Introduction. CD44 represents a family of cell-surface glycoproteins that are expressed by a wide variety of connective tissue and hematopoietic cells. CD44 is recognized as the principal cell surface receptor for hyaluronic (hyaluronic acid, HA), a major component of extracellular matrix. The binding of CD44 to its ligand is implicated in a variety of processes such as leukocyte extravasation at an inflammatory site, T lymphocyte activation, and the uptake and degradation of HA. Furthermore, critical roles for CD44 in development and tumor invasion have been proposed.

In patients with rheumatoid arthritis (RA), the amount of CD44 protein present in the synovial tissue of a rheumatoid joint correlates with the degree of synovial inflammation. All major cell types within the rheumatoid synovial tissue express CD44 at high levels, suggesting that this receptor has a regulatory function in the recruitment of inflammatory cells to the joint. Treatment of mice with anti-CD44 antibodies during experimental arthritis evoked a rapid loss (shedding) of CD44 from both leukocytes and synovial cells. Antibody-enhanced CD44 loss was accompanied by a dramatic decrease in joint swelling, and an inhibition of leukocyte recruitment to the synovium and joint space.

To address the role of CD44 in both physiological and pathological processes, we generated CD44 gene deficiency in DBA/1 mice that are susceptible to collagen-induced arthritis (CIA). In this study, we describe the effects of CD44 deficiency on the physiology of knock-out (KO) animals, as well as on inflammation-related processes including collagen-specific immune responses and leukocyte trafficking, during the development of CIA.

Methods. CD44 deficiency was generated DBA/1/LacJ mice by targeted disruption of the CD44 gene. The lack of CD44 gene, transcript and protein expression in the KO animals was confirmed using Southern hybridization, Northern blotting, and flow cytometry, respectively. The cellular composition of the immune system in wild type and CD44 KO animals was compared using antibodies to T- and B-cell markers. CIA was induced by injecting chicken type II collagen emulsified in Freund’s complete adjuvant (LPS) at day 54. The onset of arthritis was recorded, and the severity index for each animal was as expressed as a cumulative score of paw swelling. Collagen-specific antibody titers were measured by ELISA, and cellular immune responses were determined using bioassays for T-cell proliferation and interleukin-2 release.

To assess the trafficking of leukocytes, we employed an in vivo cell migration assay. Briefly, leukocytes from the spleens and lymph nodes of wild-type mice were labeled with PKH67 red fluorescent cell linker, and those from CD44 KO mice were tagged with PKH26 red fluorescent linker. Equal numbers of green and red fluorescent leukocytes were mixed and injected intravenously into wild-type mice. To study the trafficking of naive cells, a mixture of leukocytes from naïve CD44+/+ and CD44-/- donors were injected into naïve wild-type mice. To study the migration of “arthritic” cells, leukocytes from arthritic donors (CD44+/+ and CD44-/-) were injected together into arthritic wild-type animals. Leukocytes were obtained from peripheral blood of the recipients at 2 and 24 hours after administration of labeled donor cells, and from spleens, lymph nodes and bone marrow after 24 hours. The ratio of red and green fluorescence-labeled cells (R1) was determined by flow cytometry, and the results were normalized to the initial ratio (R0).

Results. CD44 KO mice lacked CD44 expression at both gene and protein levels, but this deficiency did not result in obvious developmental defects. The cellular composition of the immune system was comparable in CD44+/+ and CD44-/- mice. Upon immunization with type II collagen, wild type and KO animals displayed nearly identical cellular and humoral responses. However, the incidence (Fig. 1A) and severity (Fig. 1B) of CIA were significantly lower in CD44 KO as compared to wild-type mice, and these differences remained constant when inflammation was aggravated in both groups of mice by the injection of LPS.

Transfer of fluorescence-labeled wild type and KO leukocytes to wild-type donors revealed differences in the homing properties of CD44+/+ and -/- cells (Fig. 2). When leukocytes from naïve (normal) mice were transferred, little difference was found, i.e., the distribution of wild-type and KO cells in either the blood (Fig. 2A) or the primary and secondary lymphoid organs (Fig. 2B) of recipients was nearly identical. However, when cells from arthritic donors were transferred in the same way, a clear increase in the ratio of CD44 KO over wild-type cells was found in the blood (Fig.2A), as well as in bone marrow, lymph nodes and spleens (Fig. 2B) at 24 hours.

Discussion. This is the first study showing that deletion of CD44 in arthritis-susceptible mice results in a strong reduction in both the incidence and severity of the experimentally-induced disease. Attenuation of CIA in CD44 KO animals is not due to alterations in either the cellular composition or the function of the immune system, as these parameters are essentially the same in CD44+/+ and -/- DBA/1 mice. In vivo cell trafficking experiments have indicated that CD44-deficient cells of arthritic animals tend to remain longer in the circulation than the wild-type counterparts, and these same cells also show an increased capacity to home to lymphoid organs. Previous studies have concluded that CD44 is more involved in the extravasation of activated/inflammatory cells to the site of tissue injury than in the homing of lymphocytes to lymphoid organs. Consistent with that conclusion, our results suggest that the increased resistance of CD44 KO mice to CIA is primarily due to a reduced capacity of activated leukocytes to extravasate in non-lymphoid tissues including the synovial tissue of peripheral joints in arthritis-susceptible animals.