also been shown that Jagged-1 promotes inflammation of the epidermis, dermis and vascular regions of the skin by recruiting immune cell infiltrates (Ambler and Watt, 2010).

TNFα triggers a cascade of chemokines that attracts neutrophils, macrophages, and skin-specific memory T-cells strongly implicating TNFα in the pathogenesis of Ps and PsA (Banno et al, 2004). Therefore, the use of TNFi’s has revolutionised the treatment of PsA and Ps. An important goal for rheumatologists/dermatologists treating PsA and Ps patients is to identify markers that can (1) predict response to TNFi therapy, (2) predict remission rates and (3) predict those patients who can maintain remission following withdrawal of TNFi therapy (Hyrich et al, 2006; Nam et al, 2009). Identification of such markers could potentially lead to the development of a novel therapeutic target or even an adjuvant therapy which would ideally optimise a patient’s clinical diagnosis, therapy and outcome.
In this study we demonstrated that Notch-1 expression was significantly reduced in the epidermis of the lesional involved skin post anti-TNFα therapy. The effect of TNFα on this pathway was confirmed in K4 synoviocytes where we demonstrated that TNFα induces Notch-1 and Jagged-1 expression. TNFα is highly expressed in the inflamed skin and has been shown to increase expression of Jagged-1 in endothelial cells (Sainson et al, 2008) which is consistent with studies suggesting that while VEGF-induced vascular sprouting may utilise the Notch-1 DLL-4 ligand, pro-inflammatory cytokines such as TNFα may preferentially induce tip formation through Jagged-Notch-1 interactions (De Smet et al, 2009). In our study, Jagged-1 expression was decreased in the Ps involved skin post therapy; however these values were not significant.

In conclusion we have demonstrated increased expression of Notch-1 and Jagged-1 in PsA synovium and Ps lesional skin. Furthermore, we demonstrated that decreased levels of Notch-1 and Jagged-1 were expressed in Ps skin following Infliximab therapy, which was paralleled by improved clinical response in patients with moderate/severe skin disease. Inflammation and angiogenesis are key interrelated pathological processes in psoriasis/PsA. The precise mechanism by which the anti-inflammatory effects of anti-TNFα therapy is mediated is not clear, however this study suggests that one potential mechanism is the downregulation of angiogenesis and keratinocyte proliferation, via the Notch signalling pathway and this may represent a viable target for further novel therapies.
REFERENCES


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