A Comparative Genetic Analysis Between Collagen-Induced Arthritis and Pristane-Induced Arthritis

Peter Olofsson,1 Shemin Lu,1 Jens Holmberg,1 Tusheng Song,1 Patrik Wernhoff,1 Ulf Pettersson,2 and Rikard Holmdahl1

Objective. To compare the genetic regulation of collagen-induced arthritis (CIA) with that of pristane-induced arthritis (PIA) in rats.

Methods. A genome-wide linkage analysis of an (E3 × DA)DA backcross of rats with CIA (n = 364 male rats; the same strain combinations as previously used to determine the genetic control of PIA) was performed. The strongest loci in both CIA and PIA (i.e., Cia12/Pia4 and Cia13/Pia7) were isolated in congenic strains. Susceptibility in both congenic strains was tested in rats with CIA and in rats with PIA.

Results. We found a striking, although not complete, similarity of the arthritis-controlling loci in CIA and in PIA, as well as the previously defined loci associated with cartilage destruction, antibody production, and the acute-phase response. All major PIA quantitative trait loci (QTLs) identified in early severe arthritis were also strong regulators of CIA. The 2 strongest QTLs, Cia12/Pia4 on chromosome 12 and Cia13/Pia7 on chromosome 4, were also analyzed in congenic strains with DA or E3 as the background genome. Consistent with the results of linkage analysis, the congenic strain experiments showed that the chromosome 4 locus was more penetrant in CIA than in PIA, while the chromosome 12 locus almost completely dominated the control of PIA severity.

Conclusion. The underlying genetic control of CIA was found to have many, but not all, pathogenic mechanisms in common with PIA, despite the use of a cartilage-specific antigen (type II collagen) to induce CIA but not PIA.

Rheumatoid arthritis (RA) is a polygenic disease that primarily affects peripheral joints, with chronic inflammation, cartilage and bone destruction, and ultimately, joint deformation. The pathogenesis of RA is poorly understood, and the diagnosis is based on clinical descriptions rather than an understanding of the disease mechanisms (1). RA is believed to be the result of environmental factors in combination with a genetic predisposition to the disease. Several genetic studies of families, twins, and siblings showing familial aggregation and twin and sibling concordance have demonstrated a clear genetic component of arthritis (2,3). Inheritance of RA has been shown to depend upon the major histocompatibility complex (MHC) haplotype as well as other, hitherto-identified genes with varying influences (4,5). A recently published linkage analysis of human RA estimated the heritable component of RA to be as high as 60% (5).

Unfortunately, heterogeneity of the population hampers linkage analyses of RA in humans. Therefore, considerable effort has been invested in establishing animal models of disease that resemble RA in humans. These animal models, being inbred, have the advantage of identical genetic composition. Furthermore, since these animals can be studied under environmentally controlled conditions, inherited components of arthritis can be isolated.

There is a range of available rat models of arthritis that more or less resemble RA in humans. These models include cartilage-restricted antigen-
induced arthritis, such as type II collagen-induced arthritis (CIA) (6,7), adjuvant-induced arthritis, such as the classic Mycobacterium-induced arthritis (8), as well as oil-induced arthritis (OIA) (9) and pristane-induced arthritis (PIA) (10). In all these models, the induction protocols are different. In the CIA model, type II collagen (CII) emulsified in Freund’s incomplete adjuvant (IFA) is injected. Immunization with the collagen emulsification induces an antibody response followed by clinical manifestations of arthritis (11). In PIA and OIA, adjuvants (pristane and IFA, respectively) are injected to induce arthritis. These nonimmunogenic, adjuvant-induced diseases are believed to depend largely on the activation of T cells, as shown by α/β T cell depletion and T cell–transferable arthritis but no antibody response to any specific joint antigen (10,12–14). Cartilage-restricted antigen-induced arthritis involves an antibody-mediated component in the effector pathway (15).

We previously reported our genetic analyses of PIA in genetic segregating crosses between the arthritis-susceptible DA rat and the arthritis-resistant E3 rat (16). In those studies, we demonstrated strong linkage between arthritis phenotypes and several different chromosome regions. Eight quantitative trait loci (QTLs) (Pia1–Pia8), including the MHC region on chromosome 20, have been found to be associated with the clinical manifestations of PIA (10,16,17). Two QTLs that control the acute-phase response (Apr1 and Apr2) have also been correlated with disease (18). Taken together, all these loci have major effects on the outcome of arthritis, reflecting the complexity of the disease even in the context of only 2 genomes.

With regard to CIA in rats, most linkage analyses have been performed in the setting of bovine CII–induced arthritis and in strain combinations other than E3 with DA (19–23). Therefore, in order to make a reasonable comparison of the genetic inheritance of CIA and PIA in rats, we performed linkage analysis of CIA in the same strain combination and induced by the same collagen source that was used for the PIA studies. Hence, arthritis was induced with homologous rat CII in IFA in a backcross of 364 male (E3 × DA)DA rats. The CIA QTLs obtained were then compared with previously identified PIA-derived QTLs, thus matching the inheritance of these 2 rat models of arthritis in the same rat strains.

MATERIALS AND METHODS

Animals. Rats of the E3 and DA strains (Zentralinstitut für Versuchstierzucht, Hannover, Germany) were maintained in our animal facilities in a climate-controlled environment with 12-hour cycles of light/dark. 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 × DA)DA offspring and arthritis experiments were performed in the same pathogen-free animal facilities.

Female E3 rats were intercrossed with male DA rats to produce (E3 × DA)F1 offspring that were further backcrossed with DA rats to produce the 364 male (E3 × DA)DA rats that were used in the linkage analysis. DA.Pia4 (D12Rut28 to D12Mgh3; N10) and DA.Pia7 (D4Mit16 to D4Mgh11; N5) congenic rats were obtained through conventional backcross breeding to parental DA rats, with negative selection for all known QTLs and positive selection for microsatellite markers on chromosome 12 or chromosome 4, respectively. The same conventional backcross breeding procedure was used to develop the E3.Pia4 and E3.Pia7 congenic strains (N6). For the arthritis experiments, double-heterozygous congenic strains with the E3 background were intercrossed. The experimental animals were stratified for Cia12/Pia4 and Cia13/Pia7 genotypes in the analyses.

Induction and evaluation of arthritis. Lathyritic rat CIA was purified from Swarm rat chondrosarcoma grown in male rats that were receiving β-amimopropionitrilite monofumaratic salt in their drinking water during the tumor-growing period, as previously described (24,25). CIA was induced by an intradermal injection at the base of the tail with 150 μg of lathyritic rat CIA dissolved in 75 μl of 0.1M acetic acid and emulsified in 75 μl of IFA; for the DA.Pia7 congenic strain experiment, 75 μg of lathyritic rat CIA was used. PIA was induced by an intradermal injection at the base of the tail with 150 μg of pristane (2,6,10,14-tetramethylpentadecane; Aldrich, Milwaukee, WI). Rats were ages 8–12 weeks at the time of arthritis induction.

Arthritis development was monitored in all 4 paws and scored according to macroscopic appearance. 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). Rats were examined 1–4 times each week for 5 months after arthritis induction. Blood was collected (by cutting the tip of the tail) on days 21 and 49 after arthritis induction. To prevent coagulation, 10 μl of heparin (5,000 units/ml; Lövens Läkemedel, Malmö, Sweden) was mixed with 500–1,000 μl of blood. Plasma was separated from blood cells by centrifugation, removed, and stored at −70°C until assayed.

Determination of plasma protein concentrations. Levels of α1-acid glycoprotein were measured with a soluble competitive radioimmunoassay (26). Rat α1-acid glycoprotein (Zivic-Miller Laboratories, Zelienpolle, PA) and a polyclonal rabbit antibody against α1-acid glycoprotein (Agrisera, Vännäs, Sweden) were used.

Plasma concentrations of cartilage oligomeric matrix protein (COMP) were determined by a competitive enzyme-linked immunosorbent assay (ELISA), using similar conditions as described elsewhere for determining human COMP concentrations (27), with modifications. Rat COMP was used to coat...
the microtiter plates and to prepare the standard curve included in each plate, and a polyclonal antiserum raised against rat COMP was used as the capture antibody.

**Determination of antibodies.** Antibodies against rat cartilage in plasma were analyzed by ELISA. Briefly, 96-well plates (Costar, Cambridge, MA) were coated overnight at 4°C with 50 μl/well of phosphate buffered saline (PBS) containing 10 μg/ml of rat CII. All washings were performed with Tris buffered saline (NaCl 1.3 M, Tris 0.1M, pH 7.4) containing 0.1% Tween 20. Plasma was diluted in PBS–0.1% Tween 20 and analyzed in duplicate. Levels of bound IgG antibody were estimated after incubation with a donkey anti-rat IgG coupled to alkaline phosphatase (Jackson ImmunoResearch, West Grove, PA). Pararitninol was used as a chromogenic substrate, and the absorbance was determined with a SpectraMax instrument (Molecular Devices, Sunnyvale, CA). The relative amount of plasma antibody was determined by comparison against an anti-CII–positive control serum. Rheumatoid factor (RF) concentrations were determined in the same manner, except that the plates were coated with 8 μg/ml of rabbit IgG (Sigma-Aldrich, St. Louis, MO).

**Genotyping and linkage analysis.** DNA was prepared from toe biopsy tissues by heating the sample in 1 ml of 50 mM NaOH for 1 hour. The DNA solution was neutralized with 100 μl of 1M Tris buffer and used directly in the polymerase chain reaction (PCR) (28). Primer sequences for rat microsatellite markers defined as DxMity, DxMyh, DxRaty, and DxGotoy were obtained from Research Genetics (Huntsville, AL), and those for markers defined as DxWoyy were obtained from the Wellcome Institute for Human Genetics (Oxford, UK). All markers were assayed by PCR on a PTC-200 Thermal Cycler (MJ Research, Waltham, MA) according to standard protocol. The resulting PCR products were run on an ABI 377 DNA sequencer (Perkin Elmer, Emeryville, CA) or a MegaBACE 1000 sequencer (Amersham Pharmacia Biotech, Uppsala, Sweden), and data were analyzed with the software packages GeneScan 3.1 and either Genotyper 2.1 (Perkin Elmer) or Genetic Profiler 1.1 through comparison with amplified samples from parental strain rats.

To produce linkage maps covering the complete genome, all 364 backcross progeny were genotyped using 238 markers. More than 94% of the rat genome was within 10 cM of 1 microsatellite marker (maximum intermarker distance 23 cM). An improved linkage map based on several crosses involving E3 and DA can be found at our Internet site at http://net.inflam.lu.se.

Map Manager QTX13 software (29) was used to perform the QTL analysis and permutation tests. Chromosomal QTL maps showing the logarithm of the likelihood that a given QTL controlled arthritis or the arthritis-regulated blood phenotypes were drawn using Ogene 3.06w software (30). Threshold values for significance obtained from the permutation tests were determined by randomizing the phenotypes against the genotypes 500 times in order to calculate relevant significance levels, since permutation calculations based on the features under investigation provide a more accurate estimation of significance levels (31). For a claim of significant linkage, we used a threshold of logarithm of odds (LOD) values ≥2.8, as determined by permutation analysis of the respective traits used for the analysis of the backcross. All phenotype traits were transformed by natural logarithm in order to normalize the distribution of the analyzed data. Quantitative data for congenic strains are expressed as the mean ± SEM. Significance analysis was performed using the nonparametric Mann-Whitney U test.

### RESULTS

**Linkage analysis of CIA.** Previously published reports regarding linkages in crosses of the arthritis-susceptible DA rat with the arthritis-resistant E3 rat have focused on PIA. Linkages identified in PIA involve regulation of the onset (Pia2 and Pia3), severity (Pia4, Pia7, and Pia8), and chronicity (Pia1, Pia5, and Pia6) of arthritis, as well as the acute inflammatory response, analyzed as plasma concentrations of acute-phase proteins (Apr1 and Apr2) (10,16–18).

The clinical manifestations of CIA are similar to those of PIA, although the genetic susceptibility and pathogenesis are slightly different (32). E3 rats are resistant to both PIA and CIA. DA rats are 100% susceptible to PIA and are slightly less susceptible to CIA. Lower degrees of arthritis severity and later onset of arthritis are also observed in CIA compared with PIA. F1 offspring show complete resistance to CIA, whereas >50% of F1 offspring are affected by PIA, but with late onset and milder arthritis (16,18) (Table 1).

| Rats with CIA | No. of rats | Day of arthritis onset mean ± SEM | Maximum clinical score mean ± SEM | Incidence % |
|---------------|-------------|-----------------------------------|-----------------------------------|-------------|
| E3            | 9           | 0 ± 0                             | 0 ± 0                             | 0           |
| DA            | 18          | 22 ± 5                            | 19 ± 1                            | 78          |
| (E3 × DA)F1   | 18          | 26 ± 1                            | 8 ± 1                             | 34          |
| (E3 × DA)DA   | 364         | 26 ± 1                            | 8 ± 1                             | 34          |

* Values are the mean ± SEM. CIA = type II collagen–induced arthritis; PIA = pristane-induced arthritis.

### Table 1. Clinical arthritis in parental rat strains and segregating crosses of rats with CIA and PIA*

We used the CIA model to identify new QTLs inherited from E3 and DA rats and to verify QTLs that correlate between CIA and PIA. In this CIA linkage analysis, 364 male (E3 × DA)DA rats were genotyped using 238 markers, and linkages between genotype and clinical arthritis phenotypes (onset of disease and maximum arthritis severity) or plasma phenotypes (COMP,
Table 2. Linkages identified in the (E3 × DA)DA rat CIA experiment and previously identified PIA loci*

| Phenotype, SSLP marker | LOD | Inheritance† | CIA QTL‡ | Ref. | PIA QTL‡ | Ref. | Other QTLs |
|------------------------|-----|--------------|----------|------|----------|------|-----------|
| Maximum arthritis score |     |              |          |      |          |      |           |
| D3mit6                 | 3.5 | DA           | Cia11    | 20   | –        | –    | –         |
| D4rat109               | 4.7 | DA           | Cia3     | 22   | Pia5     | 16   | Aia3, Eau1 |
| D4eno2                 | 7.5 | DA           | Cia13    | 20   | Pia7     | 17   | Oia2, Cia4 |
| D12rat72               | 6.6 | DA           | Cia12    | 20   | Pia4     | 16   | Eae5, Eau2 |
| Arthritis onset        |     |              |          |      |          |      |           |
| D3mit6                 | 2.8 | DA           | Cia11    | 20   | –        | –    | –         |
| D4rat109               | 5.2 | DA           | Cia3     | 22   | Pia5     | 16   | Aia3, Eau1 |
| D4eno2                 | 10.8| DA           | Cia13    | 20   | Pia7     | 17   | Oia2, Cia4 |
| D6mgh10                | 2.9 | E3           | Cia20    | –    | Pia3     | 16   | –         |
| D12rat72               | 7.6 | DA           | Cia12    | 20   | Pia4     | 16   | Eae5, Eau2 |
| COMP                   |     |              |          |      |          |      |           |
| D4eno2                 | 4.0 | DA           | Cia13    | 20   | Pia7     | 17   | Oia2, Cia4 |
| D12rat72               | 3.6 | DA           | Cia12    | 20   | Pia4     | 16   | Eae5, Eau2, Apr1 |
| α1-acid glycoprotein   |     |              |          |      |          |      |           |
| D4eno2                 | 5.1 | DA           | Cia13    | 20   | Pia7     | 17   | Oia2, Cia4 |
| D12rat72               | 8.3 | DA           | Cia12    | 20   | Pia4     | 16   | Eae5, Eau2, Apr1 |
| Anti-CII IgG           |     |              |          |      |          |      |           |
| D1mgH11                | 3.3 | DA           | Cia6     | –    | –        | –    | Eae6, Eae7 |
| D20mgH4                | 5.8 | E3           | Cia1     | 22   | Pia1     | 10   | Cia1, Eae1, Oia1 |
| IgG rheumatoid factors |     |              |          |      |          |      |           |
| D5rat37                | 3.0 | DA           | Cia5     | 19   | –        | –    | Apr2      |
| D14wox12               | 3.2 | DA           | Cia7     | –    | Pia6     | 16   | Eae10     |

* The maximum arthritis score represents the maximum clinical score obtained during the experiment. Arthritis onset represents the first day of visible signs of arthritis after induction of type II collagen–induced arthritis (CIA). Plasma levels of cartilage oligomeric matrix protein (COMP), 1-acid glycoprotein, anti–type II collagen (anti-CII) IgG (reactive with rat type II collagen), and IgG rheumatoid factors were measured as described in Materials and Methods. SSLP = simple sequence-length polymorphism; PIA = pristane-induced arthritis; LOD = logarithm of odds; QTL = quantitative trait locus.
† Inheritance pattern determined as being DA or E3 promoting.
‡ Previously identified CIA QTL in the same chromosome region as the CIA linkage identified in (E3 × DA)DA linkage (see refs. 18, 22, 33, and 41–43).

The α1-acid glycoprotein, and antibody levels) were determined.

In the present study, we identified significant linkage between a locus on chromosome 3 and clinical arthritis. This arthritis-regulating locus overlaps with the previously reported Cia11 locus, which was identified in experiments using DA and BN rats (20). Other significant loci that regulate clinical arthritis have previously been reported, as follows: on chromosome 4, Cia3/Pia5 and Cia13/Pia7, on chromosome 6, Cia20/Pia3, and on chromosome 12, Cia12/Pia4 (Table 2 and Figure 1A). Four loci were identified as strong regulators of the acute-phase response (detected as plasma levels of α1-acid glycoprotein) and cartilage destruction (measured as plasma levels of COMP). The QTLs linked to α1-acid glycoprotein and COMP were found on chromosomes 4 and 12, respectively, and were co-inherited with clinical arthritis traits (Cia3/Pia5, Cia13/Pia7, and Cia12/Pia4/Apr1, respectively) (Table 2 and Figure 1B).

Plasma concentrations of IgG-RF and antibodies directed against CII were analyzed as a reflection of the B cell response to CIA. The production of RF was controlled by loci on chromosomes 5 (Ciaa5/Apr2) and 14 (Ciaa7/Pia6). Linkage to anti-CII antibodies was identified on chromosome 1 (Ciaa6/Eae6/7) and acute-phase response. Linkage with anti-CII antibodies was also identified on chromosome 20 (Ciaa1/Pia1), which contains the MHC region (Table 2 and Figure 1C).

Comparison of linked QTLs between CIA and PIA. We were unable to address female-dependent inherited regulation (i.e., Pia2 and Pia8) in the present study since only male rats were used. The remaining published PIA QTLs (i.e., Pia1, Pia3, Pia4, Pia5, Pia6, and Pia7, as well as Apr1 and Apr2) were identified using the CIA model on the (E3 × DA)DA cross. Pia5 and Pia6 are both linked to chronic arthritis in PIA; however, in the CIA backcross analysis, they were associated with arthritis severity and RF production, respectively. Whether these loci were also associated with chronic CIA could not be determined, since the analysis was performed only during the acute phase of disease (<50 days). A schematic representation of the rat genome is
depicted in Figure 2, with all linkages obtained in this CIA analysis indicated together with previously published loci from PIA linkage analyses using E3 and DA parental rats.

**Confirmation of the major loci in congenic strains.** Two of the major QTLs that regulate arthritis severity are the Cia12/Pia4 locus on chromosome 12 and the Cia13/Pia7 locus on chromosome 4 (16,17). We
produced congenic strains of the chromosome 12 region (DA.Pia4 and DA.Pia7) and the chromosome 4 region (DA.Pia4 and DA.Pia7) by transferring the *Cia12/Pia4* or the *Cia13/Pia7* locus, respectively, from the arthritis-resistant E3 strain to the arthritis-susceptible DA strain. By using both PIA and CIA, the genetic fragments in the...

**Figure 2.** Schematic genome view of the inheritance of pristane-induced arthritis (PIA) and collagen-induced arthritis (CIA) in linkage analyses of E3 and DA rats. Results of linkage analyses from the present study of CIA are shown together with previously published linkage analyses of PIA, acute-phase response, and antibody level. Thick black vertical bars show linkages obtained with the CIA model, using identical parental founder strains (E3 × DA); thick shaded vertical bars show the published quantitative trait locus regions of PIA linkage. Chr = chromosome.
Cia12/Pia4 (Figure 3) and the Cia13/Pia7 (Figure 4) regions were confirmed to have a strong ameliorating effect on arthritis. The ameliorating effect of Cia12/Pia4 was more pronounced in PIA than in CIA, whereas the opposite was observed in the Cia13/Pia7 congenic strain.

Since both these loci also were shown to regulate plasma levels of COMP (reflecting cartilage destruction) and α1-acid glycoprotein (reflecting systemic inflammation), plasma concentrations of these proteins during the acute phase of the disease were evaluated. The DA.Pia4 congenic strain, which showed the greatest difference in the presence of PIA compared with CIA, showed significantly decreased COMP and α1-acid glycoprotein levels. Unfortunately, no blood samples were obtained during the CIA experiment, and these blood proteins therefore could not be analyzed.

In the DA.Pia7 congenic strain, both COMP and α1-acid glycoprotein were significantly decreased in rats with CIA, whereas in rats with PIA, only the α1-acid glycoprotein level was decreased. To further analyze the different effects of these 2 loci on arthritis, reciprocal congenic strains were developed. Both PIA and CIA were tested in an intercross experiment between (E3.Pia4 × E3.Pia7)F1 congenic rats. We observed that in the presence of CIA, the Cia13/Pia7 locus had the most significant impact, whereas the Cia12/Pia4 locus

**Figure 3.** Clinical arthritis in collagen-induced arthritis (CIA) and pristane-induced arthritis (PIA) in DA.Pia4 and DA.Pia7 congenic rats. Arthritis severity was determined in DA rats (●; n = 13 for CIA and n = 16 for PIA), DA.Pia4 E3/DA rats (▲; n = 16 in each group), and DA.Pia4 E3/E3 rats (■; n = 7 for CIA and n = 16 for PIA). There were significant differences between DA and DA.Pia4 and DA.Pia7 congenic rats (P < 0.01 for CIA, showing a significant difference beginning on day 17; P < 0.0001 for PIA, showing a significant difference beginning on day 10, as determined by Mann-Whitney U test). Levels of cartilage oligomeric matrix protein (COMP) and α1-acid glycoprotein (AGP) were significant in the DA.Pia4 a/a (E3/E3) and DA.Pia4 a/b (E3/DA) rats compared with the DA.Pia4 b/b (DA/DA) rats (a = E3 allele; b = DA allele). The size of the Cia12/Pia4 congenic fragment is depicted in a schematic representation of chromosome 12.
DISCUSSION

There is a striking resemblance between the genetic control of PIA and the genetic control of CIA, as shown by linkage analysis and studies of congenic strains. The present study was designed to be highly discriminating. We used 364 male rats of the \((E3 \times DA)DA\) backcross and analyzed arthritis onset and severity as well as antibody levels, RF production, and acute-phase response. Since this has also been done in \(E3 \times DA\) crosses after injection with pristane (10,16–18), a careful comparison between these arthritis models could be made. In the CIA linkage experiment, we were able to identify several regions on chromosome 12 that were associated with disease susceptibility. These results provide further evidence for the genetic basis of CIA and PIA and suggest that there are shared genetic factors influencing both arthritis models.
able to identify all published PIA loci except for those related strictly to female inheritance (Pia2 and Pia8) and to chronicity (Pia5 and Pia6), since the analysis was performed on male rats during the acute phase of arthritis. However, the relative contribution of the various loci to the traits we examined differed, and we found additional loci associated with arthritis and anti-CII antibody production, reflecting the divergent pathogeneses of these diseases.

Arthritis is inducible in rats by the injection of CII emulsified in either IFA (mineral oil) or CFA, as in CIA, or by the injection of pure pristane oil, as in PIA. Therefore, the major difference between PIA and CIA is the use of CII in the immunization emulsion in CIA. CIA and PIA are clinically very similar, aside from the fact that CIA affects the phalangeal joints more profoundly than does PIA and that relapses are more prominent during chronic disease in PIA. PIA is a T cell–dependent disease, although the specificity of the autoimmune reaction is unknown (10). CIA is more complex, having the added influence of B cells due to the in vivo affinity of CII-specific antibodies for cartilage (34). Since CIA shares T cell dependence with PIA but has the additional antibody-dependent mechanism, it is expected that CIA would share some genetic linkages with PIA as well as demonstrate some specific genetic regulation. Loci identified in both CIA and PIA may therefore reflect genes involved in regulating the T cell response caused by the injection of adjuvant, while the CIA-specific QTLs may be involved in the more antigen-specific response directed against CII.

CIA loci that were found to regulate the onset and severity of arthritis (i.e., chromosomes 3, 4, 6, and 12, which are equivalent to Cia11 and Cia3/Pia5, Cia12/Pia7, Cia20/Pia3, and Cia12/Pia4, respectively) were identical in CIA and PIA, except for the Cia11 locus. These findings suggest that these loci harbor genes that control the general inflammatory response elicited by the adjuvant. The antibody QTLs in CIA found on chromosomes 20 and 14 are equivalent to Ciaa1/Pia1 and Ciaa7/Pia6, respectively. Both Pia1 and Pia6 are loci that regulate chronic arthritis. Hence, the identification of these loci with the use of anti-CII antibodies or RFs in CIA possibly reflects an antibody-regulated phase, which is observed in the chronic phase of PIA. In CIA, an immune response to joint-specific antigen (CII) is triggered soon after immunization, which possibly accounts for the involvement of these loci in acute disease. However, in PIA, where both Pia1 and Pia6 play a role strictly during the chronic phase of disease, these loci may be triggered only after exposure of cartilage protein to the immune system and breakage of immune tolerance due to the ongoing joint erosion and inflammation.

In previous linkage analyses of PIA using genetic segregation between E3 and DA rats, the Cia12/Pia4 locus on chromosome 12 was found to be the strongest regulator of arthritis in these strains (16). This locus also showed a strong effect on disease, since the E3 allele of this locus conferred 90% protection against arthritis severity in PIA (Figure 3). Recently, the Cia12/Pia4 QTL was found to confer structural polymorphism in the Ncf1 gene (35). The effect of this genetic variation resulted in differential production of free radicals by the NADPH oxidase complex (36). We hypothesized that the production of oxygen radicals by the antigen-presenting cells reduces the priming of autoreactive T cells. We found that in CIA, the effect of the Cia12/Pia4 locus was less potent, while Cia13/Pia7 had the greatest effect on clinical arthritis. The different effects of these
Mycobacterium butyricum confers disease protection in the DA strain. Moreover, been found to be regulated by previously identified and ally help unravel the pathways involved in human RA gathered from such genetic analyses in rats will eventu-
rat models of arthritis as possible. The information
regulating genes be identified and evaluated in as many
arthritis. It is therefore important that the arthritis-
susceptibility loci. The majority are involved in
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