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Expression of rabbit C-reactive protein in transgenic mice inhibits development of antigen-induced arthritis

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Expression of rabbit C-reactive protein in transgenic mice inhibits development of antigen-induced arthritis

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Objective: C-reactive protein (CRP) is a plasma protein of hepatic origin thought to play an important role in host defences. We used transgenic mice, capable of expressing high levels of rabbit CRP (serum concentration > 50 μg/mL) in response to dietary manipulation, to determine whether high levels of this acute-phase reactant can alter the course of experimentally induced monoarticular arthritis.
Method: Arthritis was induced by a single injection of methylated bovine serum albumin (mBSA) on day 0 followed by injections of interleukin (IL)-1β.
Results: In transgenic animals in which CRP expression had been suppressed (serum concentration < 10 μg/mL), inflammatory arthritis began to develop by day 4 and was fully developed by 7 days after the mBSA challenge. This arthritis was characterized by marked inflammatory cell infiltrates in soft tissues, synovitis, pannus, cartilage loss, and bone erosion. By contrast, when CRP expression was induced, resulting in serum concentrations > 50 μg/mL on the day of mBSA and IL-1β injections, the inflammatory response was dramatically reduced at day 7. These mice manifested little to no evidence of joint inflammation. This anti-inflammatory effect of CRP was seen in animals with high CRP levels on days 0–1 following immunization and did not require elevated CRP levels during the period of rapid inflammatory progression, 4–7 days after challenge.
Conclusion: CRP, expressed at the time of antigenic stimulation, effectively blocked the subsequent development of inflammatory arthritis in this model by altering the immune or inflammatory responses.

In rheumatoid arthritis (RA), blood levels of C-reactive protein (CRP), the prototypic acute-phase reactant (1, 2), are markedly elevated (3), and CRP has been localized in the synovial membrane and synovial fluid (4). CRP is normally present in human plasma at concentrations under 3 μg/mL with levels increasing as much as 1000-fold following inflammatory stimuli [reviewed in (5)].

Serum CRP levels in mice are markedly lower than in humans and rabbits, < 3 μg/mL even after inflammatory stimuli (6). Accordingly, to study the effect of CRP on inflammatory models, we established a strain of transgenic mice in which expression of rabbit CRP is driven by the transcriptional promoter for the rat cytosolic form of phosphoenolpyruvate carboxykinase (PEPCK) (7). As a result, a protein-rich diet activates the promoter, resulting in increased CRP expression, while a carbohydrate-rich diet has the opposite effect. The CRP response in humans and rabbits is similar in both magnitude and kinetics (8). CRP has similar properties in both species. CRP expressed in these transgenic mice is structurally and functionally indistinguishable from native pentameric rabbit CRP (7). Transgenic rabbit CRP activates human complement *in vitro* (9) and mouse complement *in vivo* (7).

The model of induced arthritis we used (10) is characterized by its reproducibility, rapid onset, and rapid resolution (11). Arthritis is induced with a single knee injection of methylated bovine serum albumin (mBSA), followed by footpad injections of interleukin (IL)-1β. We report that CRP elevation at the time of immunization substantially inhibited development of the inflammatory response.

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Materials and methods

Animals

PC-12 mice containing the PEPCK-rCRP transgene were produced as described previously (7). To

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suppress transgene expression to levels < 10 μg/mL, females 3–4 months of age were provided a carbohydrate-rich diet (12) for 4–7 days. To stimulate transgene expression, randomly selected animals on the carbohydrate-rich diet were shifted to an isocaloric protein-rich diet (12). Within 24 h after the dietary shift, expression of CRP in serum was typically increased to 50–150 μg/mL, high levels being maintained for 2–3 days before declining to < 10 μg/mL by day 4–5 (7).

Controls included outbred CF-1 mice (Charles River) maintained in parallel on the same diet regimens as the transgenic mice, and a transgenic line containing a PEPCK promoter-growth hormone (nonfunctional) transgene (gift from Dr Richard Hanson) with a parallel breeding history to PC12, maintained on the diet regimens as described above. Animal care and procedures for all experiments were performed according to institutional guidelines.

CRP assays

Serum levels of rabbit CRP in transgenic mice were determined from 50–100 μL blood samples collected by retro-orbital bleeding. Serum levels of CRP were determined by a radial immunodiffusion assay (13).

Antigen-induced arthritis

To induce acute monoarticular arthritis (10), groups of 4–5 animals were given a single injection of 20 μL of 10 mg/mL methylated BSA in sterile saline into the left rear knee (day 0). IL-1β (60 μL; 300 ng) was injected subcutaneously into the footpad at the same time, with repeat injections on days 1 and 2. Animals were killed on days 4, 7, and 14.

Histology

Knee joints were excised *en toto* and stored in cold 40% ethanol prior to dehydration and embedding. The tissue was embedded in methyl methacrylate and 7-μm sections were cut. Slides were treated with Goldner's trichrome stain.

Assessment

Each section was read in a blinded fashion by a veterinary pathologist and graded for five components of arthritis: soft tissue inflammation, synovitis, pannus, cartilage erosion, and bone loss (10, 14). Soft tissue inflammation was defined as the extent of inflammatory cell infiltrate into the anterior fat pad. The synovial score was based on the thickness and inflammatory cell infiltrate of the synovium. Pannus was defined as severity of synovial growth over the articular surfaces. Cartilage erosion was scored as the degradation of cartilage on tibial and femoral surfaces, and bone loss was scored by size of lesions in the tibia, femur, or both. Severity scores for each histological feature were compared between animals on the carbohydrate-rich diet and the protein-rich diet using the two-tailed Student's t-test. The experiment was performed three times with a total of 30 animals.

Results

In PC12 transgenic mice in which CRP expression had been suppressed (CRP concentration < 10 μg/mL), a mild arthritic response was noted by day 4 after mBSA challenge, becoming maximal by day 7, less severe by day 14, and fully resolved by day 28, as described previously (10). Figures 1A and 1B show representative Goldner trichrome-stained sections of typical responses 7 days after mBSA injection in an animal fed a carbohydrate-rich, CRP-suppressing diet, expressing only about 6 μg/mL CRP. These figures show clear evidence of inflammatory arthritis. There is severe periarticular and intra-articular inflammatory infiltrate throughout the joint, particularly in the soft tissues of the knee, with marked synovitis and loss of much articular cartilage. The infiltrate consists of neutrophils, mononuclear cells, and eosinophils (Figure 1B). Swelling in the joint has almost completely obstructed the synovial space in Figure 1A. In many of these mice, a multicellular inflammatory pannus with cartilage loss and bone erosion was apparent by day 7.

By contrast, little sign of an inflammatory reaction was seen in transgenic animals provided a protein-rich, CRP-inducing diet 1 day prior to the mBSA immunization (Figures 1C, D). With the exception of some mild pannus formation, morphology was indistinguishable from joints from uninjected or saline-injected control animals. In some animals expressing high levels of CRP, a mild inflammatory response was evident, although rarely as pronounced as in littermates with suppressed CRP synthesis.

We examined a total of 30 animals. Figure 2 summarizes our results. The low CRP-expressing group displayed a higher average score (solid bars) in all five categories than did the high CRP expressing group (hatched bars), four of which achieved statistical significance ($p < 0.05$): soft tissue inflammatory cell infiltrates in the fat pad, pannus, cartilage loss, and bone loss. Bone loss was not observed in any of the high CRP-expressing mice. There was no correlation between severity of arthritis and serum CRP levels, which ranged from 45 to 90 μg/mL in the CRP-expressing animals at the time of immunization.

To rule out the possibility that the anti-inflammatory effects we observed were due to the diets provided to the animals and not to CRP expression, we studied two additional control groups:

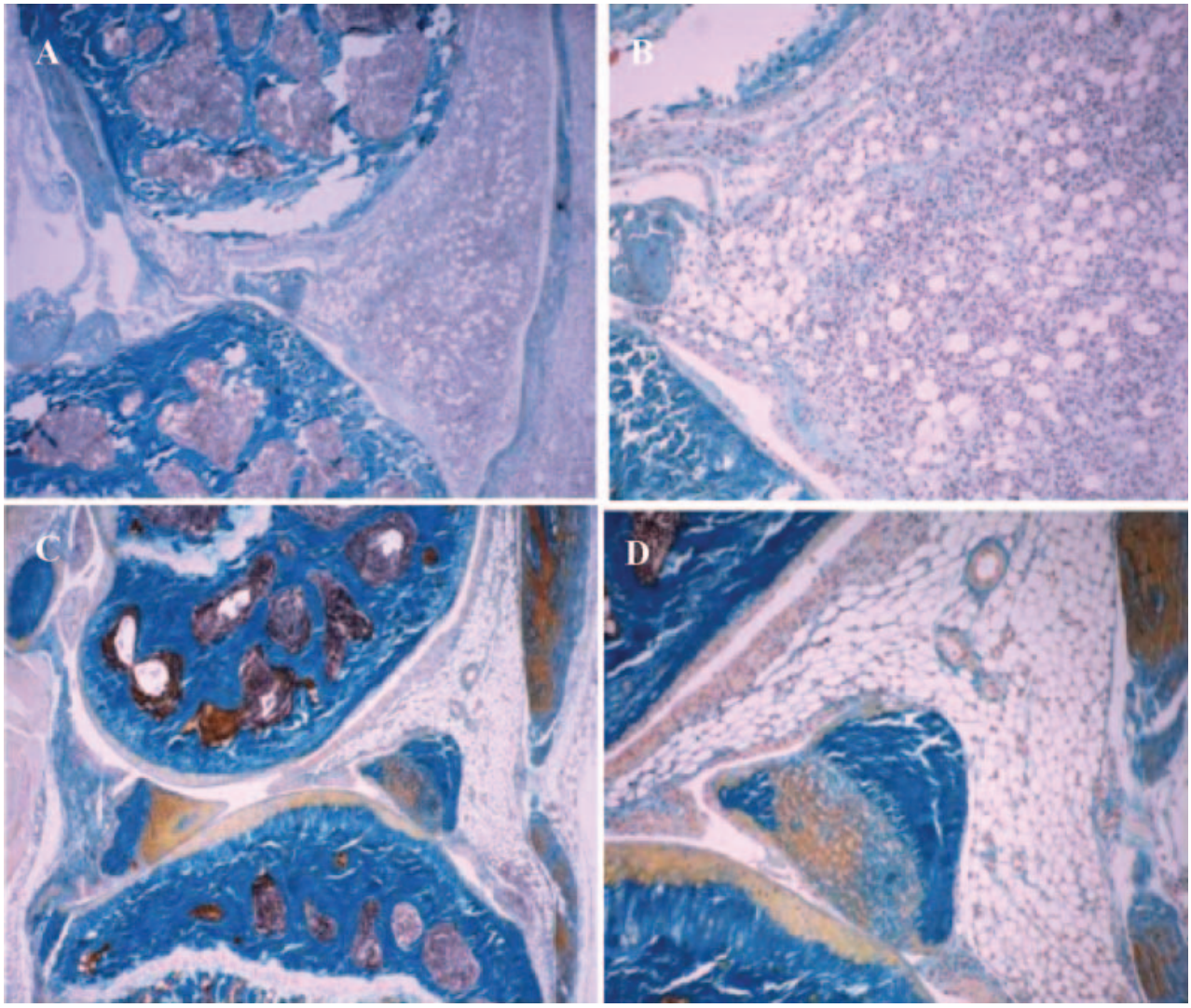

Figure 1. Sagittal section of knees in antigen-induced arthritis in PC12 transgenic mice. Arthritis was induced as described in the Materials and methods. Shown is a Goldner trichrome-stained section through the injected joint on day 7 after mBSA and IL-1β challenge. (A, B) Animal expressing low levels of CRP (6 μg/mL). (C, D) Animal expressing high levels of CRP (55 μg/mL). A and C: original magnification $\times$ 10. B and D: infrapatellar fat pad; original magnification $\times$ 25. Note the large inflammatory cell infiltrate throughout the joint in A and B.

(i) nontransgenic CF1 outbred mice were maintained on the same diet regimens as the PC12 transgenic mice and underwent the arthritis-inducing protocol described above, and (ii) a line of transgenic mice containing the PEPCK promoter linked to a non-functional growth hormone gene that had an exactly parallel breeding history to PC12. In each case, both diet groups developed comparable severe inflammatory responses, similar to those observed in the PC12 mice on the carbohydrate-rich diets.

To test the possibility that high levels of CRP in the animals on day 0 might have merely delayed rather than suppressed arthritis, three mice in each experimental group (high CRP-expressing and low CRP-expressing) were sacrificed 14 days after initial immunization. No inflammation was evident in either group, indicating that CRP was not merely delaying the inflammatory response.

The anti-inflammatory effect of CRP was noted only when its circulating concentration was maximal on the day of immunization and not several days prior to or subsequent to immunization. When PC12 animals were provided the protein-rich diet 4 days prior to or 4 days after immunization, there was no effect on subsequent development of arthritis.

Discussion

Expression of transgenic CRP at the time of mBSA immunization (day 0) effectively suppressed development of the acute erosive arthritis characteristic of this experimental model. As elevated levels of CRP could be sustained for only 2–3 days in the PC12 line when maintained on the protein-rich diet (7), circulating levels of CRP in high CRP-expressing

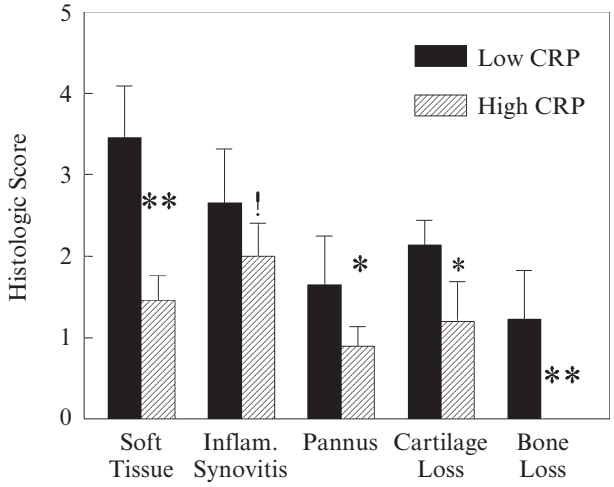

Figure 2. Semi-quantitative analysis of the histological results from 30 transgenic animals. Five histological components were scored from 0 to 5 as described previously (10, 14). Slides were prepared 7 days after the initiation of the mBSA/IL-1β protocol. Solid bars are mice on a carbohydrate-rich diet (low CRP, 2–10 μg/mL); hatched bars are mice on a protein-rich diet (high CRP, 45–90 μg/mL). Differences were evaluated with the two-tailed Student's t-test. Similar results were obtained when the data was evaluated with the nonparametric Mann–Whitney test. **$p<0.001$, *$p<0.05$, !$p<0.01$.

animals returned to baseline during the period 4–7 days post-immunization (when the maximal inflammatory reaction was observed in low CRP-expressing mice).

These findings indicate that CRP inhibits an early, time-limited afferent step in the response to mBSA/IL-1β, diminishing the subsequent inflammatory response. Neither early induction of elevated CRP (4 days before) nor induction of CRP expression 4 days following immunization inhibited development of arthritis. Elevated CRP did not simply delay the arthritic response, as no inflammation was observed at day 14 in the high CRP-expressing group.

This is the third model in which transgenic rabbit CRP has displayed net anti-inflammatory activity. Previously, we showed that CRP provided partial protection from lethal challenges with lipopolysaccharide (LPS), platelet activating factor (PAF), and the combination of IL-1β and tumour necrosis factor (TNFα) in models of septic shock (15). In addition, transgenic CRP was found to block leucocyte influx and protein extravasation in a model of pulmonary alveolitis induced by chemokines (16, 17).

The mechanism by which CRP alters the course of experimental arthritis in this model is unclear. Unlike some murine collagen-induced arthritis models that do not require mature B or T cells (18), this antigen-induced arthritis model involves T-cell activation (14, 19) and cytokine regulation (11, 20). CRP has been shown to protect mice from a T-cell-mediated autoimmune disease in a model of allergic encephalitis (21).

Our data are similar to the findings of Rodriguez et al (22), who found that early CRP treatment markedly decreased development of renal disease in MRL/lpr mice, and who presented evidence suggesting that this effect was due to induction of immunoregulatory CD25-bearing T cells. It is possible that similar mechanisms were operative in our studies.

CRP has also been reported to bind murine FcγRI and FcγRII, which are present on the surfaces of many leucocytes (23) and have been implicated in regulating the severity of antigen-induced arthritis in several animal models (24). It is theoretically possible that the effects we observed here are due to the interaction of CRP with these receptors during the progressive phase of the disease. Other anti-inflammatory effects of CRP previously defined in *in vitro* systems are the ability to induce IL-1 receptor antagonist (IL-1ra) in mononuclear cells and the ability to prevent neutrophil adhesion to endothelial cells by decreasing surface expression of L-selectin (25).

Our data provide another link between the innate immune system, of which CRP is a member, and the adaptive immune system upon which development of arthritis in this model depends. Thus, transgenic CRP appears to be interfering with the afferent arm of the adaptive immune response initiated by the mBSA/IL-1β immunization protocol.

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