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# Genetic Links Between the Acute-Phase Response and Arthritis Development in Rats

Peter Olofsson,[1] Niklas Nordquist,[2] Carina Vingsbo-Lundberg,[1] Anders Larsson,[3] Cecilia Falkenberg,[1] Ulf Pettersson,[2] Bo Åkerström,[1] and Rikard Holmdahl[1]

***Objective.*** **The acute-phase inflammatory response is closely correlated with the development of rheumatoid arthritis, but the pathophysiologic role of its specific components is largely unknown. We investigated the genetic control of the acute-phase protein response in pristane-induced arthritis (PIA), which is a chronic erosive arthritis model in rats.**

***Methods.*** **Plasma levels of the acute-phase proteins interleukin-6 (IL-6), $\alpha_1$-acid glycoprotein (orosomucoid), fibrinogen, and $\alpha_1$-inhibitor$_3$ were quantified in 3 strains of rats during the development and progression of disease: DA and LEW.1F, which are susceptible to arthritis, and E3, which is resistant. Genetic linkage analysis was performed on an $F_2$ intercross between E3 and DA to determine the genetic control of the acute-phase response in arthritis. Elevated levels of $\alpha_1$-acid glycoprotein were associated with acute inflammation, whereas levels of IL-6 were increased during the entire course of the disease.**

***Results.*** **Using these acute-phase markers as quantitative traits in linkage analysis revealed a colocalization of loci controlling the acute-phase response and regions previously shown to control the development of arthritis in chromosomes 10, 12, and 14. In addition, 2 loci that were not associated with arthritis were found to regulate serum levels of the acute-phase protein *Apr1* (acute-phase response 1) at the telomeric end of chromosome 12 and *Apr2* on chromosome 5.**

***Conclusion.*** **The PIA model in rats is a useful tool for understanding some of the pathways leading to chronic erosive arthritis. The analysis of acute-phase proteins in PIA and its application as quantitative traits for studying the genetics of arthritis will promote the understanding of the genetic regulation of the acute-phase response.**

Rheumatoid arthritis (RA) primarily affects the peripheral joints, where a chronic inflammatory synovitis often results in cartilage destruction, bone erosion, and ultimately, joint deformity and loss of function. The pathogenesis of RA is poorly understood, and the diagnosis is based on clinical descriptions rather than an understanding of the disease mechanisms (1). Diagnosis and prediction of disease progression in RA are of great importance, since early treatment may ameliorate the later development of severe erosions and joint immobilization. Several factors have been suggested to predict a more aggressive disease course. One such factor is the DR4 locus in the major histocompatibility complex (MHC) (2,3); others are rheumatoid factors, which are elevated before the onset of arthritis (4), antibodies to citrulline-composed epitopes (5), and various acute-phase proteins (6,7).

Acute-phase proteins such as $\alpha_1$-acid glycoprotein (orosomucoid), fibrinogen, and $\alpha_1$-inhibitor$_3$ are characterized by altered plasma levels as an outcome of inflammation (8,9). In arthritis patients, the serum concentration of acute-phase proteins strongly correlates not only with disease parameters such as joint swelling, but also with serum concentrations of interleukin-6 (IL-6) (10). IL-6 has been suggested to play a role in the pathogenesis of arthritis, as demonstrated by experiments with IL-6–deficient mice (11). One of the best

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[1]Peter Olofsson, MS, Carina Vingsbo-Lundberg, PhD, Cecilia Falkenberg, PhD, Bo Åkerström, PhD, Rikard Holmdahl, MD, PhD: Lund University, Lund, Sweden; [2]Niklas Nordquist, MS, Ulf Pettersson, MD, PhD: Uppsala University, Uppsala, Sweden; [3]Anders Larsson, MD, PhD: University Hospital, Uppsala, Sweden.

Address correspondence and reprint requests to Peter Olofsson, MS, Medical Inflammation Research, I11 BMC 22184, Lund, Sweden. E-mail: peter.olofsson@inflam.lu.se.

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markers of inflammatory activity in RA is the acute-phase protein $\alpha_1$-acid glycoprotein (12,13). Determination of the glycosylation subforms of $\alpha_1$-acid glycoprotein may discriminate between more severe forms of disease (14,15). The role of these different acute-phase proteins in the arthritic process is largely unknown. It is therefore important to study animal models in which mechanistic studies and complex interactions in vivo can be addressed and tested.

Various rat models of arthritis are available which more or less mimic RA in humans. These models include both cartilage protein–induced arthritis, such as collagen-induced arthritis (CIA) (16), adjuvant-induced arthritides, such as oil-induced arthritis (17), and pristane-induced arthritis (PIA) (18). We chose PIA as the model for analyzing the acute-phase response in arthritis because it best fulfills the criteria used to diagnose RA (19).

In the present study, 3 strains of rats were examined: the E3 strain, which is resistant to PIA, and the DA and LEW.1F strains, which are highly susceptible to PIA (18). To investigate the potential role of acute-phase proteins in disease mechanisms of the PIA model, we analyzed plasma levels of $\alpha_1$-acid glycoprotein, fibrinogen, $\alpha_1$-inhibitor$_3$, and IL-6, which are known to reflect the level of inflammation in arthritis (8). These proteins were measured at 16 different times, from day 0 to day 150 after pristane injection.

Based on the findings of plasma analyses, we investigated the genetic control of the acute-phase protein response in comparison with the genetic control of arthritis in an E3 $\times$ DA crossbred strain of rats. The results showed that the levels of each acute-phase protein are associated with a specific set of loci. Interestingly, most of these loci have previously been found to control various phases of arthritis.

## MATERIALS AND METHODS

**Animals.** Pathogen-free rats of the E3, DA, and LEW.1F strain (originating from Zentralinstitut für Versuchstierzucht, Hannover, Germany) were kept in animal facilities in a climate-controlled environment with 12-hour light/dark cycles. Rats were housed in polystyrene cages containing wood shavings and were fed standard rodent chow and water ad libitum. The rats were found to be free of common pathogens, including Sendai virus, Hantaan virus, coronavirus, reovirus, cytomegalovirus, and *Mycoplasma pulmonis*.

Breeding to produce (E3 $\times$ DA)$F_2$ offspring was performed in the same pathogen-free animal facility. Female E3 rats were intercrossed to produce (E3 $\times$ DA)$F_1$ offspring that were further intercrossed to produce (E3 $\times$ DA)$F_2$ rats (n = 153; 70 females and 83 males), which were used in the linkage analysis.

**Induction and evaluation of arthritis.** Arthritis was induced by an intradermal injection at the base of the tail with 150 $\mu$l of pristane (2,6,10,14-tetramethylpentadecane; Aldrich, Milwaukee, WI). Four groups of $F_2$ animals were challenged with pristane and showed only small and not significant variations in the severity and onset of arthritis (data not shown). These groups were therefore combined for analysis.

The parental E3, DA, and LEW.1F rats were challenged simultaneously with pristane. Arthritis was induced in all rats at the age of 8–12 weeks. Arthritis development was monitored in all 4 limbs, using a macroscopic scoring system. For each limb, 1 point was given for each swollen or red toe, 1 point for each swollen midfoot, digit, or knuckle, and 5 points for a swollen ankle (maximum score per limb 15). The scores of the 4 limbs were added to yield a total score for each rat (maximum total score per rat 60).

The rats were examined 1–4 times a week for 150 days after pristane injection. Blood was obtained by cutting the tip of the tail. To prevent blood coagulation, 10 $\mu$l of heparin (5,000 units/ml; Lövens Läkemedel, Malmö, Sweden) was mixed with 500–1,000 $\mu$l of blood. The plasma was separated from blood cells by centrifugation and stored at $-70$°C until assayed. Plasma from parental strains was collected on days 0, 4, 8, 12, 16, 20, 25, 30, 35, 42, 49, 60, 80, 100, 120, and 150 after pristane injection. Serum was collected from the (E3 $\times$ DA)$F_2$ rats on days 14, 35, 49, and 100.

**Determination of plasma protein concentrations.** Levels of $\alpha_1$-acid glycoprotein were measured with a soluble competitive radioimmunoassay (RIA) using rat $\alpha_1$-acid glycoprotein (Zivic-Miller Laboratories, Zelienpole, PA) and a polyclonal rabbit antibody against $\alpha_1$-acid glycoprotein (Agrisera, Vännäs, Sweden) (20). Concentrations of $\alpha_1$-inhibitor$_3$ were measured with a solid-phase RIA (21) modified as described elsewhere (22), using polyclonal rabbit antibody against $\alpha_1$-inhibitor$_3$ (Agrisera) and purified rat $\alpha_1$-inhibitor$_3$ generously provided by Dr. Jan J. Enghild (Institute of Molecular and Structural Biology, University of Aarhus, Aarhus, Denmark) as standard.

Plasma concentrations of IL-6 were determined using the IL-6–dependent cell line B9 (23). The B9 cells were cultured in Dulbecco's Glutamax medium supplemented with streptomycin, penicillin, 10% fetal calf serum, and recombinant rat IL-6 (RDI-4016; Research Diagnostics, Flanders, NJ). B9 cells washed in phosphate buffered saline (0.14*M* NaCl, 2.7 m*M* KCl, 1.5 m*M* $KH_2PO_4$, 8.1 m*M* $Na_2HPO_4$), cultured in media containing the plasma samples for 72 hours, and pulsed with $^3$H-thymidine overnight. Cells were then harvested in a Filtermate cell harvester (Packard Instruments, Meriden, CT). The incorporation of $^3$H-thymidine was determined in a Matrix 96 direct beta counter (Packard Instruments). The level of IL-6 in the plasma was determined by comparison with the standard curve of the recombinant rat IL-6 included in each plate. The detection limit of the assay was 2.5 pg/ml.

Plasma fibrinogen was determined by rate nephelometry on a Beckman Array protein system (Beckman Instruments, Brea, CA) utilizing chicken anti-human fibrinogen (Immunsystem, Uppsala, Sweden). A pool of normal rat plasma was used to produce a standard curve, which was then used to calculate the fibrinogen content in the samples (24).

**Table 1.** Clinical scoring of pristane-induced arthritis in parental rat strains

| Strain | No. of rats | Incidence of arthritis, % | Maximum clinical score, mean ± SD* | No. of affected paws, mean ± SD† | Cumulative score, mean ± SD‡ | Arthritis onset, mean ± SD days | Incidence of tail ankylosis, % |
|---|---|---|---|---|---|---|---|
| DA | 10 | 100 | 31 ± 14 | 3.7 ± 0.5 | 370 ± 282 | 14 ± 1 | 75 |
| E3 | 10 | 0 | 0 | 0 | 0 | – | 0 |
| LEW.1F | 11 | 100 | 37 ± 13 | 3.4 ± 0.7 | 423 ± 174 | 17 ± 3 | 0 |

* Arthritis severity was evaluated as described in Materials and Methods (maximum score per rat 60). The maximum clinical score is the value for each rat at the time of the most severe arthritis.
† Mean number of paws per rat with clinical signs of arthritis at the time of the most severe arthritis.
‡ Total of arthritis scores for all scoring done during the experiment.

**Immunohistopathologic analyses.** The ankle joints from the hind paws were obtained on day 150 after pristane injection, when the rats were killed. The paws were fixed in 4% paraformaldehyde, decalcified in EDTA, embedded in paraffin, sectioned, and stained with hematoxylin and erythrosin (25).

**Genotyping and linkage analysis.** DNA was prepared from tail biopsy tissues as previously described (26). Primer sequences for rat microsatellite markers defined as *DxMity, DxMghy, DxRaty, and DxGoty* were obtained from Research Genetics (Huntsville, AL), and for markers defined as *DxWoxy* from the Wellcome Institute for Human Genetics (Oxford, UK). Fluorescent-labeled primers were synthesized by Interactiva Biotechnologie (Ulm, Germany).

All markers were assayed by polymerase chain reaction (PCR) on an ABI 877 Integrated Thermal Cycler (Perkin Elmer, Emeryville, CA) according to a standard protocol, and PCR products were run on an ABI 377 DNA Sequencer (Perkin Elmer). Data were analyzed with the software packages GeneScan 3.1 and Genotyper 2.1 (Perkin Elmer) through comparison with amplified samples from parental strain rats.

To produce linkage maps covering the complete genome, all 153 $F_2$ progeny were genotyped using 323 markers. More than 95% of the rat genome was within a 10-cM distance of 1 microsatellite marker (maximum intermarker distance 28 cM). An improved linkage map based on several crosses involving E3 and DA is available at our Internet site (http://net.inflam.lu.se). Linkage analysis was performed with the MapMaker computer package (27–29). The MapMaker/QTL software used for the quantitative trait locus (QTL) analysis and chromosomal QTL maps showing the logarithm of likelihood (LOD) of the presence of a QTL controlling disease or plasma protein levels utilizes interval mapping calculations and provides determinations of the linkage inheritance pattern (dominant, additive, or recessive). For claiming significant linkage, we used a threshold of LOD $\geq$3.4 and for suggestive linkage, we used a threshold of LOD 2.8, as described previously (30), which is based on the guidelines suggested by Lander and Kruglyak (27).

Statistical analyses other than linkage analysis were performed using Student's unpaired *t*-test.

## RESULTS

**Arthritis disease course.** Clinical manifestations of arthritis after pristane injections were characterized in 3 different rat strains: E3, DA, and LEW.1F (Table 1 and Figures 1 and 2). DA and LEW.1F rats displayed 100% incidence of severe and chronic arthritis, with a mean onset occurring on day 14 and day 17, respectively. E3 rats did not develop arthritis. The development of chronic arthritis led to a reduction in normal weight gain of both DA and LEW.1F rats. In both the DA and the LEW.1F rat strains, the arthritis was symmetric.

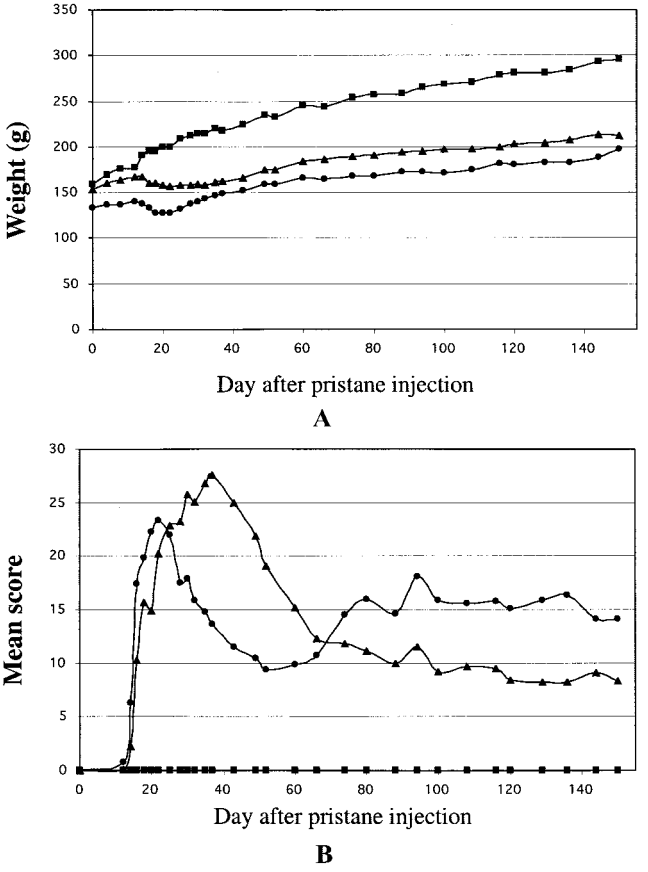



**Figure 1. A,** Mean weight and **B,** mean clinical score (maximum score for each paw 15; maximum total score for each rat 60) in pristane-injected E3 (■), DA (●), and LEW.1F (▲) rats.

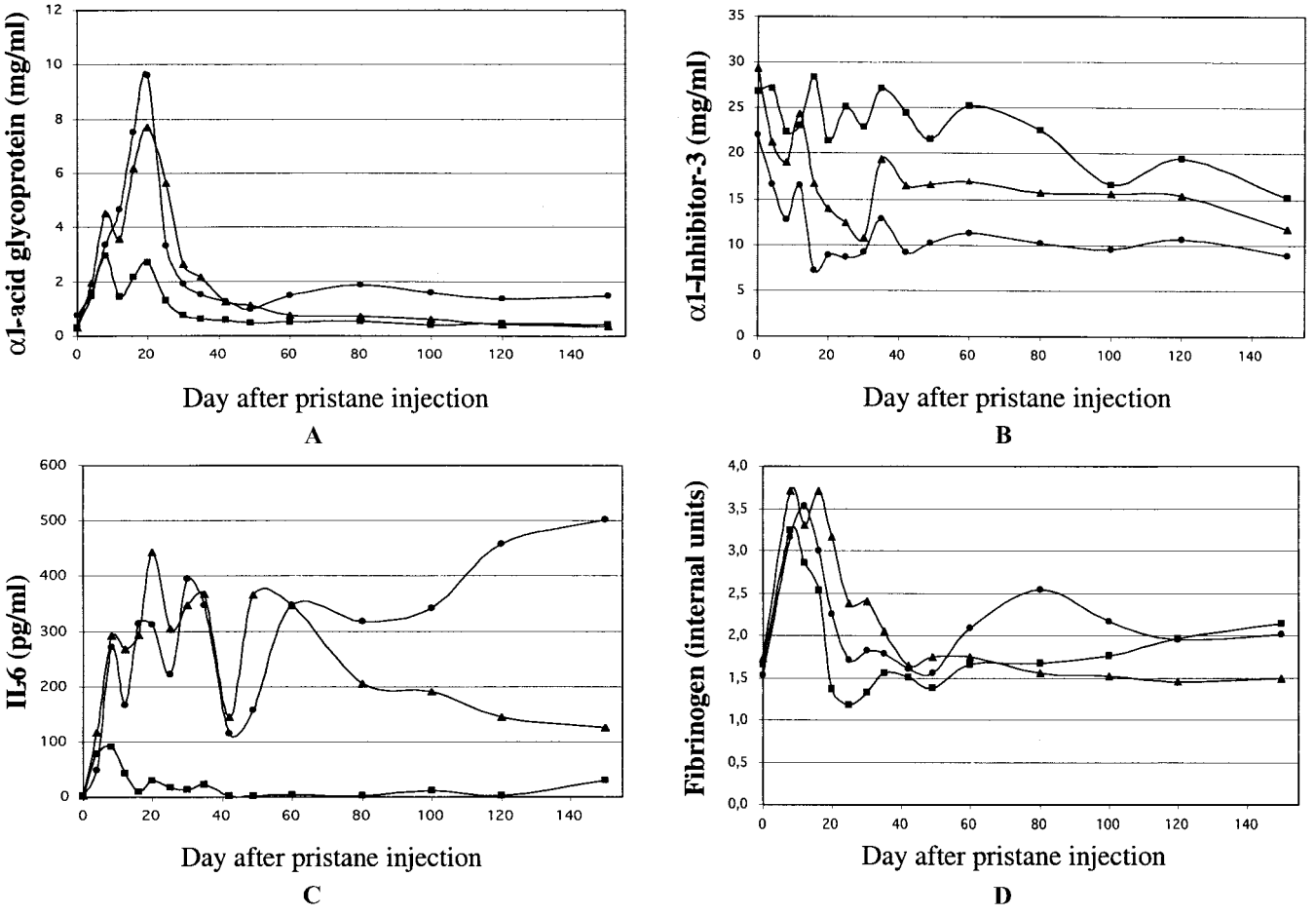


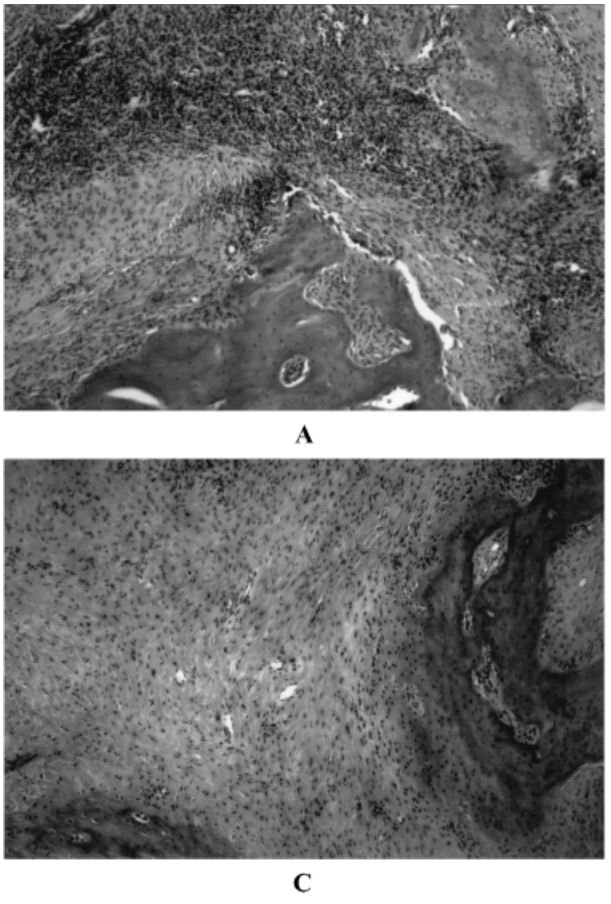

**Figure 2.** Plasma levels of the acute-phase proteins **A,** $\alpha_1$-acid glycoprotein, **B,** $\alpha_1$-inhibitor$_3$, **C,** interleukin-6 (IL-6), and **D,** fibrinogen over time following pristane injection in 3 strains of rats, E3 (■), DA (●), and LEW.1F (▲).

On average, 85% of the arthritic hind joints were symmetrically affected during the period of most severe arthritis, from day 22 to day 35 after pristane injection. The DA rats showed more severe deformity of the paws and more pronounced active chronic arthritis, with a relapsing phase starting around day 60 after pristane injection, whereas the LEW.1F rats showed a more edematous type of joint swelling and a less erosive chronic phase (Figures 1 and 2). The weight of the rats was mainly affected during the disease onset period (Figure 1). Ankylosis in the tail was observed in 75% of the DA rats but in none of the E3 or LEW.1F strains (Table 1). The ankylosis in the tail correlated with severe deformity of the arthritic paws. The most severely affected DA rats also had signs of a bent back, possibly as a result of ankylosis of the vertebral column (data not shown).

Hind paw ankles were analyzed with hematoxylin and erythrosin staining on day 150 after arthritis induction to visualize the cell infiltration and joint erosions (Figure 3). The E3 rat we examined had normal joints, with no cell infiltration or degraded cartilage. Synovial inflammation and joint erosion were seen in both the DA and LEW.1F rats. However, DA rats had a more severe pannus and greater erosions of cartilage and bone.

**Analysis of acute-phase proteins.** To investigate whether pristane injection and the development of chronic arthritis induced an acute-phase response, we determined the levels of various acute-phase proteins in blood plasma from the E3, LEW.1F, and DA strains at different times (Figure 2).

Fibrinogen levels were increased 2-fold in all strains following pristane injection and before the onset of arthritis. The arthritis onset in the DA and LEW.1F



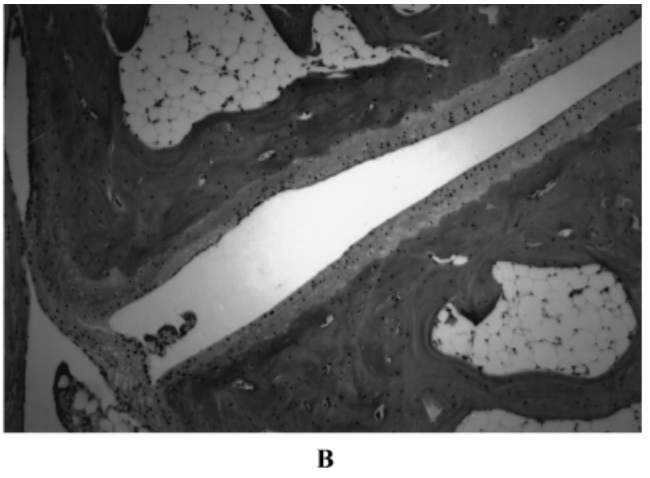




**Figure 3.** Immunohistochemical analysis of paraffin sections of the hind paw ankle joints of **A,** DA, **B,** E3, and **C,** LEW.1F rats obtained on day 150 after pristane injection. The sections from the DA and LEW.1F rats are representative of all rats with the same clinical scores. The section from the E3 rat is totally unaffected, while the sections from the DA and LEW.1F rats clearly show active inflammation. Due to the severe erosion of bone and cartilage in both the DA and LEW.1F strains, the entire joint has been engulfed in pannus, the joint is completely destroyed, and there is ankylosis, causing severe immobilization of the joint. (Hematoxylin and erythrosin stained; original magnification $\times$ 100.)

rats significantly prolonged the elevated fibrinogen level ($P < 0.01$ versus the E3 strain). In the chronic phase of the disease, the DA rats showed relapses of increased fibrinogen, possibly reflecting the relapsing arthritis in this strain. Levels of $\alpha_1$-acid glycoprotein were increased ~10-fold in the DA and LEW.1F rats, whereas the E3 strain only showed a minor increase ($P < 0.05$). The increase started at the onset of arthritis, continued to increase until maximum arthritis severity was reached, and then returned to normal levels as the acute inflammation subsided. Around day 80, the level of $\alpha_1$-acid glycoprotein was slightly elevated in the DA strain ($P < 0.05$ versus the E3 strain).

Levels of the negative acute-phase reactant $\alpha_1$-inhibitor$_3$ were reduced at arthritis onset in the arthritis-susceptible DA and LEW.1F strains, but remained constant in the arthritis-resistant E3 strain during the same time period ($P < 0.05$). The reduced levels in DA and LEW.1F rats remained low during the chronic phase of arthritis.

All strains had higher levels of IL-6 on day 10 after pristane injection (i.e., before arthritis onset). The arthritis-susceptible DA and LEW.1F strains had higher levels of IL-6 compared with the E3 strain ($P < 0.01$). The higher levels were maintained during the first period of active arthritis. During the chronic phase, the DA rats showed steadily higher levels of IL-6, whereas the LEW.1F rats showed decreasing levels, reflecting differences in the disease course.

Taken together, the results show that a strong acute-phase protein response both precedes and occurs during PIA. Interestingly, the various acute-phase proteins showed unique patterns of response during active PIA and in connection with the different types of chronic active arthritis seen in the DA and LEW.1F strains. The strain-specific acute-phase protein responses after pristane injection are most likely genetically determined, but the observed correlation with the development of arthritis could be a secondary phenomenon. To investigate the regulation of the various acute-phase proteins in PIA, we performed a genetic study to identify the specific genetic control for each of the

**Table 2.** Quantitative trait loci regulating the acute-phase protein concentration in (E3 × DA)$F_2$ intercross rats with pristane-induced arthritis

| Phenotype* | Marker | Inheritance pattern | LOD | Variance, %† | Locus | Reference‡ |
|---|---|---|---|---|---|---|
| $\alpha_1$-acid glycoprotein | | | | | | |
| d14 (M) | *D9Wox17* | Recessive | 2.9 | 25 | *Cia15*, *Ciaa3* | 32, 43 |
| d14 (M)§ | *D12Rat10* | Recessive | 6.0 | 35 | *Pia4*, *Cia12* | 30, 32 |
| d14 (M)§ | *D12Mgh11* | Recessive | 6.1 | 35 | *Apr1*¶ | Present study |
| $\alpha_1$-inhibitor$_3$ | | | | | | |
| d14 (M) | *D12Mgh11* | Recessive | 2.8 | 18 | *Apr1*¶ | Present study |
| d49 | *D1Wox25* | Additive | 3.2 | 11 | *Eae7* | 48 |
| d49§ | *D5Wox10* | Dominant | 3.7 | 8 | *Apr2*¶ | Present study |
| Interleukin-6 | | | | | | |
| d14§ | *D14Mgh3* | Recessive | 3.7 | 11 | *Pia6* | 30 |
| d14 (F) | *D1Mgh21* | Dominant | 3.0 | 19 | *Eae6* | 48 |
| d35 (M)§ | *D12Wox14* | Recessive | 6.7 | 31 | *Pia4* | 30 |
| d35 | *D18Mgh1* | Recessive | 2.8 | 12 | *Cia17* | 43 |
| d49§ | *D10Mgh9* | Additive | 3.9 | 12 | *Oia3*, *Cia5* | 34, 35 |

* Phenotypes are described in Materials and Methods. Letters in parentheses indicate that the linkage was found in only one sex (M = male; F = female).
† Extent to which the observed phenotype can be accounted for by this locus.
‡ Significant linkages found in published studies of rat arthritis models.
§ Significant linkage (logarithm of odds [LOD] score >3.4).
¶ New locus representing the acute-phase response. Two new loci were identified: *Apr1* and *Apr2*.

responding acute-phase proteins, and we compared this with the genetic control of arthritis.

**Linkage analysis.** To address the question of how the acute-phase reaction in PIA is genetically regulated, serum was taken from 153 (E3 × DA)$F_2$ intercross rats on days 14, 35, 49, and 100 after pristane injection. The loci controlling arthritis in this intercross have previously been determined (30). Serum was analyzed for the acute-phase proteins $\alpha_1$-acid glycoprotein, $\alpha_1$-inhibitor$_3$, and IL-6; because serum instead of plasma was obtained for this experiment, fibrinogen could not be analyzed. The acute-phase proteins were then used as quantitative traits in linkage analysis using the MapMaker software package (31). The QTLs identified are summarized in Table 2. In order to detect sex-specific effects, the 83 male and 70 female rats were analyzed separately.

Many of the identified loci that showed a significant LOD score (>3.4) (27) were in the same chromosomal regions as previously published arthritis QTLs in rats (30,32–35). The previously identified locus on chromosome 12 (*Pia4*) (30), which controls erosive arthritis, was found also to be associated with elevated serum levels of $\alpha_1$-acid glycoprotein on day 14 (LOD 6.0 in males only) and IL-6 on day 35 (LOD 6.7 in males only).

The chromosome 14 locus (*Pia6*), associated with chronic arthritis was found to be associated with elevated IL-6 levels as early as day 14 after pristane injection. Another locus associated with elevated IL-6 levels (on day 49) was found to be located on chromosome 10, close to the *Oia3*/*Cia5* locus associated with oil-induced arthritis and collagen-induced arthritis in crosses involving strains other than E3 (34,35).

We also found 2 significant loci that were associated with an acute-phase protein response which have not previously been observed to be associated with PIA. Linkage with $\alpha_1$-acid glycoprotein on day 14 was found on a locus near the telomeric end of chromosome 12, with an LOD score of 6.1 that was inherited in a DA recessive manner in males. This new locus was named *Apr1* (representing the acute-phase response). Another previously undetected linkage was found between $\alpha_1$-inhibitor$_3$ on day 49 and a locus on chromosome 5 (*Apr2*), which was inherited in a DA dominant manner (Figure 4). In addition, we listed in Table 2 the identified suggestive loci, several of which are colocalized with previously identified arthritis loci.

## DISCUSSION

The present genetic analysis of rat strains with variable susceptibility to chronic erosive arthritis induced by pristane shows a close correlation between the acute-phase response and some, but not all, genetic regions that control erosive arthritis. We were able to follow the acute-phase response through different phases of the disease: the initial response to the pristane injection, the process leading to the onset of arthritis, the outbreak of severe arthritis, and the development of chronic arthritis. This was achieved through a comparison of 3 different rat strains.

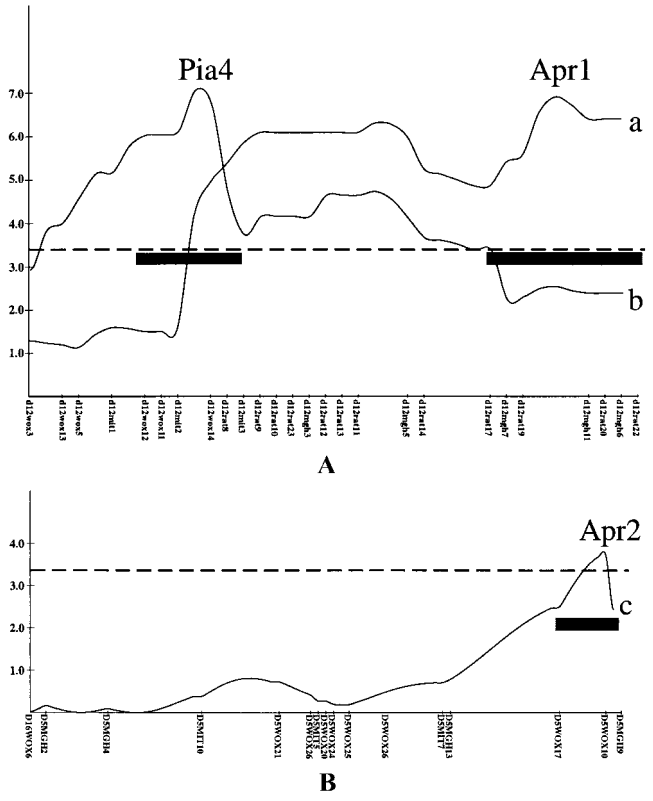



**Figure 4.** Plots of the logarithm of odds (LOD) scores for chromosomes indicating the quantitative trait loci (QTLs) identified for **A,** $\alpha_1$-acid glycoprotein d14 in males only (line a) and IL-6 d35 in males only (line b), as well as for **B,** $\alpha_1$-inhibitor$_3$ d49 (line c). The broken horizontal line at LOD 3.4 shows the threshold for a significant linkage in a fixed mode of inheritance. Solid boxes show 95% confidence intervals.

The DA strain had the most severe disease, with early onset and a chronic relapsing arthritis with severe erosions of the joints. The DA strain also experienced ankylosis of the tail. The presence of more deformities and erosions in the paws of this strain was probably a result of the intense chronic/relapsing phase of arthritis. The LEW.1F strain also developed a severe erosive arthritis with a subsequent chronic arthritis but with less severe erosions of the joints (Figures 1 and 2). The LEW.1F strain had more pronounced edematous joints during the first acute phase, but less severe erosions and deformities and no tail ankylosis. The E3 strain was resistant to PIA but had a minor acute-phase response after the pristane injection; this was identified as minor alterations in the plasma levels of acute-phase proteins (Figure 2).

The mechanism of PIA that leads to differences in susceptibility and development of arthritis is unclear, as it is in other arthritis models and in RA in humans. However, it is known that PIA is strictly dependent on activation of $\alpha/\beta$ T cells, since depletion of T cells prevents PIA and has therapeutic effects on the chronic phase if $\alpha/\beta$ T cells are depleted after the onset of disease (18). Interestingly, PIA is MHC associated and the association is not primarily with susceptibility, but rather, with chronic severity (18), as has been suggested for the association of MHC in RA (3). However, there is no clear evidence in PIA for any immune response to cartilage proteins (Olofsson P, et al: unpublished observations). This is in contrast to RA, where antibodies specific for various cartilage proteins, such as type II collagen, are detectable in a substantial proportion of patients (36) and is correlated with HLA–DR alleles (37).

Thus, in PIA, we are searching for non–antigen-specific factors that may play a role in the autoimmune response. One such process is the acute-phase protein response, which is also closely associated with RA (9) and seems to be evoked in animal models of arthritis (8). There is, however, very limited knowledge on the precise role of the various acute-phase proteins in inflammation and their relevance for arthritis.

The acute-phase response is the immediate reaction to inflammation. Three key regulators of the acute-phase response are IL-6, IL-1, and tumor necrosis factor (TNF). Hepatocytes in the liver produce the acute-phase proteins. These proteins can be divided into 2 types; type 1 acute-phase proteins ($\alpha_1$-acid glycoprotein, serum amyloid A, complement component C3, and haptoglobin) are regulated by IL-1 and TNF, and type 2 acute-phase proteins (fibrinogen, $\alpha_1$-antitrypsin, and $\alpha_2$-macroglobulin) are regulated by IL-6 (6). The glucocorticoids, which are regulated through cytokine stimulation via the central nervous system, represent another stimulatory factor of the acute-phase response. The glucocorticoids are strong up-regulators of $\alpha_1$-acid glycoprotein (6,38).

Analysis of acute-phase proteins and cytokines in the blood of rats is somewhat hampered by the lack of specific antibodies and assays. For example, we analyzed TNF$\alpha$, but the levels in the circulation were below the detection limit of the assay (data not shown). We also analyzed plasma concentrations of glucocorticoids, but the influence of daily rhythms and stress was of such importance that the data could not be interpreted (data not shown). Attempts to analyze $\alpha_2$-macroglobulin and $\alpha_1$-antitrypsin in plasma using sodium dodecyl sulfate–polyacrylamide gel electrophoresis failed because of a lack of rat-specific antibodies (data not shown). Since a

successful linkage analysis of quantitative traits largely depends on assays in which the phenotypes can be analyzed in a high throughput manner, we chose to focus on the acute-phase proteins fibrinogen, $\alpha_1$-acid glycoprotein, and $\alpha_1$-inhibitor$_3$ and the cytokine IL-6.

Fibrinogen is an acute-phase protein that is increased in plasma immediately after pristane injection. All rat strains we examined (DA, E3, and LEW.1F) showed increased levels of fibrinogen regardless of their susceptibility to PIA. This feature argues that the difference in arthritis susceptibility in these rat strains is independent of the systemic reaction to pristane that leads directly to increased levels of fibrinogen. The elevated fibrinogen concentrations seen in the DA rat during the second phase of active inflammation that starts around day 80 after pristane injection supports this hypothesis.

The $\alpha_1$-acid glycoprotein level showed a strong correlation with arthritis in the susceptible rat strains (DA and LEW.1F) and is therefore very useful as a plasma marker of active inflammation. During the period of most severe arthritis, $\alpha_1$-acid glycoprotein plasma levels were significantly different between the healthy E3 rats and the arthritis-susceptible DA and LEW.1F rats ($P < 0.05$). The function and mechanism of $\alpha_1$-acid glycoprotein regulation remain unclear. It has been suggested that the protein plays an immunoregulatory role, owing to its ability to interfere with leukocytes through binding to selectins (39). Thus, the increased levels of $\alpha_1$-acid glycoprotein may reflect a feedback mechanism that prevents the infiltration of leukocytes during inflammation.

The finding that the levels of $\alpha_1$-acid glycoprotein during the development of arthritis (day 14) were linked to the *Pia4* locus, but not other PIA loci, indicates a direct role of $\alpha_1$-acid glycoprotein in the pathogenic pathway leading to arthritis that is related to *Pia4*. The observation that the strongest linkage to the $\alpha_1$-acid glycoprotein levels was observed earlier (day 14) than the linkage to arthritis severity (day 35) suggests that it is involved in a primary mechanism in the pathogenic pathway. The observation that the $\alpha_1$-acid glycoprotein levels showed a stronger correlation in male rats but the arthritis linkage to *Pia4* was not sex specific could argue for the possibility that different genes control these traits. However, in this context it is worth noting that pregnancy, through the effects of estrogens, influences both the levels and the glycosylation of $\alpha_1$-acid glycoprotein (15). It is therefore likely that the $\alpha_1$-acid glycoprotein response has a sex-specific regulation.

Interestingly, the strong linkage to *Apr1*, which is located on chromosome 12 although it is distinct from *Pia4*, again shows that genes that are not related to arthritis control the levels of $\alpha_1$-acid glycoprotein. In rat chromosome 12, the 2 QTLs *Pia4* and *Cia12* (30,32) coincide. *Apr1* seems to be regulated by genes that are distinct from *Pia4* and *Cia12*, as shown in Figure 4. The *Apr2* region (separate from the *Pia4* locus) is also involved in antibody production in PIA (Olofsson P, et al: unpublished observations), which further strengthens the position of this QTL as being separate from the QTL region covering *Pia4* and *Cia12*.

The negative acute-phase protein $\alpha_1$-inhibitor$_3$ is a homolog of $\alpha_2$-macroglobulin that has been found only in the plasma of rodents (40). It has been shown in both CIA and *Mycobacterium*-induced arthritis that the concentration of $\alpha_1$-inhibitor$_3$ decreases during inflammation (41,42). As expected, levels of $\alpha_1$-inhibitor$_3$ were clearly decreased in the susceptible DA and LEW.1F strains compared with the E3 strain ($P < 0.005$), showing large between-strain differences during the acute, severe phase of the arthritis. However, genetic analysis showed that the levels of $\alpha_1$-inhibitor$_3$ were not associated with arthritis loci. Instead, a new locus was found on chromosome 5 (*Apr2*), and a suggestive linkage was also found with *Apr1*. The *Apr2* locus on chromosome 5 is located near a previously published QTL (*Ciaa5*) that showed suggestive linkage to antibody titers in CIA (43). This finding might indicate that QTLs for the acute-phase response which do not coincide with QTLs previously assigned to clinical disease in arthritis models might be loci that are also involved in the antibody production pathway.

It has been suggested that IL-6 is one of the most important regulators of acute-phase proteins (13). Elevated levels of IL-6 have been detected in serum in both RA patients (10) and rodent models of arthritis (44). In the mouse CIA model, IL-6 is expressed in the joints as one of the earliest markers of inflammation (45). It has been reported that IL-6–deficient mice are either not susceptible to arthritis (46) or have a delayed onset, although with a similar incidence of CIA as in wild-type control mice (11). Blockage of the IL-6 receptor with monoclonal antibodies was shown to reduce the severity of CIA in mice (47). Thus, it is clear that IL-6 is an important factor in the first acute phase of arthritis. This was also indicated in our PIA model by the elevated IL-6 levels in the susceptible rat strains. However, our findings also showed that there was a recurrence of IL-6 production associated with the relapsing phase of arthritis, which is of critical importance for comparison with RA. Interestingly, this recurrence is genetically deter-

mined, since only the DA strain, with a more pronounced relapsing arthritis, had elevated IL-6 levels, whereas the LEW.1F strain had elevated levels only during the acute phase.

The loci controlling the IL-6 levels colocalize with arthritis loci. The *Pia4* locus on chromosome 12 also controls the level of IL-6 in serum on day 35, the time point at which this locus showed the strongest association with arthritis. The levels of IL-6 at an earlier time point (day 14) were more strongly associated with another locus, *Pia6*, on chromosome 14. Surprisingly, this locus was associated with chronic arthritis rather than arthritis onset. If the same gene controls these traits, IL-6 could be an initiator of this pathway.

In summary, genetic analysis revealed that serum levels of each acute-phase protein we examined ($\alpha_1$-acid glycoprotein, $\alpha_1$-inhibitor$_3$, and IL-6) are controlled by different sets of QTLs, and that many of these coincided with QTLs for clinical phenotypes of arthritis (30,32,34,43). In addition, 2 new loci were identified (*Apr1* and *Apr2*), suggesting that part of the acute-phase response is regulated by mechanisms that are different from those controlling the clinically observable arthritis. These results show that plasma proteins, such as the acute-phase proteins, are excellent phenotypic markers for analyzing the genetics of arthritis. Clearly, the genetic control of arthritis is influenced by sex. About half of the loci identified so far have different effects in males and females (30,43).

We believe that the PIA model in rats will be a useful tool for unraveling some of the pathways that lead to chronic erosive arthritis as seen in RA. With different rat strains, this model can be used to study the different manifestations of arthritis. By the use of blood markers, such as the acute-phase protein $\alpha_1$-acid glycoprotein, a better analysis of the development of arthritis can be achieved. Since these blood markers can be used to study the genetics of arthritis, they are also of great value for the isolation of polymorphic genes in the various QTLs in congenic strains and are also accessible for corresponding analysis in humans.

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