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Combined Autoimmune Models of Arthritis Reveal Shared and Independent Qualitative (Binary) and Quantitative Trait Loci

Vyacheslav A. Adarichev,* Juan C. Valdez,† Tamás Bárdos,‡ Alison Finnegan,§ and Tibor T. Glant*∥

Collagen-induced arthritis (CIA) and proteoglycan-induced arthritis (PGIA) are murine models for rheumatoid arthritis both in terms of their pathology and genetics. Using the F2 hybrids of the CIA-susceptible, but PGIA-resistant DBA/1 mice, and the CIA-resistant, but PGIA-susceptible BALB/c mice, our goals were to 1) identify both model-specific and shared loci that confer disease susceptibility, 2) determine whether any pathophysiological parameters could be used as markers that distinguish between nonarthritic and arthritic mice, and 3) analyze whether any immune subtraits showed colocalization with arthritis-related loci. To identify chromosomal loci, we performed a genome scan on 939 F2 hybrid mice. For pathophysiological analyses, we measured pro- and anti-inflammatory cytokines (IL-1, IL-6, TNF-α, IFN-γ, IL-4, IL-10, IL-12), Ag-specific T cell proliferation and IL-2 production, serum IgG1 and IgG2 levels of both auto- and heteroantibodies, and soluble CD44. In addition to multiple CIA- and PGIA-related loci identified in previous studies, we have identified nine new CIA- and eight new PGIA-linked loci. Comprehensive statistical analysis demonstrated that IL-2 production, T cell proliferation, and IFN-γ levels differed significantly between arthritic and nonarthritic animals in both CIA and PGIA populations. High levels of TNF-α, IFN-γ, IL-2, and Ab production were detected in F2 hybrids with CIA, whereas T cell proliferation, IL-2 and IFN-γ production, and a shift to IgG2a isotype were more characteristic of PGIA. Quantitative trait loci analysis demonstrated colocalization of numerous immune subtraits with arthritis-related traits. Quantitative trait loci on chromosomes 5, 10, 17, 18, and X were found to control arthritis in both models. The Journal of Immunology, 2003, 170: 2283–2292.

To investigate loci associated with rheumatoid arthritis (RA),3 numerous studies have used animal models which have the advantage of a controlled environment and known genetic background. To date, disease-associated loci were identified in animal models for arthritis, induced by immunization with adjuvant (1), oil (2), pristane (3), bacterial wall components (4), type II collagen (5–10), and proteoglycan (11–13). Together, these studies have identified a large number of loci associated with clinical symptoms of arthritis, thus illustrating the underlying complexity of autoimmune diseases. Many of these quantitative trait loci (QTL) are colocalized to homologous chromosomal regions in different models and in different species suggesting common genetic components (4, 14–16). Presumably, certain genes associated with these loci will correspond to genes involved in RA susceptibility (17–19). Furthermore, many of these loci have been colocalized with loci uncovered in other autoimmune models or diseases (14–16), suggesting a shared or common genetic pathway in autoimmune diseases.

Although these studies have helped define the genetic relatedness and similarities of the available autoimmune models, none have successfully narrowed the genetic interval of any QTL to the point where positional cloning can be used. Thus, the central problem of the identification of the disease-responsible genes remains. The use of different genetic crosses, increasingly dense genetic maps and congenic strains, as well as the completion of the human and mouse genome projects, will likely make these goals a reality.

The approach used in this study makes use of a single F1 intercross that permits simultaneous analysis of two genetically distinct murine models of RA: collagen-induced arthritis (CIA) and proteoglycan (aggrecan)-induced arthritis (PGIA). CIA is an autoimmune model that can be generated in rats (20), mice (21), and monkeys (22). PGIA is an autoimmune murine model with 100% incidence in the BALB/c mouse strain (23–26). DBA/1 (H-2q) mice are susceptible to CIA but resistant to PGIA, whereas BALB/c mice (H-2d) are susceptible to PGIA, and resistant to CIA. To gain insight into the mechanisms of how the major clinical (disease susceptibility, severity, and onset of arthritis) and immunological traits (Ag-specific T and B cell responses and cytokine production) are influenced in this special combination of genetic background, we have generated a unique intercross of BALB/c and DBA/1 parent strains, and the F1 and F2 hybrids were immunized for either CIA or PGIA. The combination of two arthritis models using F2 hybrids of the susceptible parental strains provides an avenue for testing the hypothesis that QTL identified in one model may also be involved in a second model. Presumably, some QTL will be model-specific, while others will be shared between different models. It is our hypothesis that loci shared between different models...
are more likely to involve genetic pathways that are also shared in RA, and perhaps autoimmune diseases in general.

Our aims in this study were multifold. First, we wanted to evaluate the F2, and F3 hybrids of this novel cross in terms of arthritis incidence and severity. We have hypothesized that, while BALB/c mice are resistant to CIA and DBA/1 mice resistant to PGIA, there may be genetic components from each background that contribute to susceptibility in the disease model from which that particular mouse strain is resistant. Secondly, we wanted to test a wide range of pathophysiological and immunological markers to determine whether any of these parameters could be used as phenotypic markers associated with disease susceptibility or severity. Finally, we sought to determine whether QTL controlling clinical symptoms, or any of the pathophysiological parameters, would colocalize with QTL previously identified (7, 8, 11, 12) and, if so, how these traits modify the clinical picture of the original model.

Materials and Methods

Animals, Ags, and Immunization

BALB/c female mice (Kingston colony K51; Charles River Breeding Laboratories, Wilmington, MA) were mated with DBA/1 males (The Jackson Laboratory, Bar Harbor, ME), and the resulting F1 offspring were intercrossed to generate F2 hybrids (n = 930). Parent BALB/c females were selected to achieve 100% incidence of PGIA in the parental line (26) and DBA/1 males to the highest incidence for CIA (27). Notably, BALB/c mice were absolutely resistant to CIA, and the DBA/1 strain was previously selected to achieve 100% incidence of PGIA in the parental line (26) and DBA/1 females (Kingston colony K51; Charles River Breeding Laboratories, Wilmington, MA) were mated with DBA/1 males (The Jackson Laboratory, Bar Harbor, ME), and the resulting F1 offspring were intercrossed to generate F2 hybrids (n = 930). Parent BALB/c females were selected to achieve 100% incidence of PGIA in the parental line (26) and DBA/1 males to the highest incidence for CIA (27). Notably, BALB/c mice were absolutely resistant to CIA, and the DBA/1 strain was previously found to be resistant to PGIA (26). Mice were immunized by the standard method described with PGIA and CIA antigens. The first injection was given i.p. on day 1 and the second injection (same dose and adjuvant) was given i.p. on day 19. A third injection was given i.p. on days 29 and 39. The first and second injections were given in CFA (Difco), whereas the second and third boosters contained Ag in IFA (Difco). Mice that did not develop arthritis within 3 wk of the second collagen injection were boosted with a third injection (equally divided i.p. and into the proximal tail) and sacrificed 6 wk later. For PGIA (26), 100 μg of Ag (measured as proteoglycan core protein) was emulsified with adjuvant and injected i.p. on days 0, 7, 28, and 69. The second and fourth injections were given in CFA (Difco, Detroit, MI), whereas the second and third boosters contained Ag in IFA (Difco). Mice that did not develop arthritis within 5 wk after the fourth injection were boosted and sacrificed 4 wk later. These extra Ag injections (third in CIA and fifth in PGIA) were given in both models to provoke CIA or PGIA in all, but possibly less susceptible, F2 hybrid mice.

Assessment of quantitative and qualitative arthritis traits

Arthritis was assessed daily and the inflammation (quantitative trait). The assessment of quantitative and qualitative arthritis traits during the experimental period, was applied for each animal (12, 25, 26). However, this clinical score includes two combination events were reanalyzed. The chromosomal linkage maps and marker positions were ultimately confirmed using The Jackson Laboratory Web resource (http://www.informatics.jax.org/) and Celeria Discovery system (http://www.celeria.com/); Linkage of potential QTL to SSAP marker polymorphisms and χ2 statistics for trait-marker association was determined with Map Manager QTX version 13 using a free regression model (13, 34). For selection of QTL in the intercross, a logarithm of the odds (LOD) score value of 2.8 was used as a cut-off for suggestive linkage and a LOD score value of 4.3 was used as the threshold for significant linkage (35).

Statistical analysis

Statistical analysis was performed using the statistical software package SPSS (version 13, Chicago, IL). Clinical traits, including binary severity, and onset scores of arthritis, and basic immune parameters (Abs and T cell proliferation) demonstrated nonparametric distributions in F2 hybrid population. Therefore, we used the nonparametric Mann-Whitney U test to analyze differences between subgroups. The Spearman’s correlation coefficient was used to evaluate biases between traits. For statistical analysis of parametric data, the two-sample Student’s t test was used. The significance level was set at p < 0.05.
Table I. Incidence and severity of arthritis in a combined model of CIA and PGIA

<table>
<thead>
<tr>
<th>Model</th>
<th>Gender</th>
<th>Cross†</th>
<th>Arthritis/Immunized Incidence (%)</th>
<th>Onsetb</th>
<th>Severityb</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIA</td>
<td>Malesa</td>
<td>DBA/1</td>
<td>43/57 (75.4)</td>
<td>3.9 ± 1.1</td>
<td>3.3 ± 2.1</td>
</tr>
<tr>
<td>CIA</td>
<td>Malesa</td>
<td>F1</td>
<td>49/49 (100)</td>
<td>4.1 ± 0.7</td>
<td>6.3 ± 3.4*</td>
</tr>
<tr>
<td>CIA</td>
<td>Malesa</td>
<td>F2</td>
<td>176/453 (38.8)</td>
<td>3.0 ± 1.8*</td>
<td>8.7 ± 3.4*</td>
</tr>
<tr>
<td>CIA</td>
<td>Females</td>
<td>F2</td>
<td>33/84 (39.2)</td>
<td>3.6 ± 2.2*</td>
<td>7.8 ± 4.0</td>
</tr>
<tr>
<td>PGIA</td>
<td>Females</td>
<td>BALB/c</td>
<td>56/57 (98.2)</td>
<td>4.2 ± 0.3</td>
<td>8.2 ± 4.0</td>
</tr>
<tr>
<td>PGIA</td>
<td>Females</td>
<td>F1</td>
<td>20/49 (40.8)</td>
<td>4.7 ± 2.8</td>
<td>5.3 ± 2.7*</td>
</tr>
<tr>
<td>PGIA</td>
<td>Females</td>
<td>F2</td>
<td>108/340 (31.8)</td>
<td>3.4 ± 1.1*</td>
<td>4.5 ± 2.7*</td>
</tr>
<tr>
<td>PGIA</td>
<td>Malesa</td>
<td>F2</td>
<td>19/62 (30.6)</td>
<td>4.0 ± 2.2*</td>
<td>4.2 ± 1.9*</td>
</tr>
</tbody>
</table>

† DBA/1 males were immunized for CIA, and BALB/c females for PGIA. F1 indicates DBA/1 × BALB/c/F1 hybrids, while F2 indicates DBA/1 × BALB/c/F2 hybrids. Conversely, BALB/c mice produce Abs and a high level of T cell responses to type II collagen, but remain resistant to CIA (26). Hence, we were surprised to observe that 41% of the females (20 arthritic of 49 immunized F1 hybrid mice) were positive for arthritis with an average severity score of 5.3 ± 2.7 (Table I and Fig. 1).

The BALB/c × DBA/1 F2 hybrids tested for CIA (38.9% positive for CIA; 209 of 537) had an average severity score of 8.6 ± 3.5, which was much higher than the severity scores measured in the parental DBA/1 strain (3.3 ± 2.1; Table I). Many of these F2 hybrids with CIA developed severe arthritis, which has never been observed in any of the parental strains for either CIA or PGIA (Fig. 2). Clearly, F2 hybrids of BALB/c × DBA/1 intercross inherited certain genes from the BALB/c background, which made CIA significantly more severe (Fig. 1C). In contrast, the BALB/c × DBA/1 F2 hybrids tested for PGIA (31.6% positive for PGIA; 127 of 402) had an average severity score of 4.4 ± 2.6, which was significantly lower than the severity scores measured in the parental BALB/c strain (8.2 ± 4.0; Table I). Thus, F2 hybrids with PGIA lost some genes, which were involved in disease severity in BALB/c parents. When males and females were analyzed separately, no significant differences in clinical scores (susceptibility, onset, or severity) between sexes were detected within the groups immunized for CIA or PGIA (Table I).

Clinical traits of CIA and PGIA in F2 hybrid mice of BALB/c and DBA/1 intercross

We have hypothesized that the susceptibility to disease and arthritis severity are governed by different sets of genes. In earlier studies (11, 12), we have used an "acute" and/or "cumulative" arthritis score as a single trait, which was applied to both arthritic and nonarthritic mice with a scale from 0 to 16. However, this cumulative acute arthritis score contained a mixture of several clinical

**FIGURE 1.** Onset of arthritis during the course of immunization (A and B), and the severity score (C) in parent strains and their F1 and F2 hybrids with CIA or PGIA. Each paw was individually scored from 0 to 4 resulting in a possible maximum score per animal of 16 (26), and then the percent of arthritis incidence (from 0 to 16) is shown in a weekly scale (A and B). For comparison of various groups, scores measured 3 days after the onset are summarized and shown. The incidence and severity of each group are listed in Table I. Arrows indicate the time of Ag injection. C, Summary of the severity score in parent strains and their hybrids.
traits, thus we further dissected it into three scores: susceptibility to arthritis (binary), onset of the disease (onset), and severity of inflammation (severity). Although separation of clinical traits did not create biases among all traits, this step seemed to be a necessary procedure for correct calculations and linkage analysis of genes that might control the different features of arthritis. Indeed, differences between the three clinical traits (binary, onset, and severity) and their linkage to different mCia and Pgia loci clearly indicated the necessity of this approach. The binary (qualitative) trait (susceptibility) is insensitive to the degree of inflammation and takes only two values: either “1” or “0”. Disease severity is a separate quantitative trait varying from 1 to 16, and by definition is determined for arthritic mice only (Fig. 1C). An additional phenotype of the disease, which is possibly independent of both the binary trait and severity, is the disease onset that reflects the speed of disease progression (Fig. 1A and B).

Genome scans identified both model-specific and common QTL. In both models, the major locus for disease susceptibility was localized over the MHC region on chromosome 17 (Fig. 3). The effect of the MHC locus on clinical traits (except severity) was much more prominent in CIA than in PGIA (LOD score 21 in CIA and 6.0 in PGIA; Fig. 3). Other QTL on chromosomes 3, 5, 10, 19, and X in CIA (Fig. 3), while they were highly significant, have never reached the level of the MHC effect. In the model of PGIA, two major QTL were found on chromosome 17, one within the MHC locus and another one in the telomeric region (Fig. 3). Additional significant QTL of clinical traits were identified on chromosome 5, 6, 9, and 18. Most of the QTL identified were model-specific, and only the MHC locus on chromosome 17 seemed to be a common genetic component for both murine models of arthritis.

The nonparametric Spearman’s correlation coefficient $p$ was applied to test biases between traits in PGIA and CIA mice populations. Each correlation coefficient was characterized with significance of correlation, and the $p$ value was set to 0.05 level. As expected, an early onset tightly correlated with the arthritis score and binary trait ($r = 0.92–0.98$) in both models (not shown), whereas the onset of arthritis demonstrated a significant positive correlation ($r = 0.24$) with severity only in PGIA (Fig. 4).

Immune and pathophysiological markers in F$_2$ hybrid mice with or without CIA or PGIA

All animals of BALB/c × DBA/1 F$_2$ population were immunized either with type II collagen or proteoglycan, and ~39% of the entire F$_2$ population was susceptible to CIA and only 32% was susceptible to PGIA (Table I). Although the majority of animals did not develop arthritis, all mice were positive for Ag-specific T cell responses and serum levels of TNF-$

\[ \text{FIGURE 2. Clinical appearance (insets) and histopathology of ankle joints 3 days after the onset of arthritis. A “resistant” (A) and a highly susceptible (D) (paw score = 4) F}_2 \text{ hybrid (BALB/c × DBA/1 cross) immunized with type II collagen. B, An F}_1 \text{ hybrid with CIA (score = 2); C, an F}_2 \text{ hybrid with PGIA (score = 4). Although the onset frequently was delayed in PGIA and severity was higher in F}_2 \text{ hybrids with CIA (Table I; Fig. 1), the inflammatory cell reaction, cartilage, and bone erosions (i.e., the overall histopathological pictures) were highly comparable in all arthritic animals.} \]
and IL-12 were significantly different between the arthritic and non-arthritic animals in both CIA and PGIA, while other differences appeared to be model-specific. In the CIA model, serum levels of IL-6 and Abs (both against mouse and human type II collagen) were significantly higher in arthritic, than in nonarthritic, mice (Table II). In contrast, the ratio of IgG1 to IgG2a, and the Ag-induced IL-4 production, were significantly less in mice with PGIA than those remaining asymptomatic; both parameters reflecting a shift of Th1/Th2 balance to the Th1 direction in, or during the development of, arthritis (30, 37).

Genome-wide linkage analysis of clinical and immunological traits in F2 hybrids of BALB/c/H11003×DBA/1 intercross

The results of linkage analysis for clinical and immunological traits are summarized in Tables III and IV. A number of these QTL were identified in other studies (7–9, 11–13) and we maintained the original QTL number for a given chromosome region, even if our QTL represented linkage with a new trait. As summarized in Table III for CIA and in Table IV for PGIA, using the genetic cross of BALB/c/H11003×DBA/1, we have identified four new QTL of clinical traits in CIA (mCia10-mCia13) and three new QTL of PGIA (Pgia18-Pgia20). In addition to the earlier studies (7–9, 11–13) and in results summarized in Fig. 3, we identified five additional new QTL in CIA and five new QTL in PGIA. Many of these new immune- or cytokine-associated QTL shared the same chromosome region with clinical QTL (Fig. 3), either in the same or the other model. Taking linkage analysis data together, we identified nine new QTL (mCia10-mCia13) for mouse CIA, and eight new Pgia QTL (Pgia18-Pgia25), and the summary of these arthritis and immune QTL are presented in Fig. 3, Table III, and Table IV.

FIGURE 3. LOD score plots for individual chromosomes containing putative QTL controlling arthritis (black line), onset of the disease (green), arthritis susceptibility (blue), and severity (red). QTL identified in this study are indicated by asterisks (*). The description of these clinical scores of arthritis are in Materials and Methods. The length of each chromosome is adjusted to the same size, albeit the centimorgan length of each chromosome varies. Genetic maps were constructed using Map Manager QTX. The positions of SSLP markers used for genotyping are indicated on the x-axis of each panel with vertical sticks. The LOD score for the free regression model is given on the y-axis. Significance is set by the suggestive LOD score of 2.8 and is indicated by the horizontal dotted line on each panel. For clarity, linkage with arthritis score, onset, and binary/susceptibility traits were analyzed for the total F2 population, but the severity of the disease was calculated for arthritic mice only. In most cases, the arthritis score showed colocalization with either susceptibility or onset of the disease, but severity QTL showed distinct localization. As the penetrance of QTL was frequently affected by mouse gender, the population (males and/or females) is shown on each panel. Immunological (pathophysiological) traits linked to either CIA or PGIA are listed in Tables III and IV.
Correlation between clinical traits of arthritis and pathophysiological markers in F₂ hybrids of BALB/c × DBA/1 intercross

In an effort to identify critical immune parameters that may play a role either in CIA or PGIA, or have an effect on one or both models, statistical comparisons were made between the major overall clinical scores of arthritis (from 0 to 16) or the subtraits of severity (scores from 1 to 6), onset (scores from 0 to 5), and susceptibility (either score 0 or 1), and the immune response/inflammation-related parameters (Table V). Clinical traits of arthritis demonstrated biases with certain immune response parameters, a pattern that was specific for each animal model. Ag-specific T cell responses (proliferation and IL-2 production) demonstrated significant correlation with arthritis in both models, but the correlation was positive in CIA (p 0.19 to 0.37) and negative in PGIA (p from −0.18 to −0.45). Similarly, Ag-induced IFN-γ production showed significant correlations with the overall arthritis score in both models (p 0.21–0.25; Table V). Serum Ab levels (both hetero- and autoantibodies) were specific immune markers for CIA, but not for PGIA. On the list of serum markers tested (Table II), only IL-6 demonstrated biases with certain immune response parameters, a pattern that was specific for each animal model.

Table III. Summary of QTL in F₂ hybrid (BALB/c × DBA/1) mice immunized with type II collagen for CIA

<table>
<thead>
<tr>
<th>Chr</th>
<th>QTLc</th>
<th>cMd</th>
<th>Marker</th>
<th>Clinical Traitsc</th>
<th>Immuneological Traitsc</th>
<th>Other Traits</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>mCia4f</td>
<td>8</td>
<td>D1Mit65</td>
<td></td>
<td></td>
<td>IFN-γ (2.9)</td>
<td>This study</td>
</tr>
<tr>
<td>2</td>
<td>mCia7b</td>
<td>33–34</td>
<td>D1Mit76-D1Mit48</td>
<td></td>
<td></td>
<td>sCD44 (2.8)</td>
<td>This study</td>
</tr>
<tr>
<td>3</td>
<td>mCia2c</td>
<td>15–34</td>
<td>D3Mit221-D3Mit199</td>
<td>A (4.0), S (5.8)</td>
<td>hAb (2.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>mCia10f</td>
<td>26–41</td>
<td>D5Mit105-D5Mit304</td>
<td>A (5.0), B (3.0), S (4.1)</td>
<td></td>
<td></td>
<td>This study</td>
</tr>
<tr>
<td>6</td>
<td>mCia11f</td>
<td>61–78</td>
<td>D5Mit278-D5Mit31</td>
<td>A (7.8), S (6.1), B (4.0), O (4.2)</td>
<td></td>
<td></td>
<td>This study</td>
</tr>
<tr>
<td>8</td>
<td>mCia6c</td>
<td>28</td>
<td>D8Mit46</td>
<td></td>
<td></td>
<td>sCD44 (3.3)</td>
<td>This study</td>
</tr>
<tr>
<td>10</td>
<td>mCia8c</td>
<td>50–69</td>
<td>D10Mit30-D10Mit35</td>
<td>A (2.8)</td>
<td></td>
<td></td>
<td>This study</td>
</tr>
<tr>
<td>14</td>
<td>mCia7c</td>
<td>15</td>
<td>D14Mit60</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>mCia11c</td>
<td>18–25</td>
<td>D17Mit16-D17Mit10</td>
<td>A (16.1), O (11.2), B (20.6)</td>
<td>aAb (18.5), hAb (4.3), cT Cell (8.2), bCT Cell (6.5)</td>
<td>sCD44 (5.3)</td>
<td>7,8</td>
</tr>
<tr>
<td>18</td>
<td>mCia8b</td>
<td>50–55</td>
<td>D18Mit80-D18Mit128</td>
<td></td>
<td></td>
<td>aAb (3.4), hAb (3.2)</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>mCia2c</td>
<td>1</td>
<td>D19Mit59-D19Mit93</td>
<td>A (3.1)</td>
<td></td>
<td></td>
<td>This study</td>
</tr>
<tr>
<td>X</td>
<td>mCia3c</td>
<td>50–67</td>
<td>DXMit172-DXMit121</td>
<td>A (3.9)</td>
<td>aCT Cell (2.9), bCT Cell (2.8)</td>
<td>sCD44 (7.2), IgG1/2a (2.9)</td>
<td>This study</td>
</tr>
</tbody>
</table>

af Qualitative (binary) and quantitative (clinical and immunological) trait loci; n = 209 arthritic of 537 immunized mice.
bg Only chromosomes (Chr) which showed QTLs are listed.
ch As CIA in rats is a frequently used arthritis model, we indicated CIA loci in mice with “mCia” to avoid confusing Ciu-QTL labeling in the two (mouse vs rat) species.
1d Either center point cM position or CM flanking region for QTL peak is shown.
2e Clinical and immunological traits are detailed in Materials and Methods, Table II, and Figs. 3 and 4. Under clinical traits, A denotes the arthritis score; O, the onset; B, the binary/susceptibility; and S, severity subtraits. aAb, hAb, aCT Cell, and bCT Cell indicate the Ab or IL-2 (CT Cell-2) production in the presence of mouse (a, auto-) or human (b, hetero-) Ag (type II collagen). In each case, the number in parentheses indicates the corresponding LOD score.
3f New loci identified in this study.
showed correlation with severity (ρ 0.33), and TNF-α with arthritis scores (ρ 0.22), both in CIA only (Table V).

**Discussion**

We report in this study the first time that two separate autoimmune models have been “combined” through a single genetic cross. We were taking the advantage of a single cross in an identical environmental condition to compare the genetics of these two (CIA and PGIA) most frequently used murine models of RA. We immunized F2 hybrids of two parent strains, which are susceptible for either CIA or PGIA (most frequently used murine models of RA). We immunized were taking the advantage of a single cross in an identical environment to compare the genetics of these two (CIA and PGIA) most frequently used murine models of RA. As expected, the linkage analysis identified different localization finding from these experiments has been the observation of how strong the contribution of a resistant background can be to disease development. We were astounded by the observation that the CIA profile was faster and much more severe in the F2 hybrids than in the parental DBA/1 strain (Figs. 1C and 2). This suggests that while the BALB/c strain is resistant to CIA, it contains significant numbers of loci that can contribute to the disease, when placed in the proper background. Interestingly, the reciprocal observation was not true (Table I). This observation supports the hypothesis described above for a dominant gene in CIA controlling susceptibility, and the presence of multiple genes controlling clinical traits in PGIA. The strength of these loci is further illustrated by the suppression of the sex effect in F2 hybrids (Table I), which is typically seen in parent DBA/1 mice immunized for CIA (16) and in BALB/c mice immunized for PGIA (23, 24).

As expected, the linkage analysis identified different localization patterns for the susceptibility, onset, and severity QTL in murine models of arthritis (Fig. 3; Table III and Table IV). Although qualitative/binary trait loci, i.e., the disease susceptibility, were colocalized with the onset of arthritis in most cases, severity QTL exhibited very diverse distributions over the genome, and essentially no linkage with other clinical traits were found (Figs. 3 and 4). Only the telomeric part of chromosome 5 was significantly associated with arthritis (Fig. 3), but we also screened the entire genome, and analyzed all possible traits or substrains in all immunized animals.

Perhaps the most important finding from these experiments has been the observation of how strong the contribution of a resistant background can be to disease development. We were astounded by the observation that the CIA profile was faster and much more severe in the F2 hybrids than in the parental DBA/1 strain (Figs. 1C and 2; Table I). This suggests that while the BALB/c strain is resistant to CIA, it contains significant numbers of loci that can contribute to the disease, when placed in the proper background. Interestingly, the reciprocal observation was not true (Table I). This observation supports the hypothesis described above for a dominant gene in CIA controlling susceptibility, and the presence of multiple genes controlling clinical traits in PGIA. The strength of these loci is further illustrated by the suppression of the sex effect in F2 hybrids (Table I), which is typically seen in parent DBA/1 mice immunized for CIA (16) and in BALB/c mice immunized for PGIA (23, 24).

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pathophysiological traits in the CIA model, such as IgG1/IgG2a ratio, sCD44, and IL-2 production, but the same locus also influenced IL-1 and sCD44 production in PGIA (Pgia25).

Taking advantage of the combined model of RA we used in this study, we have analyzed overlapping immune and clinical QTL discovered for each model (Tables III and IV). Only one locus shared high significance in both models: the MHC on chromosome 17 (Pgia17, mCia1). The MHC is exceptionally important carrying the most dominant alleles for susceptibility to insulin-dependent diabetes mellitus, RA, systemic lupus erythematosus, multiple sclerosis in human autoimmune diseases, and their animal models (38, 39). Although the MHC is the most important known genetic predisposition factor for all autoimmune diseases mapped to date in humans or in corresponding animal models, the MHC alone is insufficient for disease induction, and can only partially control the progression of established disease. This seems to be also relevant for autoimmune arthritis models (11–13), and we are compelled to believe that additional QTL on chromosomes 5, 10, 18, and X are among the most important regions which control arthritis susceptibility, severity, or onset.

Loci on chromosome 5 (Pgia16, mCia10, and mCia11) are also involved in other arthritis models, such as pristane-induced arthritis (3) and CIA severity in rats (10). This region corresponds to the locus in the human genome that was shown to be involved in RA (40, 41) and type I diabetes (42). Genes that are localized in this region (40–80 cM) of mouse chromosome 5 were found to control murine lupus (43) and Lyme disease in mice (44, 45). Locus at 50–69 cm on mouse chromosome 10 (Pgia6, mCia8) was shown to be relevant for RA (38, 41), CIA in rats (9, 46), systemic lupus erythematosus in human patients and lupus-prone mice (38, 47), human type I diabetes (38, 48), and murine experimental encephalomyelitis (49). A locus on mouse chromosome 18, around 50 cm (Pgia11, mCia18) corresponds to a QTL which is involved in RA (19, 50), and susceptibility to both murine experimental autoimmune encephalomyelitis (49) and lupus (39, 49, 51). QTL on chromosome X linked to arthritis were demonstrated in this study (Pgia24, Pgia25, mCia13) and in allied conditions of rat (52) and mouse studies (53), and in human patients with RA (19, 41). Chromosome X carries gene(s) linked to numerous immune disorders such as X-linked severe combined immunodeficiency (54), Graves’ disease (55), hyper-IgM syndrome (56), Bruton-type agammaglobulinemia (57, 58), and ichthyosis vulgaris (59). Many of these loci, and some additional QTL are summarized and discussed in Refs. 38 and 39, and the online database of Mendelian inheritance in man (60).

These results helped us to confirm the long-held hypothesis that while different autoimmune models may have model-specific genes, other loci will be shared between different models. Presumably, loci shared between multiple models are more likely to be involved in autoimmune diseases in general. This hypothesis is supported in the literature as many papers have documented clustering of loci in autoimmune disease models (14–16, 38, 39, 48, 61). Consequently, we believe that this combined model of arthritis provides a powerful tool for the identification and localization of common loci.

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Analysis of chromosomal loci that control arthritis conditions and are linked to immunological and/or pathophysiological traits in mice might help to reveal true arthritis loci in different models. Assuming this hypothesis is correct, we calculated linkage for all scored immune response parameters and cytokines, even if no arthritis QTL was identified in the region for the particular model (Tables III and IV). Indeed, while clinical QTL in CIA on chromosome 5 (mCia11) and chromosome 10 (mCia8) did not show any relationships with immune parameters in our CIA population, the same chromosome regions, while not carrying clinical traits for PGIA, interlaced with IL-1 and IL-4 (Pgia16) and T cell responses (Pgia6). QTL on chromosome X (mCia13) is linked to other
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References


