Studies of Congenic Lines in the Brown Norway Rat Model of Th2-Mediated Immunopathological Disorders Show That the Aurothiopropanol Sulfonate-Induced Immunological Disorder (Aiíd3) Locus on Chromosome 9 Plays a Major Role Compared to Aiíd2 on Chromosome 10

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J Immunol 2004; 172:6354-6361; doi: 10.4049/jimmunol.172.10.6354
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Studies of Congenic Lines in the Brown Norway Rat Model of Th2-Mediated Immunopathological Disorders Show That the Aurothiopropanol Sulfonate-Induced Immunological Disorder (Aiid3) Locus on Chromosome 9 Plays a Major Role Compared to Aiid2 on Chromosome 10

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Brown Norway (BN) and Lewis (LEW) rats are powerful models of human immunopathological disorders because they show an inverse polarization of their immune responses and susceptibility to experimentally induced immunological disorders. Type 1-mediated, organ-specific autoimmune disease, such as experimental autoimmune encephalomyelitis (EAE), can be easily induced in LEW, but not in BN, rats (2). Conversely, BN rats are susceptible to Th2-dependent, gold-induced autoimmunity, and LEW rats are resistant (3). This model was initially designed to understand the mechanisms of the immunotoxic effects (skin, hematologic, pulmonary, glomerular diseases, and increase in serum IgE concentration (4, 5)) of gold salts, which are still used as disease-modifying, anti-rheumatic drugs (6, 7). After regular injections of aurothiopropanol sulfonate (Atps), BN rats show a biphasic increase in serum IgE concentration, produce anti-laminin and anti-DNA Abs, and exhibit IgG deposits in kidney vessel walls and autoimmune glomerulonephritis. This glomerulonephritis is characterized initially by linear IgG deposits along the glomerular basement membrane and then by a membranous glomerulopathy similar to that observed in some gold-treated patients (8). Serum concentrations of IgG1, a Th2-dependent isotype of Ig, are also increased, whereas IgG2b, a Th1-dependent isotype, remains in a normal range. LEW rats are resistant to these manifestations. Based on these contrasting observations, we have used these two rat strains to investigate the genetic control of Th2-mediated immunopathological disorders triggered by gold salts.

Using F2 hybrids from the BN and LEW strains, we have previously identified three quantitative trait loci (QTLs) that control the Th2-dependent immune manifestations triggered by gold salts (9, 10). These loci, now named Aiid1, Aiid2, and Aiid3 (Atps-induced immunological disorders), are located on chromosomes (c) 20, 10, and 9, respectively. Aiid1, which includes the MHC...
region, partly controls the production of anti-DNA Abs and the IgG deposits in kidney arteries. It also controls the kinetics of the IgE response to gold salts. The kinetics follow a biphasic pattern, with two peaks on days 7 and 21 in F2 hybrid rats bearing one or two BN alleles at this locus; they follow a monophasic pattern, with a single peak on day 14 in rats homozygous for the LEW allele (our unpublished observations). The other loci, Aiid2 and Aiid3, have been found to jointly control ~45% of the variance in the IgE response; they also partly control the anti-laminin Ab response and the development of glomerular IgG deposits. The correlation observed between these three traits in the LEW × BN F2 crosses strongly suggests the involvement of a single gene or a group of closely linked genes at Aiid2 and Aiid3 (10).

The construction of congenic rodent strains is a powerful approach for the dissection of polygenic diseases (12–14). Congenics derived between BN and LEW rats for the QTLs Aiid2 and Aiid3 represent new models for refining the positions of the gene(s) involved in Th2-mediated pathologies (11). In the work described in this study we have developed a series of congenic lines that allowed us to map the Aiid2 and Aiid3 QTLs at 7- and 1.2-cM intervals, respectively, as well as to dissect the phenotypic effects of these loci on susceptibility to gold-induced adverse reactions. Aiid2 was found to contain several genes that partly control the IgE response, but have no significant effect on renal pathology. Aiid3 appeared to exert a major effect on Atps-induced immunological disorders. Indeed, BN rats congenic for the 1.2-cM segment of Aiid3 from LEW rats show a 10-fold decrease in the IgE response to Atps and a dramatic prevention of glomerular IgG deposits.

Materials and Methods

Housing conditions of rats

All breeding and experimental procedures were conducted in accordance with European guidelines. Rats were bred and kept under specific pathogen-free conditions. Sanitary controls were performed every 3–4 mo according to the health-monitoring program of the Federation of European Laboratory Animal Science Associations (15).

Development of congenic lines

An initial population of F1 hybrids animals was produced by breeding female LEW with male BN rats obtained from the Janvier breeding center (Le Genest-Saint-Ise, France). Male F1 rats were subsequently crossed either to LEW female rats to transfer the Aiid2 (c10) and Aiid3 (c9) loci onto the LEW (LEW.BNc10 and LEW.BNc9 congenics) or the BN (BN.LEWc10 and BN.LEWc9 congenics) genetic background. Females of this first backcross generation were selected for subsequent repeated backcrosses to either male LEW (LEW.BN set) or BN (BN.LEW set) rats on the criterion of heterozygosity for markers selected for screening on c9 and c10 (see section on genotyping below). Male or female hybrid rats retaining heterozygosity for c9 or c10 markers were used for further backcrosses. Although carrying out selection of rats for heterozygosity of markers screened on c9 or c10, the genetic background of rats was also screened using a genetic marker-assisted protocol (16). In this breeding strategy, known as speed congenics, the genetically “best” animals, which carry the least amount of genetic contaminants of donor origin outside the targeted QTL, are selected for backcross breeding, thus decreasing the number of successive generations required to fully (99.9%) eliminate donor alleles from the genetic background of the congenics (11). From the second backcross, the genetic background of heterozygous rats at c9 loci was screened for c10 and c20 markers to select rats that share a recipient genetic background homozygous for all markers at the Aiid2 and Aiid3 loci. Intercrossing rats derived from the fourth and subsequent backcrosses allowed us to check that the introgressed chromosomal region bearing the genes that control susceptibility to gold salts were successfully maintained during the successive backcrossing procedures.

During the successive backcrosses, progenies were also selected for combination within the loci to develop simultaneously several lines, for the subsequent genetic dissection of the loci. At the eighth or ninth backcross, when the rats showed heterozygosity of the recipient genome for the set of markers used to screen the genetic background, the homozygous state for the introgressed chromosomal regions was fixed by mating two heterozygous rats and selecting progenies carrying the appropriate genotypes at the QTL. Congenic strains were subsequently maintained by brother-sister mating. To this date, LEW rats bearing BN Aiid2 (LEW.BNc10), BN rats bearing LEW Aiid2 (BN.LEWc10), and BN rats bearing LEW Aiid3 (BN -LEWc9) have been successfully produced and maintained.

Genetic markers and genotype analysis

Preparation of rat genomic DNA and genotyping determination were performed as previously described (10). The PCR products were size-fractionated on 4% agarose (ResolPhor agarose (Eurobio, Les Ulis, France) or SLE-type agarose (Kalys, Roubais, France)) and visualized under UV light after staining with 1 μg/ml ethidium bromide. Polymorphic microsatellite markers were chosen according to their position in genetic maps of the rat (17–19) (http://rgd.mcw.edu; http://well.ox.ac.uk/rat_mapping_resources). A total of 243 microsatellite markers were used for the genetic characterization of rat progenies to verify the elimination of major contaminant alleles of donor origin from the genetic background of the congenics and determine the genetic length of the QTL region introgressed. The rat genetic map was covered at ~99%, with a minimal spacing of 30 cm and an average spacing of ~13 cm between markers. Linkage maps of c9 and c10 were constructed in an F2 (LEW×BN) rat cohort (10) with 23 and 63 markers, respectively, using the Kosambi map function within the MAPMAKER/EXP 3.0b computer package (20).

Phenotype analysis

Groups of 4–10 rats, aged between 8 and 11 wk, were injected s.c. with Atps (Allocrychus; Laboratory Solvay Pharma, Suresnes, France) at 20 mg/kg body weight, three times a week for 5 wk. Rats were weighed and bled retro-orbitally under anesthesia before the first administration of Atps, then once a week until sacrifice (10). Enzyme immunoassays used to measure serum concentrations of IgE, IgG1, IgG2b, and anti-laminin Ab titers were performed as previously described (21). Kidneys were studied by immunofluorescence on snap-frozen 4-μm sections stained with FITC-conjugated antisera to rat IgG as previously described (21).

Statistical analysis

Analysis was performed using the SPSS 8.0.1F statistical package (SPSS, Chicago, IL). The significance of differences found between groups was initially derived from a Kruskal-Wallis H test and subsequently confirmed by the Mann-Whitney test. In some instances, Student’s t test was used. Pearson’s χ2 test was used to determine a difference in the genotype distribution according to the intensity of glomerular IgG.

Results

Genetic characterization of Aiid2 congenic lines

Eight LEW.BNc10 and three BN.LEWc10 congenic lines were developed. The genetic map of c10 and the genomic fragments of c10 of LEW and BN origins introgressed into the other genetic background are shown in Fig. 1. Forty-one markers were located initially derived from a Kruskal-Wallis H test and subsequently confirmed by the Mann-Whitney test. In some instances, Student’s t test was used. Pearson’s χ2 test was used to determine a difference in the genotype distribution according to the intensity of glomerular IgG.

Phenotypic analyses of Aiid2 congenic lines

A weak, but sharp, increase in the serum IgE concentration was observed in four LEW lines (LEW.BNc10-A, -B, -I, and -H) carrying BN alleles at the Aiid2 locus (Fig. 2A). In these lines the peak of the response was found on day 14 or 21. Three lines (LEW.BNc10-C, -F, and -G) showed almost no IgE response to Atps administration, as observed in the LEW parental strain. The LEW.BNc10-D line showed an intermediate phenotype (Figs. 2A and 3A). The response of rats of the BN.LEWc10-C line was identical in pattern and intensity to that of BN rats (Figs. 2B and 3A). In rats of BN.LEWc10-B and -F lines, serum IgE concentrations of the second peak were decreased, particularly on day 21 (p < 0.01). The decrease was more pronounced in the BN.LEWc10-F strain than in the BN.LEWc10-B strain (p < 0.01; Figs. 2B and 3A).
The Aiid2 chromosomal region that partly controls the IgE response can be localized in an ~7-cM interval and divided into two subregions.

Combining results from both genetic and phenotypic characterizations of the reciprocal congenic lines provided essential information for the fine mapping of the Aiid2 chromosomal region that controls the IgE response (Fig. 3). First, the Aiid2 locus can be reduced from >30 cM to a 5.5- to 7.2-cM interval between D10Rat37/D10Rat173 and D10Mco17/D10Rat83. The lower limit of the refined Aiid2 locus can be deduced from results in the nonresponder LEW.BNC10-C line and located in the ~0.2-cM region between D10Mco17 and D10Rat83 (Table I). Its centromeric limit can be deduced from results in the LEW.BNC10-I line and located in the 0.9-cM region between D10Rat37 and D10Rat173 (Table I). Results from the BN.LEWc10 congenics support the mapping of the Aiid2 locus in such a refined interval. Rats of the BN.LEWc10-C line, which are homozygous BN in this segment, show an IgE response similar to that of BN rats, whereas rats of the BN.LEWc10-F strain, homozygous LEW in this interval, show a sharp decrease in the second peak of the IgE response.

Second, the intermediate IgE responses observed in rats of both LEW.BNC10-D and BN.LEWc10-B reciprocal lines suggest that Aiid2 comprises two subregions, Aiid2a and Aiid2b (Fig. 3B). In LEW.BNC10-D rats, the presence of BN alleles at the centromeric sublocus Aiid2a is by itself responsible for a mild increase in the serum IgE concentration in response to Atps. In BN.c10-LEW-B rats, the presence of LEW alleles in Aiid2a is responsible for a mild decrease in the serum IgE concentration at the second peak of the response to Atps. As the BN.LEWc10-B line carries LEW alleles up to the marker D10Rat126, the boundary between Aiid2a and Aiid2b falls in the 0.2-cM region between D10Rat126 and D10Rat164. Our mapping of Aiid2a, therefore, spans a 4.5- to 5.8-cM region between D10Rat37/D10Rat173 and D10Rat126/D10Rat164. For Aiid2b it spans a 1.0- to 1.4-cM region between D10Rat126/D10Rat164 and D10Mco17/D10Rat83 (Fig. 3B).

Genetic characterization of BN.LEWc9 congenic rats

From the 23 polymorphic markers used to construct the genetic map of c9, 11 were located within the ~7-cM of the Aiid3 locus. In this region, there was no gap larger than 2 cM between markers. Twelve markers were located within the remaining ~80 cM of c9, with an average spacing of ~7 cM. They were used for the genetic screening of BN.LEWc9 congenic rats. Before true congenic lines were obtained, rats of the BN genetic background, homozygous for all the BN markers at Aiid1 and Aiid2, but heterozygous for the LEW Aiid3 locus, were derived from the fourth, fifth, and seventh (BN × LEW) × BN backcrosses and were brother × sister intercrossed. Their progenies were genotyped and investigated for immunological disorders triggered by Atps. Moreover, the BN.LEWc9-B congenic line, obtained after the ninth (BN × LEW) × BN backcross, was similarly investigated (Table I).

On the BN genetic background, LEW Aiid3 markedly down-modulates the Atps-induced immunopathological disorders

Results obtained with rats bred by intercrossing rats from the fourth and fifth (LEW × BN) × BN backcrosses are shown in Fig. 5. As expected, rats bearing two copies of BN alleles at the Aiid3 locus and treated with Atps showed the full pattern of immunological disorders usually observed in BN rats (nn; Fig. 5). By contrast, rats bearing two copies of LEW alleles at the Aiid3 locus showed a marked decrease in the IgE response (ll; Fig. 5A) and a total or marked prevention of glomerular IgG deposits (ll; Fig. 5B).

Significant increases in serum IgG1 concentrations and anti-laminin Ab titers were observed in LEW.BNC10-B, -E, and -I lines compared with LEW controls (Fig. 4). Moreover, the serum IgG1 concentration in the LEW.BNC10-D line was increased compared with that in LEW controls (p < 0.05), but was lower than that found in the three other lines (p < 0.01). As expected, no difference in serum IgG2b concentration was found among these congenic lines (not shown). Regarding kidney pathology, no glomerular IgG deposits were observed in any of these six LEW.BNC10 congenic lines (not shown). In BN.LEWc10 congenics, no significant difference was found in IgG1 or IgG2b concentrations, anti-laminin Ab titers, or intensity of glomerular IgG deposits compared with control BN rats (not shown).

In conclusion, these phenotypic analyses of Aiid2 congenic lines confirm the role of Aiid2 in the control of serum IgE and IgG1 concentrations and anti-laminin levels and indicate that this locus does not exert any detectable effect on renal pathology.
For both these phenotypes rats heterozygous at the Aiid3 locus showed an intermediate response, which was significantly different from the responses observed in rats homozygous BN and LEW at this locus (nl; Fig. 5). Moreover, rats homozygous LEW at the Aiid3 locus showed decreased concentrations of IgG1 and decreased titers of anti-laminin Abs compared with littermate rats homozygous BN and/or heterozygous BN/LEW at the Aiid3 locus (Fig. 5A). Thus, in contrast to the genetic control of the locus Aiid3 on IgE, which is expressed under an additive mode, its effect on IgG1 and anti-laminin Abs is expressed under a dominant BN mode. No differences in IgG2b concentrations were found between rats of different genotypes at the Aiid3 locus (not shown).

Mapping of the Aiid3 locus to an ~1.2-cM interval on c9

After backcrossing for four generations, rats were selected as heterozygous for various c9 regions due to recombination events. Their progeny issued from the fifth generation of backcross was intercrossed via brother-sister mating, and the resulting offspring were genotyped. Rats with and without recombination within Aiid3 were investigated for IgE response to Atps (Fig. 6). Among the three recombinant rats that were homozygous BN at the most centromeric markers (D9Wox24 and D9Got200) and heterozygous thereafter (recomb. a), two developed a high IgE response (>800 μg/ml). On the one hand, this response was similar to that observed in littermates that were homozygous BN for the entire Aiid3 locus (nn; mean ± SD, 902 ± 330 μg/ml). In contrast, that response was higher than that observed in rats that were heterozygous for the entire Aiid3 locus (nl; 440 ± 187 μg/ml). This result suggests that the gene(s) responsible for the phenotype of the Aiid3 locus could be localized in the c9 region centromeric to D9Got8.

Additionally, the intermediary IgE response to Atps observed in recombinant b that was homozygous LEW at D9Wox24 and heterozygous thereafter fit this interpretation. Moreover, the fact that recombinant b was homozygous LEW at D9Wox24 allowed us to exclude this marker from the Aiid3 locus and to narrow the mapping of the putative control gene(s) between D9Got200 and D9Got8. Results obtained with recombinants c, d, and e fitted this interpretation even if one of the two recombinants of the d type, which was homozygous LEW for this interval, showed low/intermediate response to Atps (270 μg/ml).

The likely localization of the control gene(s) in the 1.2-cM interval between D9Got200 and D9Got8 led us to look for rats recombining between these two markers. One rat with such a recombination was selected at the sixth backcross. Rats from its progeny were further backcrossed, and a congenic line (BN.LEWc9-B) was developed. As shown in Fig. 7, a dramatic decrease in the IgE response to Atps was found in the BN.LEWc9-B congenic line compared with the parental BN strain. IgE response to

Table 1. Chromosomal regions of donor strains introgressed into the recipient strains in congenic lines

<table>
<thead>
<tr>
<th>Lines</th>
<th>Centromeric Boundary</th>
<th>Telomeric Boundary</th>
</tr>
</thead>
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<tr>
<td></td>
<td>LEW&lt;sup&gt;a&lt;/sup&gt;</td>
<td>cM&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LEW.BNc10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>B</td>
<td>D10Rat72&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.0</td>
</tr>
<tr>
<td>C</td>
<td>D10ncol7</td>
<td>0.2</td>
</tr>
<tr>
<td>D</td>
<td>D10Rat72</td>
<td>2.0</td>
</tr>
<tr>
<td>F</td>
<td>D10Rat116</td>
<td>0.5</td>
</tr>
<tr>
<td>G</td>
<td>D10Arb4</td>
<td>4.5</td>
</tr>
<tr>
<td>H</td>
<td>D10Rat100</td>
<td>5.4</td>
</tr>
<tr>
<td>I</td>
<td>D10Rat37</td>
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<td></td>
<td>D10Mgh10</td>
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<td></td>
<td>D10Got56</td>
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<td></td>
<td>BN&lt;sup&gt;+&lt;/sup&gt;</td>
<td>cM&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>BN.LEWc10</td>
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<tr>
<td>B</td>
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<tr>
<td>C</td>
<td>D10Arb4</td>
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<td>F</td>
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<tr>
<td></td>
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<td>D10Rat42</td>
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<td></td>
<td></td>
<td>D9Got200</td>
</tr>
</tbody>
</table>

<sup>a</sup> The congenic strain is of the homozygous LEW genotype for the indicated marker.
<sup>b</sup> Distance in centimorgans (cM) between the two adjacent markers.
<sup>c</sup> The congenic strain is of the homozygous BN genotype for the indicated marker.
<sup>d</sup> When several markers are at the same position on the map, they are all indicated in this table. In the text, only the first of these markers is indicated.
Atns appeared to be decreased by 90%, as deduced from the ratio of the areas under the curve of the responses observed in the BN parental strain and in the BN.LEWc9-B congenic line. Moreover, an almost complete prevention of IgG glomerular deposits was observed (not shown). These results indicate that one gene or a closely linked set of genes located in a 1.2-cM interval between D9Got200 and D9Got8 exerts a major control on the Th2-immunopathological disorders triggered by gold salts in the BN rat.

Discussion
In previous studies we had shown that the BN rat injected with Atns is a very useful experimental model for investigating the multigenic control of Th2-triggered adverse reactions to gold salts (9, 10), for which we have defined three QTLs for susceptibility (Aiidd1, -2, and -3). In the present study, using congenic lines for Aiidd2 and Aiidd3, we first confirmed the role of Aiidd2 on c10 and that of Aiidd3 on c9 in the control of the Th2-triggered adverse reactions to the gold salt Atns. Second, we have narrowed the mapping of the Aiidd2 locus from 30 cM down to 7 cM. This region is further split into two subregions, named Aiidd2a and Aiidd2b that contribute to the phenotypic expression through an

![FIGURE 2. IgE response to Atns injections in LEW.BNc10 (A) and BNc9 (B) congenic lines. Rats were injected with Atns as described in Materials and Methods. The serum IgE concentration was determined by ELISA. Note that the scale and kinetics of the response are different in congenics from the LEW (relatively low and monophasic response) and the BN (high and biphasic response) backgrounds. Symbols represent the mean values (n = 4–9 in the various congenic lines tested). △, ■, ○, and □, strains showing no difference in the IgE response compared with the corresponding parental strain; △, ■, ○, and □, strains showing a sharp difference in response (increase in the LEW background, decrease in the BN background) compared with the parental strain; X, strains showing intermediate responses. Similar results were obtained for each congenic strain in two to five independent experiments (four to nine rats per group).](image)

![FIGURE 3. QTL mapping of the IgE response to Atns in LEW.BNc10 and BN.LEWc10 congenic lines. Rats were injected with Atns as described in Materials and Methods. A, IgE response on day 21. For each strain the height of the column indicates the mean (n = 4–9), and vertical bar shows the SEM. Separate diagrams were used because the scale of the response is dramatically different in congenics from the LEW and BN backgrounds. The significance of the differences found between congenic and parental strains of the same background, as assessed using the Mann-Whitney U test, is indicated on the top of the figure. B, Genetic maps of the congenic strains at the Aiidd2 locus. Dashed lines positioned at D10Rat43 and D10Rat133 indicate the approximate centromeric and telomeric limits of Aiidd2 as mapped in our previous work (10). Black and white colors show, respectively, the chromosomal segments belonging to the BN and LEW strains. Segments in gray indicate ambiguous regions belonging to either the BN or the LEW strain, corresponding to cross-over breakpoints between markers. The couples of dashed lines positioned at D10Rat37/D10Rat173, D10Mco4/D10Rat126, and D10Mco17/D10Rat83 indicate the limits of the two chromosomal subregions that can be defined according to the results shown in A.)

![FIGURE 4. Concentrations of IgG1 and titers of anti-laminin Abs in sera from LEW.BNc10 congenic lines. Results are shown for day 21, which corresponds to the peak of the response. Columns represent the mean of the values obtained with separate rats (n = 7–22). The error bars represent the SEM. *, p < 0.05; **, p < 0.01; ***, p < 0.001 (by Mann-Whitney U test).](image)
were studied by immunofluorescence at D35. The intensity of glomerular 
Aiid2a
set of genes carried by 
Aiid3
be very strong candidates. Third, we have found that the gene or 
tion, we still consider the genes of the cytokine cluster in 
Aiid2
apparent additive effect. Regarding potential mechanisms of ac-
results shown are those from rats intercrossed from the fourth backcross (ll, n = 5; nl, n = 7; nn, n = 6). Identical results were obtained in rats intercrossed from the fifth backcross. Results shown were obtained on day 7 for IgE and on day 14 for IgG1 and anti-laminin Abs. The height of columns indicates the mean, and the bars show the SEM. The statistical significance of the results (by Mann-Whitney U test) is indicated on top of each graph. B. Results shown are those from rats intercrossed from the fourth and fifth backcrosses (ll, n = 10; nl, n = 10; nn, n = 7). Kidneys were studied by immunofluorescence at D35. The intensity of glomerular deposits was graded from 0 to 3 (0, no deposit; 1, weak; 2, mild; 3, intense).

In our LEW.BNc10 congenic lines, the BN gene variants in the regions of 
Aiid2a
and 
Aiid2b
introgressed onto the LEW genetic background are responsible for an increase in serum IgE concentrations that reach, at most, 5–10% of the peak response observed in the BN strain. This result fits with the fact that 
Aiid2
counts for only ~13% of the variance in the IgE response. Reciprocally, in our BN.LEWc10 congenic lines, the introgressed region of the LEW 
Aiid2
locus into the BN genetic background is only responsible for a decrease in the second peak of the biphasic IgE re-
sponse. This suggests a complex regulation of the kinetics of the IgE response by 
Aiid2
in the BN strain. The results obtained in the two reciprocal series of congenic lines are consistent and independ-
ently lead to the same conclusion that the 
Aiid2
chromosomal region harbors two subregions that are both involved in control of the IgE response. We hypothesize that the effects observed in the high responder LEW.BNc10 congenic lines are the cumulative ef-
effects of BN alleles at the 
Aiid2a
and 
Aiid2b
loci. Conversely, the sharp decrease in the second peak of the IgE response observed in the BN.LEWc10-F congenic line is likely to result from the cum-
ulative effects of LEW alleles at both loci. To what extent genes in these two regions could interact between them or with other

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FIGURE 5. Segregation of serum IgE and IgG1 concentrations and anti-
laminin Abs titers (A) and of the intensities of the glomerular IgG deposits (B) with genotypes at the 
Aiid3
locus in rats of BN genomic background. Rats bred by intercrossing rats from the fourth and fifth (LEW × BN) × BN backcrosses were treated with Atps as described in Materials and Methods: ll, nl, and nn indicate rats homozygous LEW, heterozygous BN/LEW, or homozygous BN, respectively, at 
Aiid3.

FIGURE 6. IgE response to Atps injection in BN.LEWc9 rats. Progeny 
Aiid2
locus into the LEW genetic background had no detectable effect on 
titers of these autoantibodies, the introgression of the BN 
Aiid3
in our BN.LEWc9 rats, the introgressed region of the 
Aiid3
chromosomal locus on the genetic map constructed from 
genotyping 205 rats F 2 (LEW × BN) × BN backcross: nn, homozygote BN (n = 13); nl, heterozygote (n = 18); ll, homozygote LEW (n = 36; □) at 
Aiid3.
The left part of the figure shows the genotypes of the rats. □, LEW homozygous; ■, BN homozygous; △, BN/LEW heterozygous. □. Ambiguous regions belonging to either the BN or LEW strain, due to cross-over breakpoints between markers. Horizontal dotted lines indicate the precise position of the mark-
ers that cover the entire 
Aiid3
locus into the BN genetic background is only respon-
sible for a decrease in the second peak of the biphasic IgE re-
sponse. This suggests a complex regulation of the kinetics of the IgE response to Atps and for an almost complete prevention of glomerular IgG deposits.

The differences observed among the various LEW.BNc10 con-
genic lines in serum IgG1 concentrations, which reflect those found in serum IgE, was not unexpected, because both these Ig isotypes are Th2 dependent. Moreover, a significant increase in anti-laminin Ab titers was found in the three same lines. This result confirms the role of this locus in the control of this trait, which was only suggested by the previous study (10). Despite the increase in titer of these autoantibodies, the introgression of the BN 
Aiid2
locus into the LEW genetic background had no detectable effect on glomerular IgG deposits. Therefore, this locus did not appear to exert major control on the development of kidney lesions.

By contrast, a marked effect of introgression of the LEW 
Aiid3
locus into the BN background was observed on the immunopatho-
ologic disorders triggered by gold salt. An ~90% decrease in serum IgE concentrations was observed in BN rats carrying the homozygous LEW allele at the 
Aiid3
locus. An ~50% decrease was also observed in BN rats heterozygous LEW/BN at the 
Aiid3
locus, indicating an additive effect in accordance with our previous study (10). These results indicate a major effect of this locus on the
Th2-mediated immunopathological disorders triggered by gold salts and fit with the fact that \( \text{Aiid3} \) accounts for about one-third of the variance in the IgE response to gold salts (10). Among the significant effects observed on the other studied phenotypes, the prevention of IgG glomerular deposits was the most striking, thus indicating a major effect of \( \text{Aiid3} \) on this immunopathological feature. It would be of interest to test whether the human homologous chromosomal region plays a role in gold-induced membranous nephritis as well as in idiopathic membranous nephritis. In this study an additive effect was again observed as expected from our previous study (10), because BN rats heterozygous LEW/BN at the \( \text{Aiid3} \) locus show a milder prevention of glomerular IgG deposits than BN rats homozygous LEW at the \( \text{Aiid3} \) locus.

The recent release of an annotated draft of the whole rat genome sequence allowed better identification of the human and mouse regions homologous to the studied rat loci (http://ensembl.com). \( \text{Aiid2a} \) and \( \text{Aiid2b} \) are homologous to mouse c11. \( \text{Aiid2a} \) is homologous to three chromosomal regions of the human 5q31-q33 (19). This region includes a cytokine gene cluster that contains several candidate genes and, more particularly, the cytokine genes for IL-4, IL-5, IL-13, and GM-CSF, and genes encoding proteins involved in transcription or signal transduction in T cells, including IFN regulatory factor 1 and T cell-specific transcription factor 7 (22). It also contains candidate conserved noncoding sequences, such as conserved noncoding sequence 1, which involved in coordinate regulation of the expression of IL-4, IL-5, and IL-13 (23, 24). In the mouse, \( \text{Tpm1} \), a locus that controls in vitro the Th1/Th2 differentiation, has been localized in the homologous region of the cytokine gene cluster on c11 (25, 26). In humans, this region has been linked to total serum IgE concentrations in atopic patients (27, 28). Further studies have suggested the presence of three asthma/atopy loci in this human homologous region (29). \( \text{Aiid2b} \) is homologous to regions of human c1, c5, and c17. To our knowledge, these regions do not contain any obvious candidate gene likely to play a major role in the regulation of Th2-mediated responses. The centromeric chromosomal portion of the \( \text{Aiid3} \) region is homologous to regions of mouse c17 and of human c19. It contains \( \text{VAV1} \), which we perceive as a candidate gene. This guanine nucleotide exchange factor is rapidly phosphorylated after activation of the high affinity receptor for IgE (FceRI) (30) and could be implicated in the modulation of serum IgE concentrations (31). Moreover, during T cell activation phosphorylation of the linker for activation of T cells (LAT) promotes the recruitment of Vav to LAT complexes (32), and an impairment of this pathway has been implicated in the induction of Th2-type immunity in mice homologous for mutation of a single LAT tyrosine residue (33). However, it is not yet possible to establish precisely the physical map of \( \text{Aiid3} \) by comparative genomics. Rat/hamster radiation hybrid mapping is in progress to better define this region and precisely determine the genes that it contains.

The \( \text{Aiid2} \) QTL consists of a cluster of at least two regions in close proximity, each capable of contributing independently to an increased IgE response to Atps. Such a clustering of loci, which produces a phenotypic effect that favors the detection of significant QTL by linkage analysis (34), has been observed in several other systems, particularly in mouse models of lupus and type 1 diabetes mellitus (35–37). A similar clustering could exist in humans, as suggested by linkage analysis of lupus susceptibility (29, 38). This locus clustering might result from an evolutionary pressure that would favor the linkage of genes directing coordinated responses among similar functional pathways. In this context, bioinformatic tools that allow extensive analyses of both the growing genomic sequence information in human, mouse, and rat (http://hgsc.bcm.tmc.edu/projects/rat/) and microarray-based expression data will be essential for identification of the genes underlying a QTL and the analysis of their functions (39). In that respect, it will be particularly informative to compare the expression patterns of genes in parental strains and those in congenic lines derived for different chromosomal regions of the QTL and sharing the same genetic background. Such approaches have recently succeeded in identifying genes at work in a mouse model of lupus (13) and a spontaneously hypertensive (SHR) rat model of insulin resistance (14).

Our results strongly suggest that the QTLs \( \text{Aiid2a/b} \) and \( \text{Aiid3} \) are central to the Th1/Th2 balance and, as such, may be common determinants of allergic and Th2-type autoimmune diseases. In the rat a genome-wide search for loci controlling the susceptibility to EAE, an experimental model of multiple sclerosis, in an F2 (LEW×BN) population has identified a locus that overlaps with \( \text{Aiid2} \) (40). Similarly, \( \text{Aiid3} \) overlaps with \( \text{Eae4} \), a locus controlling both the susceptibility to EAE and the expression of IFN-\( \gamma \) in cells infiltrating the spinal cord, in F2 rats issued from a Dark Agouti (DA)×BN cross (41). Allelic variants of genes at these loci on c10 and c9 could therefore be implicated in the control of T cell polarization to either a Th1 or a Th2 type of immune response. Such genes could have pleiotropic effects, influencing the clustering of immune dysfunction in the BN/LEW models of immune-mediated diseases. This hypothesis fits with the observation that BN rats are prone to develop Th2-mediated diseases, but are resistant to the development of EAE, whereas LEW and DA rats are prone to develop Th1-mediated autoimmune diseases, but are resistant to immunological disorders induced by gold salts (1). Recent studies suggested that regions associated with control of the balance between the CD45RC\textsuperscript{high} and CD45RC\textsuperscript{low} Th subsets overlap on c9, c10, and c20 with regions associated with susceptibility to gold salt-induced immunological disorders in BN rats and with susceptibility to EAE in LEW or DA rats (42) (our unpublished observations). In that respect, patients suffering from multiple sclerosis show significantly decreased prevalence of Th2-mediated allergic diseases (43, 44).

Congenic and recombinant congenic lines that are under development for extended genetic dissections of the loci \( \text{Aiid2a}, \text{Aiid2b}, \) and
and Aid3 will be the tools of choice for further functional investigations. They will be used to decipher the chromosomal regions that control the cellular and molecular mechanisms at play in control of the Th2-mediated disorders triggered by gold salts so as to ultimately identify the genes involved. Knowledge of the genes that control these adverse reactions and understanding of the cellular and molecular mechanisms they control could provide new perspectives on the prevention and treatment of allergic diseases.

Acknowledgments

We thank Sylvie Appolinaire, Patrick Aregui, Marie-Andrée Daussion, Éliane Pelissou, Caroline Segui, Magali Toulouse, and Aline Tridon (Zootecnic Unit, Institut Fédératif de Recherche 30) for careful handling of the rats, and Dr. Etienne Joly for critical reading of the manuscript.

References


