RNAI MEDIATED SILENCING OF HNRP-A2 DECREASES COLLAGEN-INDUCED ARTHRITIS BY INHIBITING PROLIFERATION AND CYTOKINE PRODUCTION OF CELLS OF THE MONONUCLEAR PHAGOCYTIC SYSTEM

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Background Heterogeneous nuclear ribonucleoprotein A2 (hnRNP-A2) belongs to a heterogeneous family of nuclear proteins, importantly involved in mRNA trafficking, translational and transcriptional processes. Recent evidence let suggest that hnRNPAs post-transcriptionally modulate expression of inflammatory mediators such as cyclooxygenase-2, tumour necrosis factor α (TNFα), interleukin 1β (IL-1β) and inducible nitric oxide synthase by affecting mRNA stability and translation.

Strong upregulation of hnRNP-A2 at sites of inflammation and the generation of antibodies and autoreactive T cells against hnRNP-A2 in rheumatoid arthritis patients and various arthritis models points towards a potential involvement of this protein in arthritis pathogenesis.

Objective To gain more insight into the role of hnRNP-A2 in chronic inflammation the authors have investigated the impact of hnRNP-A2 silencing in vivo on arthritis development in mice with collagen-induced arthritis (CIA).

Animals and methods Expression of hnRNP-A2 in tissues and cellular distribution was analysed by flow cytometry and immunoblotting. To study the effect of hnRNP-A2 silencing in J77.4 cells, proliferation was measured by 3H-thymidine incorporation and cytokine production was analysed by ELISA. Arthritis was induced in DBA/1 mice by immunisation with chicken collagen dissolved in complete Friedns adjuvant. For silencing of hnRNP-A2 expression, siRNA containing lipopolaxes were used, which were injected intravenously once a week. Control animals were treated with unspecific siRNA/lipoplexes or phosphate-buffered saline. Silencing efficiency was analysed by immunoblotting and real-time PCR. Arthritis was measured by an established clinical scoring system, inflammation and bone erosions were analysed by histomorphometry.

Results HnRNP-A2 was highly expressed in lymphoid organs such as lymph-nodes, spleen and thymus. Among cells of the immune system monocytes/macrophages showed the strongest expression of hnRNP-A2. Silencing of hnRNP-A2 in a monocytic cell line diminished the proliferative capacity of transfected cells.

Silencing of hnRNP-A2 in vivo by using siRNA packed in lipoplexes revealed a 60–70% silencing efficiency in lymph nodes and spleen of injected mice. Interestingly, incidence of arthritis in those mice, which were injected with hnRNP-A2 specific siRNA/lipoplexes, was only 20% as compared to 70% and 80%, respectively, in the control groups. Arthritis scores and weight loss differed significantly from control animals. Histological analysis of paws confirmed that both inflammation and erosion were significantly reduced in animals treated with hnRNP-A2 specific siRNA. Serum levels of cytokines typically produced by cells of the mononuclear phagocytic system such as TNFα, IL-23 and IL-1 were strongly reduced.

Conclusion In vivo silencing of hnRNP-A2 in CIA largely prevents induction of disease presumably by affecting the mononuclear phagocyte system thereby diminishing the inflammatory immune response.