Localization of Quantitative Trait Loci Regulating Adjuvant-Induced Arthritis in Rats: Evidence for Genetic Factors Common to Multiple Autoimmune Diseases

Yutaka Kawahito, Grant W. Cannon, Pércio S. Gulko, Elaine F. Remmers, Ryan E. Longman, Van R. Reese, Jianping Wang, Marie M. Griffiths and Ronald L. Wilder

*J Immunol* 1998; 161:4411-4419; ;
http://www.jimmunol.org/content/161/8/4411

References
This article cites 51 articles, 15 of which you can access for free at: http://www.jimmunol.org/content/161/8/4411.full#ref-list-1

Subscription
Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Localization of Quantitative Trait Loci Regulating Adjuvant-Induced Arthritis in Rats: Evidence for Genetic Factors Common to Multiple Autoimmune Diseases

Yutaka Kawahito,2* Grant W. Cannon,† Pércio S. Gulko,* Elaine F. Remmers,* Ryan E. Longman,* Van R. Reese,† Jianping Wang,* Marie M. Griffiths,† and Ronald L. Wilder3*

Adjuvant-induced arthritis (AIA) in rats is a widely used autoimmune experimental model with many features similar to rheumatoid arthritis (RA). To identify potential genetic regulatory mechanisms in RA, we conducted genome-wide linkage analysis in F2 progeny of arthritis-susceptible Dark Agouti (DA) and relatively resistant Fischer 344 (F344) inbred rats. We compared the data with our previously reported investigation of collagen-induced arthritis (CIA), which was expanded in the follow-up study reported in this work. We found two quantitative trait loci (QTLs) in common, i.e., Aia1/Cia1 on chromosome 20, which includes the MHC, and Aia3/Cia3 on chromosome 4. We also identified a second unique QTL in AIA, Aia2, on chromosome 4. Interestingly, the QTL region on chromosome 4 (Aia3/Cia3), like the MHC, appears to be involved in several other autoimmune diseases in rats, including insulin-dependent diabetes, thyroiditis, and experimental autoimmune uveitis. Moreover, an analysis of conserved synteny among rats, mice, and humans suggested that Aia2 and Aia3/Cia3, like Aia1/Cia1, contain candidate genes for several autoimmune/inflammatory diseases in mice and humans, including diabetes, systemic lupus erythematosus, inflammatory bowel disease, asthma/atopy, multiple sclerosis, and RA. The rat models appear to provide a powerful complementary approach to identify and characterize candidate genes that may contribute to autoimmune diseases in several species. The Journal of Immunology, 1998, 161: 4411–4419.

Rheumatoid arthritis (RA) has a complex multifactorial etiopathogenesis, influenced by age, gender, hormonal, and environmental factors (1). Susceptibility to RA is also influenced by genetic factors, as indicated by many studies showing higher rates of disease concordance in monozygotic than dizygotic twins, higher incidence in offspring of RA patients, etc. (2–4). The genetic contribution to RA susceptibility is estimated to be as much as 60%, of which the HLA DRB1 locus is thought to account for 30 to 50% (5, 6). Identification of genetic loci regulating RA is complicated by genetic heterogeneity and incomplete penetrance, as well as the nongenetic factors noted above (7, 8). Genetic analysis of well-defined experimental models of erosive arthritis has the potential to markedly accelerate the genetic analysis of RA.

Experimental models of arthritis in rats, such as adjuvant-induced arthritis (AIA) (9–17) and collagen-induced arthritis (CIA) (18–22), have been used extensively in studying the roles of autoimmunity and inflammation in the pathogenesis of joint disease. Susceptibility/severity of disease varies significantly among inbred strains. For example, inbred Dark Agouti (DA) rats are markedly susceptible to CIA and AIA, as well as several other autoimmune diseases, whereas inbred Fischer 344 (F344) rats are relatively resistant (23, 24). Most studies indicate that both non-MHC genes and RT1 MHC class II genes are associated with the susceptibility to CIA and AIA in DA rats (25–28). Recently, our groups, employing a genome-wide scan involving 98 polymorphic markers, localized five QTLs that regulate CIA in (DA × F344)F2 progeny. One QTL (Cia1) is located on chromosome 20 and includes the MHC. The other QTLs (Cia2-5) are located on chromosomes 1, 4, 7, and 10, respectively. In addition, a region on chromosome 8 is suggestive for linkage (29). We, therefore, were interested in determining whether AIA in (DA × F344)F2 progeny is regulated by the same loci.

To determine whether the QTLs previously identified in CIA were also operative in another autoimmune model of arthritis and to identify additional QTLs, we conducted a genome scan to identify QTLs that regulate AIA in (DA × F344)F2 progeny. We have also expanded our previous investigation of CIA (29), and compared the revised CIA analysis with our new AIA analysis. Although CIA and AIA have many clinical similarities, fundamental differences in their pathophysiology are clearly evident and provide an ideal opportunity for defining and comparing the genes that control arthritis susceptibility and severity. This comparison is particularly interesting because AIA predominantly involves T cell-mediated mechanisms, whereas CIA requires both humoral and cellular immunity (11–23). Identification of one or more loci common to both models would suggest the
existence of genes that regulate the development of autoimmune inflammatory arthritis in response to diverse stimuli. We report the identification of a non-MHC locus on chromosome 4, in addition to the MHC, that regulates both AIA and CIA. We also discuss evidence that these loci may also be involved in multiple additional autoimmune diseases in rats, mice, and humans.

Materials and Methods

Rats

Specific pathogen-free Fischer 344 (F344/NHsd) rats were obtained from Harlan Sprague-Dawley (Indianapolis, IN), and Dark Agouti (DA/Bk) rats from Bantin & Kingman (Fremont, CA). Using the convention of (female × male) to indicate strain parentage, F1 progeny were generated at the Veterinary Medical Unit, Salt Lake City VAMC from four mating pairs: two (F344 × DA) and two (DA × F344). F2 progeny were subsequently generated using eight mating cages (sib × sib) established with the F1 progeny of one (F344 × DA) mating pair and one (DA × F344) mating pair. The progeny derived from (DA × F344) and (F344 × DA) matings were evaluated for arthritis incidence and severity separately, but no statistically significant differences were detected. Analyses with the combined data are described.

Induction and evaluation of AIA and CIA

For AIA, CFA was prepared by suspending heat-killed Mycobacterium butyricum (Difco, Detroit, MI) in incomplete Freund’s adjuvant (IFA) at 7.5 mg/ml. CFA-induced arthritis was stimulated by injection of 0.1 ml of the CFA emulsion intradermally at the base of the tail, as previously described (16, 17).

For CIA, the rats were injected intradermally on the back with 2 mg/kg bovine type II collagen, prepared as previously described (18–22). In brief, collagen was suspended in 0.1 M acetic acid at 1 mg/ml and emulsified in an equal volume of IFA (Difco). Seven days after the initial injection, a booster injection of 100 μg bovine type II collagen emulsified in IFA was injected intradermally at the base of the tail.

For clinical evaluation of AIA and CIA, we used a scoring system as follows. The severity of arthritis in the wrist, midforepaw, ankle, and midfoot was scored for each extremity using the following scale: 0, no arthritis; 1, minimal swelling; 2, medium swelling; 3, severe swelling; and 4, severe swelling and non-weight-bearing.

The presence of arthritis in the three joints of each of the lateral four digits, counting from lateral to medial (two interphalangeal, and metatarso-phalangeal or metacarpophalangeal), was scored as: 0, swelling absent; 1, swelling present.

The total score for each extremity was calculated by summing the scores of the individual joints of each extremity with arthritis. The maximum score for each extremity was, therefore, 20, and the maximum total joint score was 80.

Scores were obtained twice per week by a single observer unaware of the genetic constitution of the animals. The maximum arthritis score for each animal was determined as the highest score during the 6-wk observation period. These scores are highly correlated with histologic or radiologic evaluations of disease severity. All experimental groups contained control rats of both parental strains. Scores in the text are indicated by mean ± SD in the text.

Genotypic analysis

DNA was prepared by standard procedures (30), and genotypes were determined by PCR amplification of polymorphic DNA fragments containing simple sequence repeats. For the genome-wide QTL analysis, 150 markers were used to genotype animals from the phenotypic extremes (severely affected versus mildly affected animals) of the F2 progeny (Table 1). The markers were selected to give a spacing interval of less than 20 cM. The map of these markers covered 1535 cM, which is estimated to be more than 95% of the rat genome. The sequences of the PCR primers and amplification protocols are described in ARB Rat Genetic Database (31), Wellcome Trust/Oxford University Rat Genetic Database (32), and Whitehead Institute/MIT Rat Genetic Database (33). Primers for most of these markers are available from Research Genetics (Huntsville, AL). Linkage maps were generated using the MAPMAKER/EXP version 3.0b (34, 35).

Statistical analysis

Nonparametric analysis of disease susceptibility and severity was evaluated with the Mann-Whitney test. Linkage of individual simple sequence length polymorphism (SSLP) markers to disease susceptibility/severity was evaluated by a χ2 test of independence using a 2 × 2 contingency table comparing allele frequencies in the high and low arthritis score animals (36), i.e., homozygous DA progeny contributed two DA alleles to the allele frequency, homozygous F344 progeny contributed two F344 alleles, and heterozygous progeny contributed one DA and one F344 allele. Significant cosegregation of marker alleles with a QTL, which controlled arthritis severity, and its mode of inheritance were also evaluated with the MAP-MAKER/QTML program, version 1.1b (37). We used the arcsin square root as a variance stabilizing transformation for normalizing the phenotypic distribution (38). As recently suggested (39), the threshold for suggestive linkage is a LOD score of 2 corresponding to a p value of 2.4 × 10−8, and the threshold for significant linkage is a LOD score of 3.4 corresponding to a p value of 7.2 × 10−4 in dominant inheritance mode. In free inheritance mode, the threshold for suggestive linkage is a LOD score of 2.8 corresponding to a p value of 1.6 × 10−3, and the threshold for significant linkage is a LOD score of 4.3 corresponding to a p value of 5.2 × 10−5.

Comparable maps of the Aia2 and Aia3/Cia3 homologous regions among rats, mice, and humans

The rat map was constructed from data obtained in this study. Locations of additional genes on this map were estimated from data in ARB Rat Genetic Database (31). The mouse map and the human cytogenetic map were extracted from data in Mouse Genome Database (40) and Genome Database (41), respectively. The most likely positions of various autoimmune disease susceptibility loci were deduced from our new data and review of the literature (42–53).

Results

Phenotypic expression of AIA and CIA

As shown in Figure 1, we compared the incidence and severity of CIA (as previously reported, 29) with AIA in parental DA, parental F344, F1, and F2 rats during 6 wk following immunization. DA rats were extremely susceptible to induction of severe CIA and AIA, exhibiting maximum arthritis scores (see Materials and Methods) of 62 ± 10 (mean ± SD) for CIA and 60 ± 22 for AIA. However, F344 rats were highly resistant to CIA and moderately resistant to AIA (mean maximum score: CIA = 0; AIA = 8 ± 6). The incidence of disease in F1 progeny in both models was 100%, but the mean severity was different in the F1 progeny than in the two parental strains (mean maximum arthritis score of F1 progeny: CIA = 40 ± 14; AIA = 50 ± 4). The F2 population showed a wide range of maximum arthritis scores: CIA (0–73, n = 502), AIA (0–78, n = 546). But, 23 and 5% of the F2 population failed to develop CIA and AIA, respectively.

Association of arthritis susceptibility and severity with MHC genotype

We have previously reported highly significant cosegregation of CIA susceptibility/severity with several MHC markers, including Tnfa (a marker for TNF: Cia1, LOD score = 78.5), which is located within the class III region of the rat MHC (29). For our new study of AIA, we genotyped 80 severely (arthritis index >64) and 80 mildly affected (arthritis index <17) rats from the (DA × F344)F2 progeny (n = 546, Expt. 1) with SSLP markers spanning rat chromosome 20 (Table 1). Cosegregation of genotype with phenotype was noted over a 30-cM interval on this chromosome, with the strongest association between markers D20Arb8 and D20Arb2 (RT1.N1) (Table 1). This interval includes MHC class I, II, and III genes. Interval analysis using MAPMAKER/QTML confirmed a strong QTL in this region (LOD score = 17.9 at Tnfa). Its inheritance was consistent with dominant mode, i.e., the DA allele acted dominantly to promote disease severity. We have named this locus Aia1. To facilitate comparison of AIA with CIA, we extended our previous analysis of CIA (29) by analyzing the same SSLP markers spanning rat chromosome 20 that we used for the AIA analysis. We cannot separate Aia1 from Cia1 (Fig. 2, a and b).

To evaluate the effects of the MHC on susceptibility and severity in both AIA and CIA, the variance in arthritis score at the Tnfa
locus was examined. Because the entire MHC region is within a 3-cM genetic interval (54), recombination within the MHC is infrequent. Therefore, the Tnfa-a genotype closely reflects the genotype of the entire MHC region. The MHC region influenced susceptibility differently in these two models. For CIA, in (DA × F344)F2 progeny, the MHC genotype had a relatively strong effect on disease susceptibility. For CIA, 43.4, 94.6, and 93.7% of rats with 0, 1, or 2 DA alleles at the Tnfa locus developed arthritis (Fig. 3a, c, and e). For AIA, however, the MHC genotype had much weaker effects on disease susceptibility, i.e., 90.4, 96.2, and 96.8% of rats with 0, 1, or 2 DA alleles at the Tnfa locus developed arthritis (Fig. 3, b, d, and f). On the other hand, MHC genotype clearly correlated with disease severity in both models. For CIA, the mean severity scores of arthritis-affected rats with 0, 1, and 2 DA alleles at the Tnfa locus were 12 ± 15, 35 ± 16, and 42 ± 16, respectively. For AIA, the mean severity scores of arthritis-affected rats with 0, 1, and 2 DA alleles at the Tnfa locus were 34 ± 20, 48 ± 16, and 50 ± 17, respectively. In F2 rats with 0 DA alleles, the susceptibility and severity in AIA were significantly greater than those of CIA (Mann-Whitney test: p < 0.01). In other words, F2 progeny with 0 DA MHC alleles typically developed mild disease, but the range of arthritis scores was wide in AIA and was more restricted in CIA than in AIA. Furthermore, F2 progeny with 1 or 2 DA MHC alleles developed more severe disease than progeny that were homozygous for the F344 MHC alleles in both AIA and CIA, but the range of arthritis scores was wide. In summary, the MHC genotype clearly influenced disease expression for both AIA and CIA, but this genotype information alone for any individual F2 rat was an imprecise prognostic factor for determining disease susceptibility or severity.

Non-MHC loci regulating AIA and CIA

To complete the genome scan of AIA, we examined 80 severely and 80 mildly affected F2 rats with AIA for chromosomes 1, 4, 7, 8, and 10, which contain Cia2–5, as well as a suggestive locus on chromosome 8. Maximum arthritis scores were greater than 64 in...
80 severely affected rats and were lower than 17 in 80 mildly affected rats. For the remainder of the genome scan, we evaluated 40 severely (arthritis index 66) and 40 mildly affected (arthritis index 17) F2 rats. For markers (e.g., chromosomes 3, 6, 15, and 19) that showed trends for nonrandom genotype distributions (p < 0.05) after genotypic analysis of 40 severely and 40 mildly affected F2 rats, additional genotyping was done to include the 80 severely and 80 mildly affected F2 rats. We found evidence of genotype-phenotype cosegregation with multiple chromosome 4 SSLP markers (Table I). The strongest association was with chromosome 4 markers between D4Arb26 and D4Arb16 (Ampp) (D4Mgh15, p = 2.4 x 10^-5) (Table I). Since this significance level, by definition (39), is suggestive for linkage, we conducted a replication experiment with a second group of (DA x F344)F2 progeny (n = 110, Expt. 2). In this replication experiment, we genotyped severely affected (n = 28, arthritis index 64) and mildly affected (n = 24, arthritis index <17) rats that had arthritis scores equivalent to those in experiment 1. We again detected cosegregation

---

### Table I. Associations of markers with AIA in the (F344 x DA)F2 cross, experiment 1 (n = 546)

<table>
<thead>
<tr>
<th>Chr</th>
<th>Markers</th>
<th>Distance</th>
<th>Severe affected</th>
<th>Mildly affected</th>
<th>Genotype</th>
<th>Chi-square</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>D4Arb6</td>
<td>11.7</td>
<td>65 89 78 80</td>
<td>65 78 78 80</td>
<td>0.68</td>
<td>23 75 85</td>
<td>0.035</td>
</tr>
<tr>
<td>1</td>
<td>D4Arb31</td>
<td>14.6</td>
<td>67 69 71 80</td>
<td>64 71 78 80</td>
<td>0.84</td>
<td>67 78 74 80</td>
<td>0.027</td>
</tr>
<tr>
<td>1</td>
<td>D4Arb32</td>
<td>18.5</td>
<td>87 62 78 77</td>
<td>87 78 72 77</td>
<td>0.62</td>
<td>87 78 72 77</td>
<td>0.027</td>
</tr>
<tr>
<td>1</td>
<td>D4Arb33</td>
<td>7.9</td>
<td>72 76 80 84</td>
<td>72 76 80 74</td>
<td>0.65</td>
<td>72 76 80 74</td>
<td>0.027</td>
</tr>
</tbody>
</table>

---

The strongest association was with chromosome 4 markers between D4Arb26 and D4Arb16 (Ampp) (D4Mgh15, p = 2.4 x 10^-5) (Table I). Since this significance level, by definition (39), is suggestive for linkage, we conducted a replication experiment with a second group of (DA x F344)F2 progeny (n = 110, Expt. 2). In this replication experiment, we genotyped severely affected (n = 28, arthritis index >64) and mildly affected (n = 24, arthritis index <17) rats that had arthritis scores equivalent to those in experiment 1. We again detected cosegregation

---

### Table I.

<table>
<thead>
<tr>
<th>Chr</th>
<th>Markers</th>
<th>Distance</th>
<th>Severe affected</th>
<th>Mildly affected</th>
<th>Genotype</th>
<th>Chi-square</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>D9Wmn23</td>
<td>15.2</td>
<td>32 48 43 37</td>
<td>32 48 43 37</td>
<td>0.68</td>
<td>32 48 43 37</td>
<td>0.027</td>
</tr>
</tbody>
</table>

---

The strongest association was with chromosome 4 markers between D4Arb26 and D4Arb16 (Ampp) (D4Mgh15, p = 2.4 x 10^-5) (Table I). Since this significance level, by definition (39), is suggestive for linkage, we conducted a replication experiment with a second group of (DA x F344)F2 progeny (n = 110, Expt. 2). In this replication experiment, we genotyped severely affected (n = 28, arthritis index >64) and mildly affected (n = 24, arthritis index <17) rats that had arthritis scores equivalent to those in experiment 1. We again detected cosegregation
with chromosome 4 markers, but the strongest association was with D4Arb24 (p = 4 × 10^{-4}) (Table II). Because the chromosomal segment associated with AIA extended over multiple markers and an interval of approximately 50 cM, we suspected that this chromosome contained more than 1 QTL. We, thus, further analyzed our combined data from experiment 1 and 2 (n = 656) for chromosome 4 using MAPMAKER/QTL (Fig. 2d). An interval between D4Arb26 and D4Arb30 showed significant linkage with a LOD score of 5.8 in the dominant inheritance mode. We have named this locus Aia2. Aia2 is a unique locus for AIA because additional genotyping with markers spanning the genomic interval containing Aia2 of the phenotypically highest and lowest 15% of the CIA F2 population with at least 1 DA MHC allele did not reveal a corresponding CIA regulatory locus (Fig. 2, c and d). A second interval between D4Arb30 and D4Arb16 had a maximum LOD score of 3.9 in free inheritance mode. Since this QTL was detected in both experiments 1 and 2 (Tables I and 2), we have defined this locus as Aia3. Aia3, like Cia3, was strongly linked to D4Arb24 (Fig. 2, c and d), suggesting that this region of chromosome 4 contains a QTL that regulates both AIA and CIA. Both Aia3 and Cia3 appear to act additively.

Our analysis of AIA also suggested phenotype:genotype cosegregation (p < 0.05, Table I) with one SSLP marker on chromosome 3, and two markers on chromosome 15. The genomic interval containing these markers had a maximum LOD score of 3.9 in free inheritance mode. Since this QTL was detected in both experiments 1 and 2 (Tables I and 2), we have defined this locus as Aia3. Aia3, like Cia3, was strongly linked to D4Arb24 (Fig. 2, c and d), suggesting that this region of chromosome 4 contains a QTL that regulates both AIA and CIA. Both Aia3 and Cia3 appear to act additively.

FIGURE 2. Log-likelihood plots for CIA and AIA QTLs on chromosomes 20 and 4 (n = 656). Log-likelihood values for the presence of a QTL were determined by an interval mapping technique (MAPMAKER/QTL). The most likely position for each QTL, determined by its two LOD support interval (99% confidence interval), is indicated by the closed bar above the plot. a, Cia1 on chromosome 20; b, Aia1 on chromosome 20; c, Cia3 on chromosome 4; d, Aia2 and Aia3 on chromosome 4. 

Comparative maps of Aia2 and Aia3/Cia3 among rats, mice, and humans

To examine homologies between mouse and human chromosomal regions and the chromosomal regions associated with Aia2 and Aia3/Cia3 on rat chromosome 4, we constructed comparative maps of these three species. The region including Aia2 and Aia3/Cia3 on rat chromosome 4 was homologous to mouse chromosome 6 and human chromosomes 7p, 7q, 2p, 3p, 3q, 10q, and 12p (Fig. 4).
linkage analytic techniques, including 150 SSLP markers, we identified both MHC (Aia1) and non-MHC (Aia2, Aia3) loci that regulated AIA in F2 progeny of DA and F344 strains. We also found an interval on chromosome 15, which was suggestive for linkage. Interestingly, the putative disease-enhancing QTL on chromosome 15 was derived from the F344 genetic background. We compared these data with our previously reported investigation of CIA (29), which was also extended in the current study to 150 SSLP markers (98 markers were examined in the earlier report), to facilitate comparison with AIA. Aia2 on chromosome 4 and the suggestive locus

FIGURE 3. The effect of DA MHC alleles on CIA and AIA severity. The data are shown as histograms for CIA (a, c, e) and AIA (b, d, f). (DA × F344)F2 hybrid rats with 0 Tnfa DA alleles (a, b), 1 Tnfa DA allele (c, d), and 2 Tnfa DA alleles (e, f). The CIA and AIA F2 populations include 502 and 546 progeny, respectively.

Table II. Associations of markers on chromosome 4 with AIA in the (F344 × DA)F2 cross (replication study), experiment 2 (n = 110)

<table>
<thead>
<tr>
<th>Chr</th>
<th>Markers</th>
<th>Gene Symbol</th>
<th>Distance (cM)</th>
<th>Severe (F344-DA)</th>
<th>Mild (F344-DA)</th>
<th>x^2</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>D4Arb13</td>
<td></td>
<td></td>
<td>12.1</td>
<td>17</td>
<td>11</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>D4Arb18</td>
<td></td>
<td></td>
<td>11.4</td>
<td>15</td>
<td>13</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>D4Arb26</td>
<td></td>
<td></td>
<td>11.7</td>
<td>13</td>
<td>15</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>D4Mgh15</td>
<td></td>
<td></td>
<td>2.6</td>
<td>12</td>
<td>16</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td>D4Arb11</td>
<td></td>
<td></td>
<td>6.2</td>
<td>12</td>
<td>16</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td>D4Arb30</td>
<td></td>
<td></td>
<td>12.9</td>
<td>11</td>
<td>17</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td>D4Arb21</td>
<td>Fabp1</td>
<td></td>
<td>1.3</td>
<td>12</td>
<td>16</td>
<td>20</td>
<td>4</td>
</tr>
<tr>
<td>D4Arb24</td>
<td></td>
<td></td>
<td>13.5</td>
<td>10</td>
<td>18</td>
<td>21</td>
<td>3</td>
</tr>
<tr>
<td>D4Arb16</td>
<td>Ampp</td>
<td></td>
<td>13.4</td>
<td>9</td>
<td>19</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td>D4Arb4</td>
<td>Caln1a1</td>
<td></td>
<td>11.5</td>
<td>8</td>
<td>20</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>D4Arb2</td>
<td>Prp</td>
<td></td>
<td>11.7</td>
<td>14</td>
<td>14</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>D4Arb1</td>
<td>lapp</td>
<td></td>
<td>—</td>
<td>13</td>
<td>15</td>
<td>11</td>
<td>13</td>
</tr>
</tbody>
</table>

a Distances between loci are described in centiMorgans (Kosambi).
b F344 indicates the number of F344 alleles.
c DA indicates the number of DA alleles.
d p values were calculated by a x^2 test of independence using a 2 × 2 contingency table.
on chromosome 15 appear to be unique to AIA. Aia1 and Aia3, however, mapped to the same locations on chromosomes 20 and 4, respectively, that Cia1 and Cia3 were previously mapped (29). The chromosomal regions containing Cia2, Cia4, Cia5, and Cia6* (p, region on chromosome 8 associated with suggestive linkage) do not appear to be involved in regulating AIA.

Although the Ags that initiate RA remain elusive, several subtypes of HLA DR4, including the shared epitope in many ethnic groups, are associated with RA (55, 56). The effect of HLA class II genes on the expression of RA is controversial. Some studies support an association with susceptibility and others with severity (55–59). We examined the effects of the MHC on susceptibility and severity in both AIA and CIA. Our study shows that the MHC region influences susceptibility and severity differently in these two models. For CIA, in (DA3F344)F2 progeny, MHC genotype had a relatively strong effect on disease susceptibility. For AIA, however, MHC genotype had a much weaker effect on disease susceptibility. Moreover, MHC genotype clearly exerted strong regulatory effects on disease severity in both models. But, in F2 rats with 0 DA alleles, CIA severity was milder than that of AIA. In both models, we noted many rats that were F344 homozygous at the MHC locus developed arthritis, and, conversely, many rats that were DA homozygous at the MHC locus failed to develop arthritis. In other words, our data demonstrate that non-MHC genotypes also contributed significantly to determining susceptibility and severity. Other studies have also indicated that DA non-MHC genes enhance arthritis susceptibility (25–29).

Among the non-MHC QTLs, Aia3/Cia3 are especially interesting because they map to overlapping genomic regions and may be the same locus. Moreover, these loci are potentially of more general interest. Iddm1 and Lyp (lymphopenia), in the progeny of diabetic BB rats, cosegregate with this genomic region (47). The Lyp/Iddm1 locus is also associated with the risk of development of autoimmune thyroiditis, independent of diabetes (60). It is also interesting that RA, diabetes, and thyroid disease cluster in families of some RA patients (61, 62). Aia3/Cia3 also appear to have a homologue in experimental autoimmune uveitis in the rat. In (F344 × LEW)F2 rats, a locus very close to Aia3/Cia3 on chromosome 4 has been identified (45). It remains to be definitively demonstrated whether or not Aia3, Cia3, Iddm1, Lyp, and the chromosome 4 QTL in experimental autoimmune uveitis are allelic, but in any event, rat chromosome 4, in addition to the MHC, appears to contain important genes that regulate several forms of autoimmune disease.
humans, to examine the possibility of identifying overlaps with autoimmune diseases in other species and to identify candidate genes (Fig. 4). Rat chromosome 4 and mouse chromosome 6 show strong linkage conservation. Interestingly, the region homologous to Aia3 in the rat includes loci on mouse chromosome 6 controlling Ab production (63) and Bordetella pertussis histamine sensitization (43), which cosegregates with susceptibility to experimental autoimmune orchitis (64). In addition, 2 loci regulating diabetes in nonobese diabetic mice (D6Nids1/Idl and D6Mit52/Idl), and a locus regulating systemic lupus in mice (D6Mit25/Lbw4) have been mapped to this region (48, 49, 53). The human homologues of the rat Aia2 and Aia3 regions are located on chromosomes 2, 3, 7, 10, and 12 (Fig. 4). Of significant interest, these homologous regions contain candidate genes for many other autoimmune/inflammatory diseases in humans, such as multiple sclerosis (50–52), inflammatory bowel disease (44), and atopic/bronchial asthma (42). Moreover, the Aia3 homologous region is also linked to recently described candidate loci on human chromosome 3q for rheumatoid arthritis (RA2) and insulin-dependent diabetes mellitus (IDDM9) (46). Human chromosome 12 may also have a RA candidate locus (46). Numerous candidate genes are predicted to map in this region of rat chromosome 4 and include neuropeptide Y, TCR Vβ loci, the κ-light chain of Ig, CD8, max dimerization protein, TGF-α, IL-5R α-chain, histamine receptor H1, TNF receptor 1, CD27, CD4, CD9, and the CD69 Ag (Fig. 4).

Thus, our data raise the possibility that like Aia1/Cia1, both Aia2 and Aia3/Cia3 and their homologues in other species will be important in many forms of autoimmune disease. In humans, autoimmune disease frequency is increased significantly in first degree relatives of patients with idiopathic inflammatory myopathies (65). A recent review of published mapping studies also suggested that autoimmune disease regulatory loci tend to cluster within and among species (66). In other words, it appears increasingly likely that many autoimmune disorders share genes that together act as polygenic risk factors.

In summary, our data indicate that at least three genetic loci contribute to disease development in AIA. Their homologues may also be involved in multiple autoimmune diseases in humans, including RA. Aia1 and Aia3 are likely to be identical to Cia1 and Cia3, respectively, and probably influence multiple additional autoimmune diseases. The development of QTL congenic strains involving these loci will provide a powerful approach to identify and characterize the biologic function of the underlying genes. Moreover, our data suggest the possibility of ultimately developing therapies directed at biochemical pathways common to multiple related autoimmune diseases.

Acknowledgments

We are appreciative of the excellent technical assistance of Shawna McCall.

References

41. In The Genome Database. Available at http://gdbwww.gdb.org/.