**Introduction.** Therapeutic strategies inhibiting tumor necrosis factor α (TNFα) activity in both rheumatoid arthritis (RA) and experimental autoimmune arthritis models have proven very successful. Although the etiopathogenesis leading to RA is still not understood, several studies support the concept that activated CD4+ T cells play an important role in initiating and perpetuating the chronic inflammation characteristic of this disease. CD4+ T cells are divided into two subsets based on their effector function and cytokine secretion profile, with Th1 cells producing the proinflammatory cytokines IL-2, TNF, and IFNγ, while Th2 cells secrete IL-4, IL-5, IL-10, IL-13. It has been suggested that in RA, pathophysiological processes influencing the immune response may be driven by activated Th1 cells with insufficient Th2 cell differentiation to downmodulate the ongoing inflammation. TNF is a macrophage derived cytokine with multiple inflammatory and immunoregulatory properties. Elevated levels of TNFα have been found in the sera and synovial fluid of RA patients, suggesting it plays an important role in the pathogenesis of RA. Studies suggest that neutralization of TNFα down regulates the production of IL-1, IL-6, IL-8 and GM-CSF, thereby improving the arthritic disease process. Our objective was to evaluate whether the anti-inflammatory action of tumor necrosis factor receptor (TNF-R) gene therapy could ultimately influence the reactivity of autoimmune lymphocytes in collagen-induced arthritis (CIA), an established experimental model of RA.

**Methods.** Induction and assessment of CIA: Female DBA/1 mice were injected with 100µl of CII emulsified in Freund’s adjuvant, intradermally at base of tail. Mice developing arthritis were divided on day of disease onset into two groups receiving peri-articular injections into the arthritic paw of either (1) 100µl of media alone (control group) or (2) 100µl of media containing 1.6x10⁷ pfu/ml of retroviral vector encoding human TNF-R (MOIN-sTNF-Rc-Ig) (treated group). An established arthritis scoring system was used to clinically evaluate disease daily: 0- normal appearance and flexion, 1- erythema and edema, 2- visible joint distortion, 3- ankylosis detectable on flexion.

**Measurement of circulating levels of human TNF-R and antibodies to collagen type II in sera:** Systemic levels of human TNF-R and anti-CII Ig, IgG, IgG1, IgG2a levels and IgG1:IgG2a ratio was measured by ELISA in mice sera obtained 3, 7, 14, 21 and 49 days after disease onset.

**Histology:** Hematoxylin and eosin staining was used to determine the extent of joint damage in front and rear paws in the two groups. Slides were evaluated for the presence of synovitis, pannus formation, marginal erosions, architectural changes (mostly subluxation), and destruction.

**Statistical Analysis:** All comparisons were done between treated and control mice using SPSS statistical software. Group comparisons were performed by the two-tailed independent t-test. More than two means were compared using one-way ANOVA, and p values <0.05 were considered to be statistically significant.

**Results.** A significant decrease in clinical severity 14-35 days post treatment was observed in TNF-R treated mice versus the control mice (p<0.05) as shown in Figure 1. Histological analysis revealed that the degree of synovitis, erosion and overall joint destruction was significantly reduced not only in the injected, but also in the contralateral and ipsilateral joints of the TNF-R treated animals compared to controls. TNF-R treated animals showed an overall lowered anti-CII IgG response relative to media treated controls. The changes in anti-CII IgG titers were not significantly different between the treated and control groups. However, seven days following disease onset, anti-CII IgG2a response was markedly reduced (p<0.05) in TNF-R treated animals, consequently leading to a significant (p<0.005) alteration in the anti-CII IgG1: IgG2a ratio in favor of Th2 driven IgG1 (Figure 2). Circulating levels of human TNF-R decreased from 16.7pg/ml three days post therapy to 12.7pg/ml seven days following treatment, and thereafter was undetectable. This decrease in the contrakinine levels may account for the failure to sustain the therapeutic effects on the immunological parameters of the disease, although beneficial effects on clinical disease parameters were sustained to five weeks post administration.

**Discussion:** These results suggested that presence of circulating levels of human TNF-R together with the shift in the anti-CII IgG1:IgG2a ratio towards IgG1 early in the disease leads to a clinical improvement in arthritis up to 35 days following disease onset. The amelioration in arthritis starts diminishing 35 days following disease onset probably because a single injection of TNF-R at disease onset may not be effective in blocking the disease beyond 35 days. Therefore, local TNFR gene therapy may ameliorate CIA by down regulating the inflammatory Th1 driven IgG2a rather than up regulating the anti-inflammatory Th2 driven IgG1 response. Subsequently, this may block the progression of inflammation and control the spread of the arthritogenic process to the un.injected contralateral and ipsilateral joints. Further, these results imply that retroviral mediated local TNF-R gene therapy has excellent therapeutic potential in treatment of RA beyond simple anti-inflammatory effects.