



Effect of locally administered Syk siRNA on allergen-induced arthritis and asthma

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ABSTRACT

New approaches for the treatment of inflammatory disorders such as rheumatic arthritis (RA) and inflammatory lung disease (asthma) are needed because a significant population of patients do not experience sustained relief with currently available therapies. The tyrosine kinase Syk plays a crucial role in inflammatory signaling pathways and has gained much attention as a potential target for treatment of inflammatory disorders. We have shown that our Syk siRNA injected directly into limb joints of arthritic mice, diminishes joint swelling and reduces levels of Syk kinase and inflammatory cytokines in joint tissue. Further, our Syk siRNA, administered via nasal instillation, inhibits recruitment of inflammatory cells to the bronchoalveolar fluid of allergen-sensitized mice. We propose that targeting Syk via localized application of Syk siRNA provides an opportunity for specific knockdown of Syk kinase with minimal potential for systemic effects.

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1. Introduction

Inflammatory responses in autoimmune diseases such as rheumatic arthritis (RA) and inflammatory lung disease (asthma) have been attributed in part to the ability of locally produced immune complexes to stimulate inflammatory effector cells and the production of inflammatory mediators (Nigrovic and Lee, 2006; Hirano, 2002; Monach et al., 2004; Hartwig et al., 2010). Subsequent recruitment of additional immune responding cells exacerbates inflammation and cell damage. The tyrosine kinase Syk is a 72 kDa cytosolic protein tyrosine kinase expressed in many different cell types, including cells involved in joint inflammation and lung disease (Turner et al., 2000; Riccaboni et al., 2010; Scott, 2011). In these cells, Syk is a key mediator in immunoreceptor and other signaling cascades and has of late received much attention as a therapeutic target in immune and inflammatory conditions (Scott, 2011; Boers, 2011; Hueber and McInnes, 2009; Ulanova et al., 2005a; Sanderson et al., 2009). It is an attractive target because inhibition of Syk can suppress numerous overlapping inflammatory pathways simultaneously (Pine et al., 2007). For example, inhibition of Syk diminishes the release of inflammatory mediators (such as cytokines and superoxide) that follows the ligation of Fcγ receptors by IgG-immune complexes on the surface of inflammatory cells, including macrophages and neutrophils (Stenton et al.,

2000, 2002). In RA, inhibition of Syk also decreases the release of cytokines by fibroblast-like synoviocytes (Cha et al., 2006). Other studies have demonstrated a crucial role for Syk in signaling by such diverse cellular stimuli as IL-1, IL-6, liposaccharide, IgE, TNF and β1-integrin (Yamada et al., 2001; Arndt et al., 2004; Matsubara et al., 2006; Ulanova et al., 2005b, 2006). Syk in lung cells also plays an important role in the release of inflammatory mediators and the recruitment of inflammatory cells to bronchoalveolar fluid (Stenton et al., 2000, 2002; Ulanova et al., 2005b).

siRNAs have generated much interest as a therapeutic tool because of their ability to specifically interfere with the expression of individual genes (Thompson, 2002; Grünweller and Hartmann, 2005). We previously reported that knock down of Syk by our Syk siRNA produced important inflammation-related functional consequences including down regulation of ICAM-1 expression and diminished IL-6 and NO production (Ulanova et al., 2005b, 2006). Syk siRNA has also been reported to downregulate FcεRI-induced signal transduction, TNFα release and degranulation in RBL cells (Sanderson et al., 2010). We have now demonstrated that our Syk siRNA, injected directly without carrier into leg joints of arthritic mice, diminishes joint swelling. We also observed a reduction in the levels of Syk kinase, inflammatory cytokines and activated MAPKs in the joint tissue.

Having also observed that inhibition of Syk by aerosolized antisense to Syk reduces the number of inflammatory cells in the bronchoalveolar lavage fluid of allergen-sensitized mice (Stenton et al., 2000, 2002), we propose that localized administration of our Syk siRNA without carrier has the potential to provide an efficient non-systemic approach for treatment of RA and inflammatory lung disease.

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2. Materials and methods

2.1. Reagents

Anti-Syk and anti- β -actin antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA) and anti-pMAPK antibodies were purchased from Cell Signaling Technology, Inc. (Danvers, MA). Anti-IL-6, IFN- γ and TNF- α antibodies were obtained from Abcam, Inc. (Cambridge, MA) and anti-IL-4 antibody was purchased from Abnova (Taipei, Taiwan). Serum from KRN/H2g7 mice was kindly provided by Dr. Robert Eisenberg, University of Pennsylvania (Philadelphia, PA).

2.2. Animals

Six to ten-week-old male and female C57/BL6 mice were used throughout the study, and all protocols were performed in accordance with National Institutes of Health guidelines and with the approval of the University of Pennsylvania Animal Use Committee.

2.3. Syk siRNA

Nine siRNAs were designed to target the following regions of human Syk kinase: A: GAAGCCCTTCAACCGGCC; B: CCTCATCTGGGAATATGTG; C: ACCTCATCAGGG AATATGT; D: TATGTGAAGCAGAC ATGGT; E: TCAAATCATACTCCTTCCC; F: GCC TGGCCACAGAAAGTCC; G: TCATACTCCTTCCCAAAGC; H: GGGAA-GAATCTGAG CAAAT; I: GGGCATGAGTGATGGGCT. The siRNAs were chemically synthesized at Dharmacon Research Inc. (Lafayette, CO) and Shanghai GenePharma Co, Ltd. (Shanghai, China).

2.4. Cell culture and transfections

293 cells (human embryonic kidney cells) and MOLM-14 cells (human acute monocytic leukemia cells) were maintained in RPMI 1640 supplemented with 10% FBS, 100 U penicillin, 100 mg/ml streptomycin, and 4 mM glutamine at 37 °C in 5% CO₂. The 293 cells were transfected with siRNA using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's specifications. Briefly, 2×10^4 cells (in 96-well plates) or 5×10^5 cells (in 6-well plates) were incubated with siRNA (100 nM)/Lipofectamine 2000 complex at 37 °C for 48 h before harvesting. MOLM-14 cells were transfected with siRNAs using the AMAXA nucleofactor system (Cell Line Nucleofactor Kit V, AMAXA Biosystems, Gaithersburg, MD). Nucleofection was performed according to the manufacturer's recommendations using 2×10^6 cells and 3 μ g siRNA. Controls included mismatch siRNA and saline.

2.5. Mouse model of serum-induced arthritis

KRN/H2g7 mice develop severe inflammatory arthritis, and serum from these mice causes a similar arthritis in a wide range of mouse strains, due to auto-antibodies recognizing glucose-6-phosphate isomerase (GPI) (Kyburz and Corr, 2003; Tsuboi et al., 2011). To induce limb joint inflammation, C57/BL6 mice were injected intraperitoneally (ip) with 300 μ l of KRN/H2g7 serum (days 1 and 3). The paws of the mice were scored for inflammation daily using microcalipers to assess ankle thickness on a scale of 0–4. 0 = no swelling/redness; 1 = mild, but definite redness/swelling; 2 = moderate redness/swelling; 3 = redness/swelling of entire paw including digits; 4 = maximally inflamed limb, involvement of multiple joints. Controls were mice injected with 300 μ l saline.

To test the effect of Syk siRNA on serum-induced arthritis, C57/BL6 mice were divided into treatment groups of five and injected ip with 300 μ l of KRN/H2g7 serum on days 1 and 3. 20 μ l of either saline or saline/Syk siRNA (25 μ g siRNA/joint) was injected

directly into the leg joints of the mice on days 1, 3, 5 and 8. For the carrier groups, mice received polyethylenimine (PEI)/Syk siRNA or PEI/saline.

To analyze the levels of Syk protein in the joints of the mice by Western blot, the leg joints of the arthritic mice were excised on day 8, the time point of the largest difference between Syk siRNA and saline-treated mice. Joint tissue was homogenized in RIPA lysis buffer containing the protease and phosphatase inhibitors, 5 mM EGTA, 3 mM sodium orthovanadate, 2 mM phenylmethylsulfonyl fluoride, 10 μ g/ml aprotinin and leupeptin (Sigma–Aldrich), and proteins in the high speed supernatant were separated by electrophoresis. Syk levels on Western blots were probed using anti-Syk antibody. The Syk protein bands were scanned by densitometer and values for Syk were normalized to the densities of β -actin controls. Cytokines and pMAPKs in the joint homogenates were similarly evaluated on Western blots using appropriate antibodies.

2.6. Mouse model of acute lung inflammation

Mice were injected through the tail vein with 1.25 mg of anti-OVA antibody (250 μ l total volume) (Rockland, Gilbertsville, PA). Immediately following the injection of antibody, 75 μ g of OVA (MP Biomedicals, Solon, OH) was administered intranasally. The OVA (50 μ l total volume) was administered dropwise into each nostril over a 30 s period using a micropipette. Recruitment of inflammatory cells to the bronchoalveolar lavage fluid was determined after 24 h. Red blood cells in the lavage fluid were removed by brief hypotonic lysis and inflammatory cells in the BAL fluid were quantified on cytopsins stained with Hema-3 (Fisher Scientific, Pittsburgh, PA). To assess the effect of Syk siRNA on lung inflammation, mice were given 10 μ g of Syk siRNA (total volume 50 μ l) intranasally on three consecutive days prior to induction of inflammation. Twenty four hours after the third dose of siRNA, lung inflammation was induced as described above.

3. Results

3.1. Syk siRNA: *in vitro* studies

At a concentration of 100 nM, five of the 9 siRNAs (B, C, D, H, and I) knocked down Syk mRNA levels 50–60% in 293 cells (Fig. 1A). The same five siRNAs produced decreases in Syk protein levels (Fig. 1B). The Syk siRNA targeted to Syk mRNA sequence B (CCTCATCAGG GAATATGTG) was selected for further studies because of its effectiveness in knocking down expression of Syk and because its targeted mRNA sequence is conserved in mice, rats, monkeys and humans. We verified that Syk siRNA B effectively inhibits Syk protein expression in several cell lines and cell types (e.g. MOLM-14 cells, Fig. 1C). The term 'our Syk siRNA' in this paper refers to Syk siRNA B.

3.2. Effect of Syk siRNA on Syk levels and serum-induced inflammation in arthritic mice

In the KRN/H2g7 mouse model of antibody-induced arthritis, joint swelling occurs as a result of inflammation following the deposition of IgG immune complexes (Kyburz and Corr, 2003; Tsuboi et al., 2011). Having observed that knock down of Syk in cultured cells inhibits inflammation-related pathways (Ulanova et al., 2005b, 2006), we examined the effect of our Syk siRNA in mice injected with serum from KRN/H2g7 mice. C57/BL6 mice were injected ip on days 1 and 3 with KRN/H2g7 serum. Swelling of leg joints was detectable by day 3, with inflammation peaking at day 8 (Fig. 2A and B). Inflammation diminished gradually to become virtually undetectable by day 16.

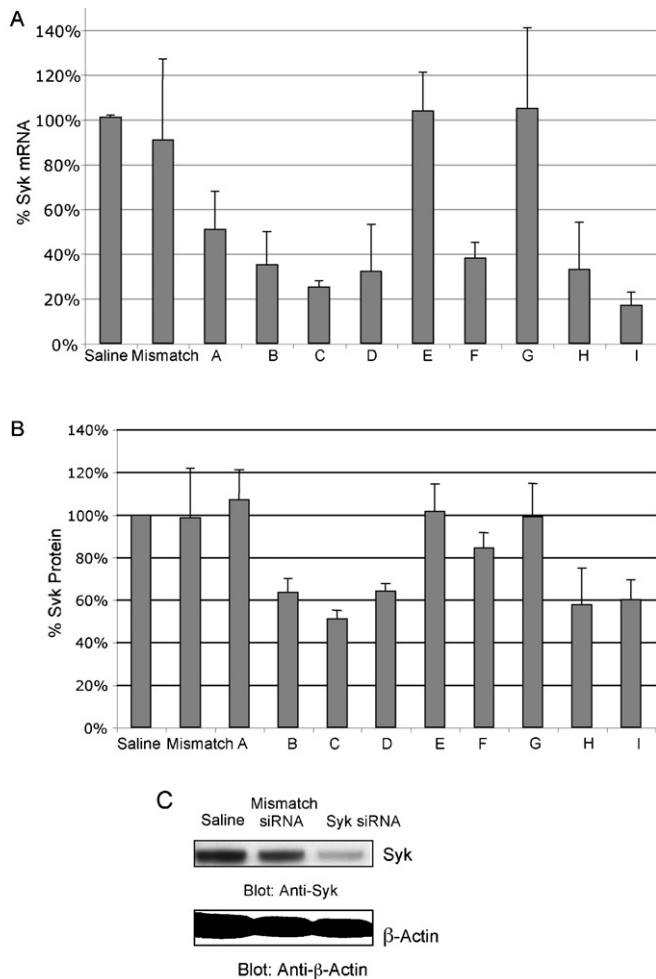


Fig. 1. Evaluation of the ability of Syk siRNAs to reduce levels of Syk mRNA (A) and Syk protein levels (B) in 293 cells. (C) Western blot. Effect of Syk siRNA B on Syk kinase levels in MOLM-14 cells. Lane 1, saline-treated cells; lane 2, mismatch Syk siRNA-treated cells; lane 3, Syk siRNA-treated cells. Top panel: anti-Syk blot indicating that Syk kinase levels are diminished in cells treated with our Syk siRNA. Bottom panel: anti- β -actin blot indicating that equal amounts of protein were loaded in each lane.

The time course for joint inflammation following serum injections is shown in Fig. 2B. A potential limiting factor for siRNA therapeutics is efficient delivery to target cells. We therefore examined the effect of our Syk siRNA delivered to leg joints with and without carrier. The red tracing indicates the effect on limb swelling of naked Syk siRNA injected directly into the limb joints of arthritic mice. We also injected mice with Syk siRNA + the carrier polyethylenimine (PEI) (aqua tracing). Controls were saline-treated mice (dark blue tracing) and mice exposed to PEI-saline (yellow tracing).

Injection of naked Syk siRNA directly into the limb joints of KRN/H2g7 serum-treated mice reduced joint swelling at all time points examined (red tracing compared to dark blue tracing, saline-treated mice). Decrease in swelling was already well evident at day 3 with the greatest effect at day 8. At day 8, the arthritis score was diminished from 10 for saline-treated mice to 4 for the Syk siRNA-treated mice. Mice injected with Syk siRNA alone or with Syk siRNA + PEI displayed similar inhibition profiles ($p=0.18$ on day 8), indicating that an siRNA carrier is not required for the inhibitory effect on joint swelling by this Syk siRNA.

To determine the levels of Syk kinase in the Syk siRNA-treated mice, we excised the hind limb joints from arthritic mice on day 8, the time point at which there was the largest difference in joint

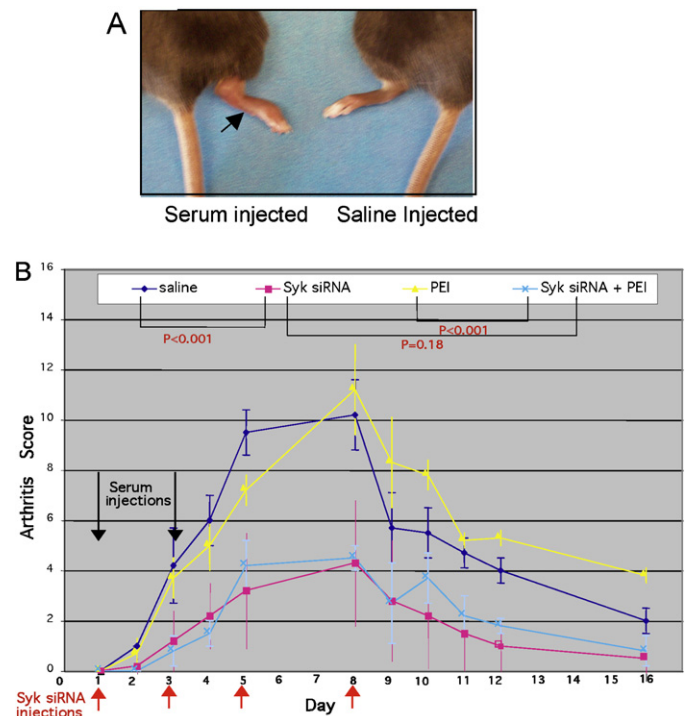


Fig. 2. (A) Representative images of hind paws from a KRN/H2g7 serum-treated mouse and a control mouse. BLK6 mice injected with serum develop visible joint inflammation which peaks at day 8. (B) Time course for limb inflammation in KRN/H2g7-treated BLK6 mice. The red tracings represent the Syk siRNA-injected mice, the aqua tracings represent the Syk siRNA plus PEI-injected mice, the dark blue tracings represent the saline-injected mice and the yellow tracings represent the PEI-injected mice. Serum injection times are indicated by black arrows. Red arrows indicate the injection regimen for Syk siRNA, Syk siRNA/PEI and controls. p values appear above tracings.

inflammation between Syk siRNA- and saline-treated mice, and examined Syk levels by Western blot. As expected, reduction in joint swelling was co-incident with diminished Syk levels. In the joints of Syk siRNA-treated mice at day 8, Syk levels were diminished by 72% (Fig. 3). Thus, the same concentration of Syk siRNA that diminished Syk kinase produces clear and positive effects on inflammatory symptoms in our RA model.

3.3. Effect of Syk siRNA on cytokine levels in serum-induced arthritis in mice

The importance of inflammatory cytokines in the pathogenesis of RA has long been recognized (Christodoulou and Choy, 2006; Houssiau, 1995). Our Syk siRNA reduced the levels of the inflammatory cytokines IL-6, TNF- α and IFN- γ in the day 8 joint tissue of serum-treated mice (Fig. 4A–C). Scanning and normalization to actin densities indicated that IL-6 levels were diminished by 70%, TNF- α levels by 65% and IFN- γ levels by 70%. In contrast, levels of the anti-inflammatory cytokine IL-4 were not decreased (Fig. 5). Our observations on the effects of Syk siRNA on pro-inflammatory cytokines are consistent with studies employing other Syk inhibitors (Sanderson et al., 2010).

3.4. Effect of Syk siRNA on MAP kinases in serum-induced arthritis in mice

Mitogen-activated protein kinases (MAPKs) comprise a family of highly conserved serine/threonine protein kinases that have been implicated in the regulation of key cellular processes including inflammatory responses in a variety of rheumatic diseases (Cha

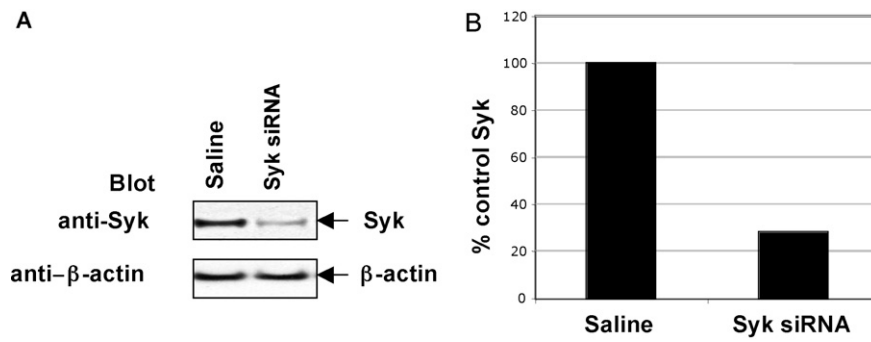


Fig. 3. Effect of Syk siRNA on Syk kinase levels in the joints of KRN/H2g7 serum-treated (arthritic) mice. Hind leg joint homogenates were obtained from mice at day 8 and Syk levels were evaluated on Western blots. (A) Western blot; (B) Evaluation of Syk levels (% Syk kinase). The densities of the Syk bands were normalized to the density of the β-actin control bands. Controls are saline-treated mice.

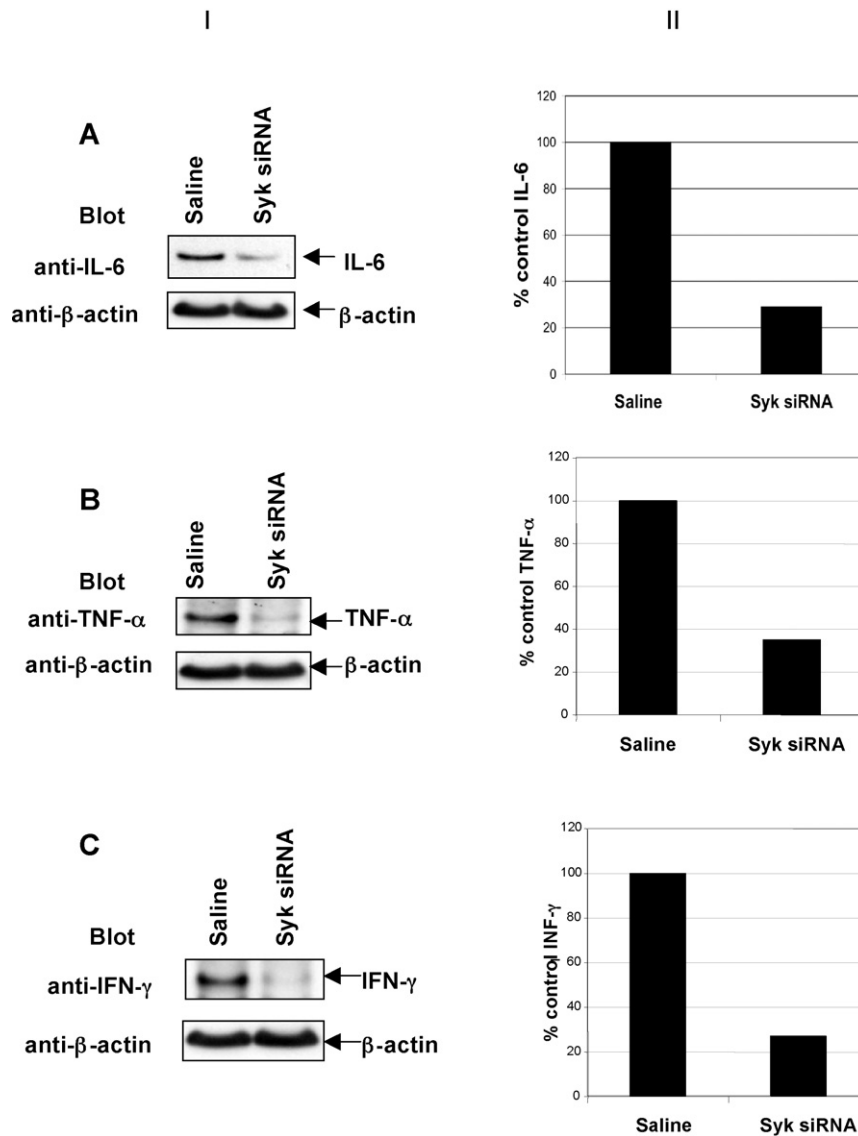


Fig. 4. Effect of Syk siRNA on inflammatory cytokine levels in the joints of KRN/H2g7 serum-treated mice. Hind leg joint homogenates were obtained from mice at day 8 and protein levels were evaluated on Western blots. Column I: Western blots. A. IL-6; B. TNF-α; C. INF-γ. Controls are saline treated mice. Column II: Evaluation of cytokine levels (% inflammatory cytokines). A. IL-6; B. TNF-α; C. INF-γ. The densities of the cytokine bands were normalized to the density of β-actin control bands.

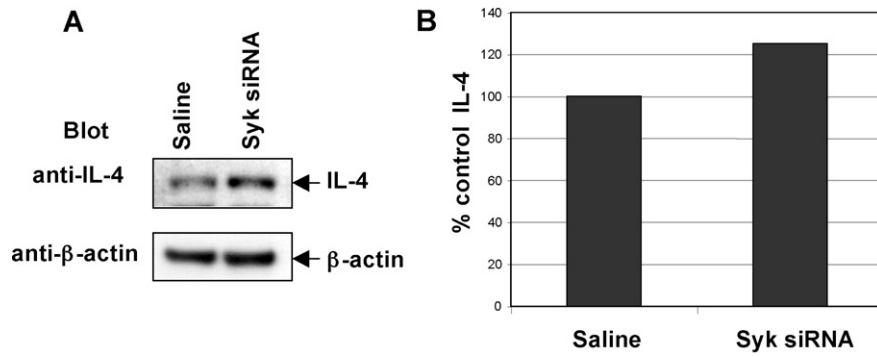


Fig. 5. Effect of Syk siRNA on IL-4 levels in the joints of KRN/H2g7 serum-treated mice. Hind leg joint homogenates were obtained from mice at day 8 and protein levels were evaluated on Western blots. Controls are saline treated mice. (A) Western blots. (B) Evaluation of IL-4 levels (% control IL-4). The blot density of the IL-4 band was normalized to the density of the β-actin controls.

et al., 2006; Thalhamer et al., 2008; Thiel et al., 2007; Schett et al., 2000). We examined the effect of Syk siRNA on levels of phosphorylated (p)-Erk, p-Jnk, and p-38 (Fig. 6). Blockage of Syk expression with our Syk siRNA diminished the level of p-Erk by 70%, with lesser effects on the levels of p-Jnk and p-38.

3.5. Effect of Syk siRNA on pulmonary inflammation in mice

To study the effect of our Syk siRNA on lung inflammation, mice were administered naked Syk siRNA via nasal instillation on three consecutive days before induction of inflammation. Syk siRNA,

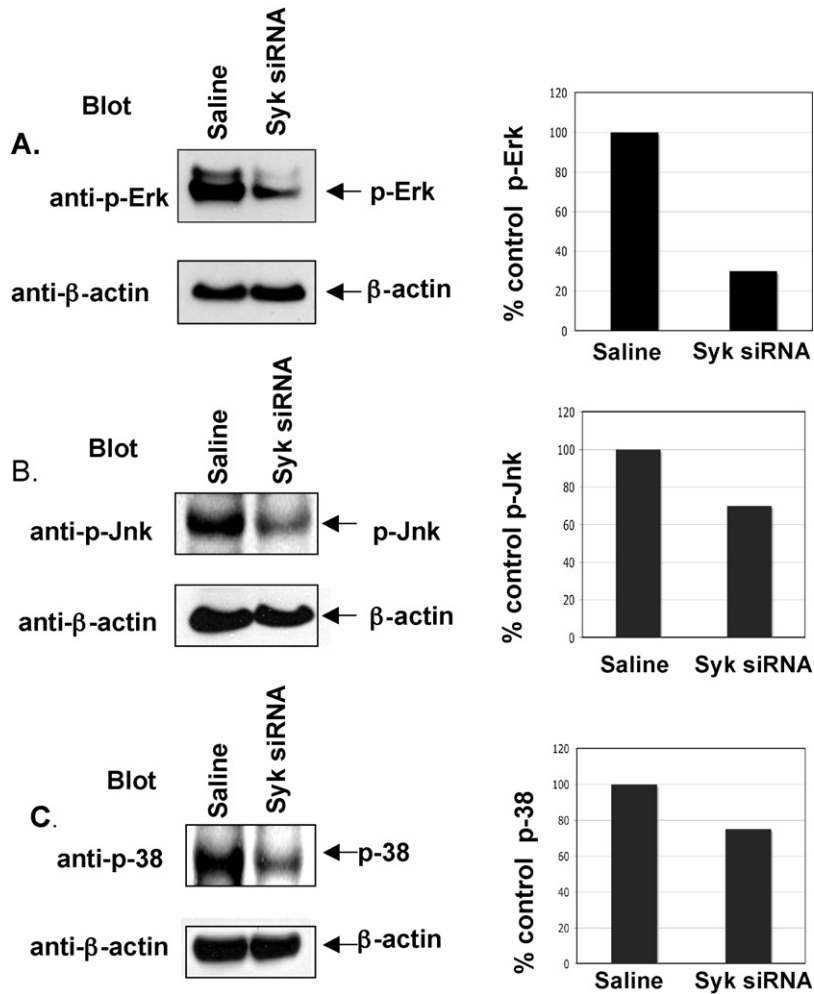


Fig. 6. Effect of Syk siRNA on levels of phosphorylated MAP kinase levels in joint tissue. Column 1: Western blots probed with phosphospecific antibodies as indicated. A. p-Erk; B. p-Jnk; C. p-38. Column 2: Evaluation of p-MAPK levels (% p-MAPKs) in Syk siRNA-treated mice. A. p-Erk; B. p-Jnk; C. p-38. The band densities were normalized to the densities of β-actin controls.

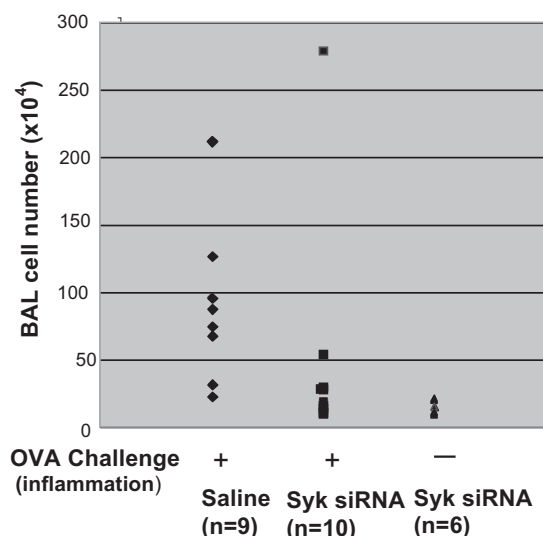


Fig. 7. Effect of Syk siRNA on immune complex-induced lung inflammation. Mice in which immune complexes were induced in vivo (OVA challenge) were treated with saline or Syk siRNA (10 μ g). After 24 h, lung lavage was performed and inflammatory cells in BAL fluid determined. Negative controls are mice not receiving the OVA challenge.

administered intranasally, diminished recruitment of inflammatory cells to BAL fluid compared to saline treatment (Fig. 7). The effect was dose dependent, effective at both 10 μ g and 1 μ g, but not effective at 0.1 μ g. In this inflammation model, recruitment of inflammatory cells to the BAL fluid was down-regulated by more than 50% at the 10 μ g dose level.

4. Discussion

Syk kinase has come to be regarded as an attractive target for anti-inflammatory therapy because of its effects on a multitude of inflammatory pathways (Riccaboni et al., 2010; Scott, 2011; Boers, 2011; Hueber and McInnes, 2009; Ulanova et al., 2005a; Sanderson et al., 2009). Syk is involved in the activation, accumulation and proliferation of inflammatory cells (including macrophages, neutrophils and mast cells) and the subsequent production and activation of biologically active molecules (Turner et al., 2000; Riccaboni et al., 2010). In RA, Syk also contributes to the inflammatory process by promoting the release of a broad array of proinflammatory mediators by resident synovial cells (Cha et al., 2006). Syk has also been implicated in induction of airway inflammation, a complex process involving resident lung cells, recruited inflammatory cells and secreted inflammatory bioactive factors (Murphy and O'Byrne, 2010; Zimmermann et al., 2003; Barnes, 2008; Busse and Lemanske Jr., 2001).

Cytokines are key mediators of inflammation, and pro-inflammatory cytokines are found in abundance in both the joint and blood of RA patients with active disease (Christodoulou and Choy, 2006; Houssiau, 1995; Guerne et al., 1989; Woodrick and Ruderman, 2010; Neuratha and Finotto, 2011). In our studies, local administration of our Syk siRNA, at the same concentration of Syk that decreased levels of Syk, diminished paw swelling, and reduced levels of IL-6, TNF- α and IFN- γ in joint tissue (Figs. 3 and 4).

In RA, TNF- α and IL-6 are known promoters of inflammatory responses and cartilage degradation (Isomäki and Punnonen, 1997), while IFN- γ has been implicated in both pro- and anti-inflammatory pathways (Isomäki and Punnonen, 1997;

Kelchtermans et al., 2008). Our observation that Syk siRNA diminishes IFN- γ levels (Fig. 4C) at concentrations that diminish paw swelling indicates that IFN- γ plays a pro-inflammatory role (mediated by Syk) in this animal model of RA.

There are also indications that the anti-inflammatory effect of Syk siRNA derives from interactions in both pro- and anti-inflammatory cytokine pathways. In contrast to IL-6, TNF- α and IFN- γ , levels of IL-4, an anti-inflammatory cytokine (Woods et al., 2001; Lubberts et al., 2000) were not decreased (Fig. 5). Studies in rodents with collagen or adjuvant-induced arthritis have shown that local overexpression of IL-4 reduces joint damage and other components of the arthritic process (Woods et al., 2001; Lubberts et al., 2000). The reduction of inflammation by IL-4 has been attributed to its ability to abrogate production of pro-inflammatory cytokines. In chronic inflammations such as RA, it is thought that an imbalance between pro- and anti-inflammatory mediators, contributes to cellular damage and destruction of cartilage and bone (Isomäki and Punnonen, 1997).

Mitogen-activated protein kinases (MAPKs) are subject to cytokine responses and are involved in the regulation of cytokines in a variety of rheumatic diseases (Cha et al., 2006; Thalhimer et al., 2008; Thiel et al., 2007; Sweeney and Firestein, 2006). All three MAPK families (Erk, Jnk and p38) are expressed in the synovial tissue of RA patients and are present in their active phosphorylated forms (Thiel et al., 2007; Schett et al., 2000; Han et al., 1999). In cultured RA fibroblast-like synoviocytes, treatment with the small molecule Syk inhibitor R-788 or with piceatannol, a chemical Syk inhibitor, was reported to diminish Syk-dependent phosphorylation of Erk1/2 and Jnk (Cha et al., 2006). Our Syk siRNA also impacts MAPK pathways, producing a large decrease in levels of p-Erk (Fig. 6). The inhibition of recruitment of inflammatory cells to bronchoalveolar fluid in our mouse model of lung inflammation (Fig. 7) is another indication of the ability of our Syk siRNA to impact a critical aspect of an inflammatory cascade.

Although orally available small molecules designed to inhibit Syk kinase (Brasemann et al., 2006; Pine et al., 2007) have opened new options for treatment of inflammatory diseases conditions such as RA and asthma, in clinical trials for RA, the positive changes in inflammatory response components produced by oral administration of R-788 were accompanied by significant adverse events, including hypertension, neutropenia and diarrhea (Weinblatt et al., 2008, 2010; Genovese et al., 2011; Rivera and Colbert, 2010). These undesirable side effects may reflect lack of pathway specificity, a disadvantage exhibited by many small molecule inhibitors. Lack of specificity is especially likely in the case of candidate small molecule tyrosine kinase inhibitors (Cha et al., 2006; Weiss et al., 2007).

The use of RNAi technology (siRNAs) (Thompson, 2002; Grünweller and Hartmann, 2005) to deliver treatment in localized inflammation may provide an alternative to small molecule Syk inhibitors. The mode of action by which siRNAs affect protein function differs from that of small molecule inhibitors. Small molecule inhibitors block the function of proteins by binding to targeted sites in the protein while siRNAs bind to complementary mRNA target sequences and knock down protein levels by interfering with translation of RNA to protein. Inhibition of protein function at the RNA level by siRNAs may be advantageous for specificity of outcome because proteins from cells treated with a small-molecule inhibitor may still retain irrelevant protein–protein interactions (Weiss et al., 2007). In general, off-target effects by siRNAs can be avoided by careful sequence design.

A potential limiting factor for siRNA therapeutics is efficient delivery to target cells. Our results indicate that localized delivery of naked Syk siRNA allows effective targeting to Syk in joint and lung tissue. Direct joint injection of our Syk siRNA without a

carrier decreased paw swelling and reduced the release of inflammatory effectors in arthritic mice. Nasal instillation of our Syk siRNA without carrier also reduced the recruitment of inflammatory cells to the BAL fluid of sensitized mice (Fig. 7). In addition to direct targeting of diseased tissue, localized delivery of siRNAs has the advantage of decreasing potential for off target systemic effects and for increasing the potential for sustained suppression of inflammation (Sanderson et al., 2010; Jorgensen and Apparailly, 2010). Further, the administration of naked siRNA is desirable because it avoids potential detrimental side effects from carrier systems.

Another concern in the use of siRNAs in therapeutics has been that double stranded siRNA, as part of its role in cellular defense against viral infection, can itself induce inflammatory mediator release (Sarkar and Sen, 2004). We have demonstrated that our Syk siRNA diminishes levels of inflammatory mediators including TNF- α and IFN- γ in the joints of arthritic mice (Fig. 4). The induction of the interferon-responsive gene IFIT1 is used as a marker for the anti-viral response and has been used to examine whether Syk siRNAs affect the cellular IFN response (Sanderson et al., 2010). That this Syk siRNA sequence does not induce a cellular IFN response has also been reported by Sanderson et al. These observations suggest that our Syk siRNA does not of itself induce inflammatory responses.

Taken together, our studies in murine model systems demonstrate the potential efficacy of our Syk siRNA delivered locally as an anti-inflammatory agent for treatment of rheumatoid arthritis and lung inflammation (e.g. asthma). Clinical trials are in progress to determine the efficacy and tolerability of our Syk siRNA as a maintenance agent for asthma patients and as a treatment for inflammatory disorders including immune complex mediated arthritis.

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