High resolution mapping of an arthritis susceptibility locus on rat chromosome 4, and characterization of regulated phenotypes

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Received March 3, 2003; Revised and Accepted July 2, 2003

The rat Natural Killer cell gene Complex (NKC) encodes molecules that can regulate immunity. It is located within an interval on DA rat chromosome 4 (RNO4) that is linked to immune-mediated inflammatory joint diseases, including oil-induced arthritis (OIA). We aimed to test the hypothesis that NKC regulates arthritis, by performing advanced mapping of arthritis and additional phenotypes induced by an intradermal injection of incomplete Freund’s adjuvant-oil. Reciprocal transfer of RNO4 intervals established that alleles from DA confer arthritis susceptibility to inbred LEW.1AV1 and PVG.1AV1 rats, whereas LEW.1AV1 and PVG.1AV1 alleles confer resistance to inbred DA. Subcongenic strains with PVG.1AV1 alleles introduced on DA allowed mapping of disease predisposition to 0.8 cM on the cytogenetic band 4q42, within the quantitative trait locus oil-induced arthritis-2 (Oia2), but outside the NKC. Alleles in Oia2 regulated arthritis in an additive fashion, and determined arthritis incidence, severity and day of onset, in both males and females. Besides macroscopic joint-inflammation, Oia2 also regulated other oil-induced phenotypes, including lymphoplasia and plasma levels of the inflammation marker a1-acid glycoprotein. The high-impact Oia2 region harbors gene sequences similar to human C3AR1, Ribosomal protein L7, DNAJA2, C-type lectins, C1s and CD163. These candidate disease genes may be of general interest, given that rat 4q42, and the syntenic mouse 6F2 and human 12p13 regions are linked to several inflammatory diseases, including rheumatoid arthritis.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic and destructive inflammatory joint disease, which affects approximately 1% of populations worldwide. Complex interactions between genetic and environmental factors are thought to contribute to arthritis development, as indicated by the concordance rates in monozygotic and dizygotic twins, which are 15 and 4%, respectively (1,2). Definition of the underlying genetic factors may enable development of strategies to prevent and cure RA, but so far, only major histocompatibility complex (MHC) genes have been conclusively linked to the disease, and have been estimated to account for ~30% of the genetic influence (3,4). The remaining genetic influences are believed to be exerted by several genes, which, in combination with the clinical heterogeneity of RA, is a major obstacle for genetic dissection of RA. Experimental arthritis provides opportunities to both control and manipulate genetic and environmental effects, in order to facilitate their identification and characterization. For example, poly-arthritis can be triggered in disease-prone rat strains by a wide range of non-immunogenic, but immunostimulating adjuvants (5,6), including oils (5,7). This incited a Swedish epidemiological survey, which now provides evidence that oil exposure increases the relative risk for RA (8). In order to genetically dissect models that display the pathological effects of oil exposure, we initiated a search for genes that regulate arthritis triggered by incomplete Freund’s adjuvant (IFA), an oil that induces T-cell mediated arthritis in DA rats, but not in other inbred rat strains (7,9–11).
Initial genetic analyses of oil-induced arthritis (OIA) in DA rats and MHC congenic rat strains revealed that susceptibility to OIA is determined by DA genes located both within and outside the MHC (Oia1) (10,12). Subsequent genome-wide linkage analysis of a cross between DA and MHC-identical but arthritis-resistant LEW.1AV1 identified a chromosome 4 region, designated Oia2, that was linked to arthritis susceptibility (13). Subsequent genome scans in pristane-induced arthritis (PIA) and collagen-induced arthritis (CIA) identified arthritis-regulating DA alleles in overlapping intervals, which were designated Pia7 and Cia13, respectively (14,15). The Oia2/Pia7/Cia13 region contains many genes that exert important functions in the immune system. Among these candidate disease genes, the natural killer cell gene complex (NKC) was particularly noted (13,15–17), because it regulates an aberrant NK cell activity of DA rats in natural toxicity, i.e. inability to lyse intravenously injected allogenic lymphocytes (18–20). It was therefore conceivable that the general and high disease susceptibility of DA rats could relate to aberrant function of leukocytes expressing DA alleles in the NKC. As a first test of this hypothesis, we demonstrated that PVG alleles, which are linked to normal NKC mediated natural cytotoxicity (20), were also linked to arthritis-resistance in a DA/DA × PVG.1AV1 back-cross (16). Here we aimed to further test the hypothesis by fine-mapping of Oia2 in relation to NKC, and also to functionally characterize the influence of Oia2 on arthritis development and other adjuvant oil-induced phenotypes.

First, congenic strains were produced to determine if Oia2 from DA could confer arthritis susceptibility when transferred to LEW.1AV1 and PVG.1AV1, and if Oia2 from LEW.1AV1 and PVG.1AV1 can confer arthritis resistance to DA. Second, Oia2 was fine-mapped in relation to NKC using two sets of independently derived congenic and recombinant strains with PVG chromosome 4 alleles introduced on the DA genome in both homozygous and heterozygous form. Third, Oia2 regulation of oil-induced phenotypes beside arthritis were determined, such as changes in plasma levels of α1-acid glycoprotein (AGP), body weight and weight of lymph nodes, thymus, liver and spleen. Finally, candidate disease genes in Oia2 were identified. Homologous chromosomal regions and genes were determined in mouse and human, and reported disease linkage(s) were compiled in the three species.

RESULTS

Evaluation of arthritis susceptibility in Oia2 congenics compared with parental strains

As reported previously, LEW.1AV1 (11–13,21) and PVG.1AV1 (11,16) were completely resistant to induction of OIA, whereas DA were susceptible (Table 1). The first signs of inflammation typically occurred 11–14 days post injection (d.p.i.) in ankles or metatarsal joints. The inflammation progressed, often symmetrically, to include larger paw areas and additional types of joints, including finger joints. However, inflammation never enganged an entire paw (score <4), and severity was moderate or low (score <13). Arthritis in more detrimental models (often score 16) can become chronic, but in OIA, disease subsides before 30 d.p.i., leaving no or limited signs of disease at 40 d.p.i., approximately.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Number of rats</th>
<th>Incidence (%)</th>
<th>Day of onset (mean ± SD)</th>
<th>Severity (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEW.1AV1</td>
<td>12</td>
<td>0.0</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>LEW.1AV1.C4(DA)</td>
<td>13</td>
<td>15.4</td>
<td>16 ± 0.0</td>
<td>3.0 ± 1.4</td>
</tr>
<tr>
<td>PVG.1AV1</td>
<td>29</td>
<td>0.0</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>PVG.1AV1.C4(DA)</td>
<td>16</td>
<td>68.8</td>
<td>20.2 ± 11.9</td>
<td>1.4 ± 0.5</td>
</tr>
<tr>
<td>DA</td>
<td>27</td>
<td>96.3</td>
<td>14.0 ± 5.5</td>
<td>8.3 ± 3.0</td>
</tr>
<tr>
<td>DA.C4(LEW)</td>
<td>14</td>
<td>0.0</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>DA.C4(PVG)</td>
<td>30</td>
<td>10.0</td>
<td>31.7 ± 10.2</td>
<td>1.0 ± 0.0</td>
</tr>
<tr>
<td>DA.C4(PVG/DA)</td>
<td>7</td>
<td>71.4</td>
<td>16.0 ± 4.5</td>
<td>2.0 ± 1.7</td>
</tr>
<tr>
<td>DAa</td>
<td>5</td>
<td>100.0</td>
<td>12.2 ± 0.4</td>
<td>n.d.</td>
</tr>
<tr>
<td>DA.C4(PVG)a</td>
<td>7</td>
<td>0.0</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>DA.C4(PVG/DA)a</td>
<td>13</td>
<td>62.0</td>
<td>14.9 ± 2.4</td>
<td>4.5 ± 2.9</td>
</tr>
<tr>
<td>DA.C4(DA)a</td>
<td>13</td>
<td>100.0</td>
<td>14.3 ± 1.8</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

Inbred and congenic strains were injected with IFA and monitored for arthritis incidence, day of onset and severity. Two congenic strains with PVG Oia2 alleles introgressed on DA genome were produced and tested independently, in Sweden, and in Norway. n.a., not applicable, cannot be determined in healthy rats. n.d., not accurately determined, because of treatment with prednisolone or early euthanasia.

In rats made congenic for chromosome 4 (C4) intervals, as depicted in Figure 1, arthritis incidences of 0–10% in DA.C4(LEW) and DA.C4(PVG) demonstrated that OIA resistance was transferred together with LEW or PVG C4 intervals into the DA strain. Conversely, incidences of 15% in LEW.1AV1.C4(DA) and 69% in PVG.1AV1.C4(DA) demonstrated transfer of OIA susceptibility together with DA C4 intervals into the LEW.1AV1 and PVG.1AV1 strains (Tables 1 and 2). After injection of the more arthritogenic oil squalene, the arthritis incidence was increased to 43% in LEW.1AV1.C4(DA) (3/7 males, 3/7 females) as compared with 14% in LEW.1AV1 (4/22 in females and 1/14 in males, combined data from two previous studies) (22,23).

Evaluation of macroscopic arthritis in DA made congenic for Oia2/NKC from PVG, and mapping of arthritis susceptibility in recombinant strains

DA rats carrying PVG and/or DA alleles in C4 intervals, harboring Oia2/NKC (Fig. 1), were independently produced in Sweden (S) and Norway (N), and were tested in a standardized and collaborative manner. Both DA.C4(PVG)/S and DA.C4(PVG)/N displayed almost complete arthritis resistance, and heterozygotes (PVG/DA) were also markedly less susceptible than DA. In the Norwegian experiments, incidences were 0/7 in DA.C4(PVG), 8/13 in DA.C4(PVG/DA) and 13/13 in DA.C4(DA). Two recombinant (R) strains were tested, DA.C4R1/N and DA.C4R2/N, both covering the NKC (Fig. 2). The incidences were 7/7 in DA.C4R2(PVG/DA)/N, 3/3 in DA.C4R2(PVG/DA)/N and 3/3 in DA.C4R2(DA)/N demonstrating lack of arthritis-regulation. In contrast, DA.C4R1 (PVG)/N, which covers a larger interval centromeric of NKC, displayed low incidence, 3/12, demonstrating arthritis regulation. Taken together, this provides evidence that the structural gene associated with OIA susceptibility is situated outside the
NKC. These results were corroborated and extended in the Swedish study, where analysis of arthritis data for recombinants DA.C4R3-R16 (Table 2), in relation to alignment of congenic intervals along rat chromosome 4 (Fig. 2), narrowed the arthritis-regulating region to an ~1 cM interval, hereafter defined as *Oia2*, between the microsatellite markers D4Got126 and D4Got136 (Fig. 2).

Positioning of *Oia2* on a linkage map

The chromosomal positions of microsatellite markers, used to define *Oia2* in recombinant strains, were determined by PCR analyses on genomic DNA obtained from a panel of (DA × PVG)F1 × DA rats, previously used for mapping of the *NKC* on rat chromosome 4 (20) (Fig. 3). The rat *NKC* spans 1 to 2 Mbp, with the *NKR-P1* gene cluster situated at the centromeric end of the complex. Linkage mapping showed that the *Oia2* interval, as defined in recombinants, lies ~0.2 cM centromeric to *Nkrp1b*, with D4Got136 defining the telomeric end of the interval, and with a distance of 0.8 cM between D4Got136 and D4Got126 (Fig. 3). No crossovers were detected between D4Rat90, D4Got 136 and Eno2, whereas results from the Stockholm recombinant strains show that D4Rat90 lies proximal to D4Got136, i.e. within the *Oia2* interval.

Excluding the presence of arthritis-regulating PVG alleles outside the *Oia2* interval in the congenic strains

In the Swedish mapping material, we compared all inbred DA (n = 48) with each group of ‘essentially DA’ that were used as controls, i.e. the progeny from crosses between recombinants and DA that lacked PVG alleles within the *Oia2* region. None of these groups of ‘essentially DA’ deviated from inbred DA. Comparing all ‘essentially DA’ as a group (n = 127) with inbred DA yielded almost identical phenotype data: incidence 97 and 96%; day of onset 13.9 and 14.6; severity 6.8 for both groups. The results demonstrate that ‘essentially DA’ do not contain arthritis-regulating PVG alleles in their genomes.

Extended analysis of macroscopic arthritis in relation to gender and *Oia2* haplotype

Analysis of sex influence in ‘essential DA’ revealed phenotype differences between 59 males and 68 females (M versus F): severity 7.4 versus 6.2 (P = 0.010); day of onset 13.4 versus 14.3 (P = 0.048); incidence 97% for both groups. Gender influence has not been reported before, most likely because the effect is small and requires large numbers of animals to be detected. The gender difference was similar, but not significant, in 24 male and 24 female inbred DA (Table 3). We then determined the impact of *Oia2*, defined according to genotype in the D4Got126–D4Got136 interval, on arthritis phenotypes. All DA.C4/S and DA.C4R3-16/S animals were categorized as being *Oia2*(PVG/PVG), *Oia2*(PVG/DA) or *Oia2*(DA/DA), yielding groups consisting of 55, 71 and 56 animals, respectively. Phenotype data for the respective groups were compiled and compared, yielding the following results (Table 3): incidence 91.5, 54.9 and 97.8%; severity, 2.0, 2.6 and 6.7; and day of onset, 20.8, 16.3 and 13.7. Repeated analysis in relation to gender demonstrated that *Oia2* influences arthritis in both males and females, with regulation more pronounced in males.

Evaluation of additional adjuvant-oil induced phenotypes, beside macroscopic arthritis

DA.C4R3(PVG) were compared with DA and PVG.1AV1 concerning macroscopic arthritis (Fig. 4A), changes in body weight (Fig. 4B) and plasma levels of the inflammation marker AGP (Fig. 4C), an acute phase protein that increases during inflammation and acute phase reactions. The analysis revealed an arrested body weight gain in DA that lasted from arthritis onset to recovery, i.e. 10–30 d.p.i. DA.C4R3(PVG) followed the DA body weight curve closely, although they did not
Table 2. Susceptibility to oil-induced arthritis of DA.C4(PVG) recombinant animals

<table>
<thead>
<tr>
<th>Strain</th>
<th>Number of rats</th>
<th>Incidence (%)</th>
<th>Severity (mean ± SD)</th>
<th>Day of onset (mean ± SD)</th>
<th>Susceptibility (P-value)</th>
<th>Experiment no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1 (PVG)N</td>
<td>12</td>
<td>25**</td>
<td>N.D.</td>
<td>13.7 ± 1.5</td>
<td>n.a.</td>
<td>1</td>
</tr>
<tr>
<td>R2 (PVG)N</td>
<td>7</td>
<td>100</td>
<td>N.D.</td>
<td>12.4 ± 1.3</td>
<td>n.a.</td>
<td>1</td>
</tr>
<tr>
<td>R2 (PVG/DA)N</td>
<td>3</td>
<td>100</td>
<td>N.D.</td>
<td>17.0 ± 6.1</td>
<td>n.a.</td>
<td>1</td>
</tr>
<tr>
<td>R3 (PVG)</td>
<td>37</td>
<td>5***</td>
<td>3.5 ± 2.1</td>
<td>14.0 ± 0.0</td>
<td>&lt;0.0001</td>
<td>2</td>
</tr>
<tr>
<td>DA</td>
<td>15</td>
<td>93</td>
<td>5.1 ± 4.1</td>
<td>14.9 ± 5.9</td>
<td>n.a.</td>
<td>2</td>
</tr>
<tr>
<td>R4 (PVG/DA)</td>
<td>9</td>
<td>100</td>
<td>5.8 ± 1.8</td>
<td>14.4 ± 2.2</td>
<td>n.s.</td>
<td>3</td>
</tr>
<tr>
<td>R4 (DA)</td>
<td>9</td>
<td>89</td>
<td>6.3 ± 2.9</td>
<td>14.8 ± 3.3</td>
<td>n.s.</td>
<td>3</td>
</tr>
<tr>
<td>R5 (PVG/DA)</td>
<td>9</td>
<td>22*</td>
<td>3.0 ± 0.0*</td>
<td>17.0 ± 2.8</td>
<td>0.006</td>
<td>4</td>
</tr>
<tr>
<td>R5 (DA)</td>
<td>6</td>
<td>83</td>
<td>7.2 ± 2.2</td>
<td>14.4 ± 1.3</td>
<td>n.s.</td>
<td>4</td>
</tr>
<tr>
<td>R15 (PVG/DA)</td>
<td>11</td>
<td>100</td>
<td>5.4 ± 3.0</td>
<td>14.9 ± 3.4</td>
<td>n.s.</td>
<td>5</td>
</tr>
<tr>
<td>R15 (DA)</td>
<td>10</td>
<td>80</td>
<td>6.6 ± 1.6</td>
<td>13.3 ± 1.0</td>
<td>n.s.</td>
<td>5</td>
</tr>
<tr>
<td>R6 (PVG/DA)</td>
<td>11</td>
<td>45**</td>
<td>4.0 ± 1.9*</td>
<td>14.0 ± 1.4</td>
<td>0.0005</td>
<td>6</td>
</tr>
<tr>
<td>R6 (DA)</td>
<td>12</td>
<td>100</td>
<td>6.7 ± 2.1</td>
<td>13.3 ± 1.0</td>
<td>n.s.</td>
<td>6</td>
</tr>
<tr>
<td>R14 (PVG/DA)</td>
<td>10</td>
<td>100</td>
<td>7.5 ± 2.4</td>
<td>12.6 ± 1.0</td>
<td>n.s.</td>
<td>7</td>
</tr>
<tr>
<td>R14 (DA)</td>
<td>10</td>
<td>100</td>
<td>7.3 ± 2.5</td>
<td>13.4 ± 1.3</td>
<td>n.s.</td>
<td>7</td>
</tr>
<tr>
<td>R16 (PVG/DA)</td>
<td>12</td>
<td>100</td>
<td>6.8 ± 2.2</td>
<td>13.0 ± 1.3</td>
<td>n.s.</td>
<td>8</td>
</tr>
<tr>
<td>R16 (DA)</td>
<td>11</td>
<td>100</td>
<td>6.5 ± 3.2</td>
<td>13.6 ± 1.5</td>
<td>n.s.</td>
<td>8</td>
</tr>
<tr>
<td>R12 (PVG/DA)</td>
<td>7</td>
<td>43*</td>
<td>3.0 ± 1.7</td>
<td>12.7 ± 1.2</td>
<td>0.007</td>
<td>9</td>
</tr>
<tr>
<td>R12 (DA)</td>
<td>9</td>
<td>100</td>
<td>5.6 ± 2.8</td>
<td>14.3 ± 2.1</td>
<td>n.s.</td>
<td>9</td>
</tr>
<tr>
<td>R9 (PVG/DA)</td>
<td>12</td>
<td>75</td>
<td>1.8 ± 1.1*</td>
<td>15.4 ± 2.3</td>
<td>0.02</td>
<td>10</td>
</tr>
<tr>
<td>R9 (DA)</td>
<td>9</td>
<td>100</td>
<td>4.9 ± 3.5</td>
<td>14.3 ± 3.5</td>
<td>n.s.</td>
<td>10</td>
</tr>
<tr>
<td>R9 (PVG)</td>
<td>4</td>
<td>25*</td>
<td>1.0 ± 0.0</td>
<td>20.0 ± 0.0</td>
<td>0.01</td>
<td>11</td>
</tr>
<tr>
<td>DA</td>
<td>6</td>
<td>100</td>
<td>4.3 ± 2.8</td>
<td>16.3 ± 2.3</td>
<td>n.s.</td>
<td>11</td>
</tr>
<tr>
<td>R13 (PVG/DA)</td>
<td>11</td>
<td>100</td>
<td>5.3 ± 2.5</td>
<td>13.3 ± 1.8</td>
<td>n.s.</td>
<td>12</td>
</tr>
<tr>
<td>R13 (DA)</td>
<td>12</td>
<td>100</td>
<td>6.0 ± 2.8</td>
<td>13.2 ± 2.2</td>
<td>n.s.</td>
<td>12</td>
</tr>
<tr>
<td>R8 (PVG/DA)</td>
<td>8</td>
<td>100</td>
<td>8.3 ± 3.5</td>
<td>14.4 ± 4.9</td>
<td>n.s.</td>
<td>13</td>
</tr>
<tr>
<td>R8 (DA)</td>
<td>13</td>
<td>100</td>
<td>8.0 ± 2.9</td>
<td>13.8 ± 3.9</td>
<td>n.s.</td>
<td>13</td>
</tr>
<tr>
<td>R11 (PVG/DA)</td>
<td>14</td>
<td>64**</td>
<td>1.8 ± 2.0***</td>
<td>19.0 ± 4.4***</td>
<td>&lt;0.0001</td>
<td>13</td>
</tr>
<tr>
<td>R10 (PVG/DA)</td>
<td>11</td>
<td>55*</td>
<td>4.2 ± 3.2***</td>
<td>17.2 ± 5.4*</td>
<td>0.001</td>
<td>13</td>
</tr>
<tr>
<td>R10 (DA)</td>
<td>10</td>
<td>100</td>
<td>9.2 ± 1.7</td>
<td>12.8 ± 1.5</td>
<td>n.s.</td>
<td>13</td>
</tr>
<tr>
<td>R7 (PVG/DA)</td>
<td>18</td>
<td>89</td>
<td>6.3 ± 3.4</td>
<td>14.8 ± 3.2</td>
<td>n.s.</td>
<td>14</td>
</tr>
<tr>
<td>R7 (DA)</td>
<td>7</td>
<td>100</td>
<td>5.4 ± 3.1</td>
<td>16.9 ± 5.3</td>
<td>n.s.</td>
<td>14</td>
</tr>
</tbody>
</table>

Inbred DA and DA.C4(PVG) recombinants were injected with IFA and monitored for arthritis incidence, day of onset and severity. Groups with recombinant animals are compared, within each experiment, with inbred DA or with littermates lacking PVG alleles. *P < 0.05, **P < 0.01 and ***P < 0.001. SD and P-values for severity and day of onset were analyzed with t-test. P-values for incidence were analyzed with Fisher’s exact, two-tailed, test, except for R11, which was analyzed with Pearson’s chi-square test, since the control group was derived from the two control groups for R8 and R10.

Table 3. Impact on arthritis phenotypes and Oia2 alleles introduced on DA, compared with inbred DA

<table>
<thead>
<tr>
<th>Arthritis phenotypes</th>
<th>Gender</th>
<th>DA</th>
<th>DA with Oia2 alleles from DA</th>
<th>DA with Oia2 alleles from PVG and DA</th>
<th>DA with Oia2 alleles from PVG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence (%)</td>
<td></td>
<td>100.0 (24/24)</td>
<td>94.7 (18/19)</td>
<td>45.5 (15/33)</td>
<td>7.7 (2/26)</td>
</tr>
<tr>
<td>Day of onset (mean ± SD)</td>
<td></td>
<td>14.6 ± 6.8</td>
<td>13.3 ± 1.5</td>
<td>16.7 ± 4.3</td>
<td>25.0 ± 15.6</td>
</tr>
<tr>
<td>Severity (mean ± SD)</td>
<td></td>
<td>6.4 ± 3.5</td>
<td>6.4 ± 2.6</td>
<td>2.6 ± 2.0</td>
<td>1.3 ± 0.6</td>
</tr>
</tbody>
</table>

Inbred DA (n = 48) and 16 DA.C4(PVG)/S recombinants (n = 172) were injected with IFA and monitored for arthritis incidence, day of onset and severity. Arthritis phenotypes were then calculated for subgroups defined by gender, or by Oia2 genotype in the interval D4Got126-D4Got136 (as deduced in Fig. 2).

Inbred arthritis. In contrast, there was no pronounced arrest in body weight gain in arthritis-resistant PVG.1AV1. In this strain, levels of AGP were increased at 5 and 10 d.p.i., demonstrating an early systemic response to the injected adjuvant oil. However, the levels were considerably higher in DA at 5 d.p.i., and remained elevated until 30 d.p.i., approximately. In DA.C4R3(PVG), AGP levels were intermediate at 5 d.p.i., and remained elevated until 30 d.p.i., which is markedly longer than in PVG.1AV1. This indicates that a gene within Oia2 regulates AGP levels. However, the arrested body weight gain
and prolonged elevated AGP levels in arthritis-resistant DA.C4R3(PVG) indicate that these phenotypes do not relate directly to macroscopic disease.

AGP plasma levels and relative weights of liver, thymus, spleen and lymph nodes (adjusted for body weight, and expressed as g tissue/kg body weight) were then compared between male DA.C4R9(PVG) and DA before and after injection of adjuvant oil (10 d.p.i.). Before oil exposure there were no clearcut differences between the strains, except lower AGP levels and slightly larger thymuses in DA (Table 4). After injection of adjuvant oil, both strains displayed elevated AGP, increased relative weight of lymph nodes and decreased relative liver and thymus weights. DA.C4R9(PVG) displayed the same physiological changes, except that the relative splenic weights were increased in DA and reduced in DA.C4R9(PVG) rats. Furthermore, the changes in AGP levels (DA versus R9: 4.4 versus 2.0), lymph node weights (4.5 versus 2.9) and thymic weights (0.49 versus 0.65) were far more pronounced in DA than in DA.C4R9(PVG) rats. The results demonstrate systemic effects of intradermal injections of small doses of mineral oil, and that the effects of DA versus PVG allele on an otherwise uniform background are quantitative rather than all or none, except for changes in relative weights of spleens.

**Definition of Oia2 and syntenic chromosome regions in mouse and humans**

As mapped here, Oia2 is a 0.8 cM region rat chromosome 4 (RNO4), defined by the microsatellite markers D4Got126 and D4Got136. Database searches reveal that D4Got126 corresponds to FoxJ2, and that D4Got136 is located close to, and centromeric of, Pex5. The region between FoxJ2 and Pex5 spans 1.2 Mb at the cytogenetic position 4q42, and contains a limited number of candidate genes and gene-like sequences, as depicted in Figure 5. The syntenic mouse region on 6F2 is highly homologous, whereas the corresponding human genes,
with one exception, seem to be dispersed into a larger region at the cytogenetic position 12p13.31–12p13.32. The rat gene sequence similar to DnaJ (DNAJA2) is located at human 16p11.

DISCUSSION

It was recently demonstrated, for the first time, that introduction of an arthritis-promoting QTL on an arthritis-resistant genome can confer arthritis susceptibility (23). Here we provide additional examples, demonstrating that OIA can be induced in both PVG.1AV1 and LEW.1AV1 when they are made congenic for Oia2 from DA. In the reciprocal congenic strains, we established that DA rats made congenic for Oia2 from LEW or PVG, were almost completely arthritis-resistant.

In the PVG.1AV1 to DA strain combination, DA.C4(PVG), two independently derived congenic strains displayed OIA resistance, and both were used to produce independent recombinants for mapping of Oia2 in relation to the major candidate disease locus NKc. In both sets of recombinants, the arthritis-regulating locus mapped centromeric of NKc, which rejects the hypothesis that Oia2 equals a gene in the natural killer cell complex (13). High-resolution mapping delimited Oia2 to a 0.8 cM interval located at the cytogenetic position 4q42, ~1–2 cM centromeric of Eno2, at the same position as peak markers in the genome-scan where Oia2 was first identified (13).

Interestingly, our mapping results in DA.C4(PVG)/S recombinants suggest that Oia2 is the only locus with a detectable impact on OIA. This is notable because the DA.C4(PVG)/S strain harbors a large PVG interval, centromeric of Oia2, which overlaps with five DA quantitative trait loci (QTL) identified in other arthritis models. This raises the question why these QTLs were not detected. It is possible that the QTLs are disease-specific and regulate other arthritis models than OIA. Alternative explanations are that PVG alleles influence disease-phenotypes that are not monitored here (such as chronicity), or they exert weak effects that escaped detection, or PVG alleles are identical to DA, in which case they would not influence any phenotype in our experimental system.

For Oia2, alleles at this locus exerted additive or co-dominant effects in both males and females. Thus, one PVG allele at Oia2 in DA reduced arthritis incidence, delayed the day of onset, and decreased severity, while two PVG alleles conferred almost complete arthritis resistance. We next determined additional oil-induced phenotypes (22,24,25) that could discriminate susceptible DA from resistant homozygous congenics. This revealed that both strains displayed a period of weight loss and arrested weight gain, and had a prolonged acute phase response, which was possibly less pronounced in congenic rats. Relative weights of liver and thymus were decreased, whereas draining lymph nodes were hyperplastic. These results establish that dramatic physiological changes take place also in resistant congenic rats. However, the acute phase response and the lymph node hyperplasia were more pronounced in DA. The latter may indicate a higher level of regional immune activation (24,25), considering that T cells from lymph nodes can transfer OIA (26,27). Another notable result was that the relative weights of spleens were increased in DA but decreased in congenics.

Altogether, our results demonstrate that Oia2 regulates some, but not all, adjuvant oil-induced phenotypes. In addition, we have recently reported that PVG alleles in the region defined by DA.C4R3(PVG) also regulate arthritis induced with the cartilage autoantigen collagen type II emulsified in IFA (CIA), as well as arthritis induced by other adjuvant oils such as pristane and squalene (SIA) (28). This suggests that Oia2 regulates arthritis induced both by specific and non-specific activation of the immune system. We propose, therefore, that Oia2 is identical to the overlapping QTLs Pia7 and Cia13. If this is correct, arthritis-resistance alleles should be present in the strains used to define these loci: E3 for Pia7; F344 for Cia13; LEW, PVG and BN (unpublished data) for Oia2. This functional information will be important when candidate genes are being sequenced.

Beside the impact on arthritis, Oia2 has previously been linked to phenotypes in an experimental model for multiple sclerosis, i.e. myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis (MOG-EAE) (29). In accordance, we have recently demonstrated that DA.C4R3(PVG) differs from DA in MOG-EAE (30). This regulation of an additional organ-specific inflammatory disease may relate to adjuvant-specific use of IFA together with MOG for induction of EAE (IFA is routinely used together with MOG to break tolerance and induce pathogenic
autoimmunity). However, it is also possible that Oia2 regulation of different diseases reflects broader actions of genes involved in inflammatory responses, or containment of inflammatory responses. A general role in inflammation and autoimmunity may be supported by the fact that the region on rat 4q42, and the syntenic regions on 12p13 in human and 6p2 in mouse have been linked to several inflammatory diseases and associated phenotypes (31), including arthritis (13–15,22, 32–34), systemic lupus erythematosus (35), spontaneous diabetes (36,37), atherosclerosis (38,39), encephalomyelitis (29,40), asthma or airway responsiveness (41,42) and allergy (43–45).

As defined here, Oia2 harbors a limited number of candidate disease genes encoding molecules with functions in the immune system. This includes C3a anaphylatoxin receptor, Ribosomal protein L7, which is a target of autoantibody responses in rheumatic diseases (46), and CD163, which scavenges hemoglobin/haptoglobin complexes and may lead to activation in terms of secretion of pro-inflammatory cytokines after receptor cross-linking on the surface of macrophages (47,48). Another gene sequence shows similarity to DnaJ, a 40kDa heat shock protein (HSP 40) that may regulate inflammatory responses. This molecule may also be a target of crossreactive autoimmune responses (49,50), since some microbial DnaJ contain an amino acid sequence that constitutes a suggested ‘shared epitope’ (51,52) of HLA DRB1 alleles associated with RA. Interestingly, the Oia2 region also encodes several C-type lectin superfamily members (CLECSF) (53) that are likely to regulate functions of macrophages and dendritic cells in immune responses and inflammation.

It is possible that only one of the described genes regulates phenotypes mapped to Oia2 and syntenic regions, but it is also possible that more than one gene may be of general importance in inflammatory diseases. We suggest, therefore, that the described high-resolution mapping of candidate disease genes offers a valuable shortcut to detection of disease pathways and genetic disease associations in human diseases, including RA.

### MATERIALS AND METHODS

#### Animals

Inbred PVG.1AV1 originated from Harlan UK Ltd (Oxon, UK), while inbred DA and LEW.1AV1 originated from Zentralinstitut für Versuchstierzucht, Hannover, Germany. Congenic and recombinant strains were derived from these strains by standard congenization procedures, essentially as described by Greenhouse (54). Animals were bred, kept and used under specific pathogen-free conditions, as determined by a health monitoring program (FELASA) at the National Veterinary Institute in Uppsala. They were reared in climate-controlled environment with 12 h light/dark cycles, housed in polystyrene cages containing wood shavings, and had free access to standard rodent food and water. Inbred strains and congenic strains were established in parallel, in Sweden and Norway: in Sweden, at the Biomedical Center in Uppsala or the Center for Molecular Medicine, Karolinska Institutet, Stockholm; in Norway, at The Institute of Basic Medical Sciences, University of Oslo. All procedures involving animals were performed according to guidelines provided by the central Board for Animal Experiments at the Swedish and Norwegian Departments of Agriculture, and were approved by the ethics committees of Stockholm North and Oslo.

### Congenic and recombinant strains

The congenic and recombinant strains were made by transfer of chromosome 4 (C4) intervals (Figs 1 and 2) between strains, as follows: LEW.1AV1 containing a C4 interval from DA [backcrossed (bc) for 10 generations, including the first intercross generation], i.e. LEW.1AV1.C4(DA) (bc10); PVG.1AV1.C4(DA) (bc10); and DA.C4(LEW) (bc10). DA.C4(PVG) were produced in both Norway (N) and Sweden (S), i.e. DA.C4(PVG)/N (bc11) and DA.C4(PVG)/S (bc10), respectively. Recombinants (R) were made from DA.C4(PVG)/S by at least three additional backcrosses (Bc13, or more), and from DA.C4(PVG)/N as follows: DA.C4R1(PVG)/N (bc15); and DA.C4R2(PVG)/N (bc13).

#### Genetic analysis

Tailtips were collected, and genomic DNA was purified according to a standard protocol (55). Genotypes were determined by PCR amplification of tandemly repeated sequences (microsatellites) that are polymorphic between the parental strains, essentially as previously described (56), except 32P-P-γ ATP was used to label one of the primers in each pair allowing genotypes to be determined by autoradiography. Markers used for genotyping are depicted in Figures 1 and 2, and where purchased from Research Genetics (Huntsville, AL). Genotypes are denoted DA for DA homozygotes, and PVG or
LEW for PVG and LEW homozygotes, respectively. Heterozygotes are designated with both alleles, e.g. PVG/DA.

**Arthritis induction and evaluation**

Arthritis was induced by an intradermal injection of 200 μl of IFA (Difco, Detroit, USA) at the base of the tail of anesthetized animals that were sex- and age-matched for each experiment.

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for severity and susceptibility, but not for incidence and day of onset.

Determination of phenotypes other than macroscopic arthritis

To monitor oil-induced fluctuations of AGP, individual plasma samples were prepared from blood obtained from tailtips. Then, AGP levels were determined in samples from different strains and time-points using a radial immunodiffusion kit (Cardiotec, Louisville, KY). One test per sample was used, since the coefficient of variation is less than 4% in repeated measurements of identical aliquots of the same test specimen, according to the manufacturer. To monitor oil-induced changes in size of organs, animals were sacrificed, and inguinal lymph nodes, spleen, thymus and liver were dissected out and weighed.

Statistical methods

Susceptibility was analyzed using non-parametric Mann–Whitney U-test. Severity, day of onset, AGP levels, weights of organs were analyzed using Student’s t-test. The chi-square test was used for analysis of incidence. P-values below 0.05 were considered significant.

ACKNOWLEDGEMENTS

This project was supported by Astra Zeneca, by a grant from the ‘Network for Inflammation Research’ funded by the Swedish Foundation for Strategic Research, and by grants from the Swedish Medical Research Council, the Swedish Rheumatism Association, King Gustav V 80th Birthday Jubilee Foundation, Åke Wibergs Foundation, Alex and Eva Wallström Foundation and Nanna Svartz Foundation. J.C.L. is a recipient of a research fellowship from the Swedish Medical Research Council.

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