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Two Major Interacting Chromosome Loci Control Disease Susceptibility in Murine Model of Spondyloarthropathy

Anikó Végvári,‡ Zoltán Szabó,‡ Sándor Szántó,§ Andrew B. Nesterovitch, Katalin Mikecz, Tibor T. Glant, and Vyacheslav A. Adarichev

Autoimmune spondylitis was induced in BALB/c mice and their MHC-matched (BALB/c × DBA/2)F1 and F2 hybrids by systemic immunization with cartilage/intervertebral disk proteoglycan (PG). As in human ankylosing spondylitis, the MHC was the major permissive genetic locus in murine PG-induced spondylitis (PGIS). Two major non-MHC chromosome loci with highly significant linkage were found on chromosomes 2 (Pgis2) and 18 (Pgis1) accounting for 40% of the entire F2 trait variance. The dominant spondylitis-susceptibility allele for Pgis2 locus is derived from the BALB/c strain, whereas the Pgis1 recessive allele was present in the disease-resistant DBA/2 strain. The Pgis1 locus significantly affected the disease-controlling Pgis2 locus, inducing as high incidence of spondylitis in F2 hybrids as was found in the spondylitis-susceptible parent BALB/c strain. Additional disease-controlling loci with suggestive linkage were mapped to the chromosomes 12, 15, and 19. Severity of spondylitis in F2 mice positively correlated with serum levels of amyloid A, IL-6, and Pg-specific Abs, and showed negative correlation with Ag-induced T cell proliferation, IFN-γ, IL-4, and TNF-α production. A major locus controlling serum IL-6 was found on chromosome 14 near osteoclast differentiation factor Tnfsf11. Locus on chromosome 11 near the Stat3 and Stat5 genes controlled serum level of the Ig IgG2a isotype. The two major genetic loci Pgis1 and Pgis2 of murine spondylitis were homologous to chromosome regions in human genome, which control ankylosing spondylitis in human patients. Thus, this animal model of experimentally induced spondylitis might facilitate the identification of spondylitis-susceptibility genes in humans. The Journal of Immunology, 2005, 175: 2475–2483.

C hronic inflammation of the axial skeleton and sacroiliac joints is the hallmark of ankylosing spondylitis (AS) in human patients. The incidence of AS is 0.1–0.3% in the Caucasian population; it is higher in Native Americans and lower in African-Americans (1–4). Although the etiology of the disease is unknown, environmental and genetic components have been implicated as predisposing factors. To date, the strongest genetic risk factor is the HLA-B27 allele among the MHC class I genes (5). Although 92% of Caucasian patients with AS carry HLA-B27, the genetic background, spondylitis, sacroiliitis, and enthesitis can be induced in unmodified susceptible mouse strains (BALB/c and C3H) using systemic immunization with certain constituents of cartilage and/or intervertebral disks (IVDs), such as proteoglycan (PG), link protein, or versican (15–18). Clinical symptoms, histopathological and x-ray abnormalities, and the progression of the disease in PG-immunized mice, are very similar to those in human AS. As in human patients, inflammation in mice occurs first in sacroiliac joints occupied with enthesitis, and then expands upwards involving multiple IVDs (15, 19).

We have mapped non-MHC genetic loci associated with clinical or immunological parameters of the disease in F2 hybrid mice from spondylitis-susceptible BALB/c and a virtually spondylitis-resistant DBA/2 cross, and found two major quantitative trait loci (QTL) with significant linkage to genes on mouse chromosomes 2 and 18. The first murine locus is homologous to human chromosomes 5q and 18q, both of which have significant linkage with AS found in British and European kindreds (6, 9, 20). The second locus overlaps with the cluster of IL-1 genes and is homologous to an AS locus described in human studies (8).

Materials and Methods

Animals, Ag, and immunization

Inbred female BALB/c and male DBA/2 mice were purchased from the National Cancer Institute and mated to generate F1 and then F2 offspring. All animal experiments were approved by the Institutional Animal Care and Use Committee of Rush University Medical Center. All mice were

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3 Abbreviations used in this paper: AS, ankylosing spondylitis; IVD, intervertebral disk; PG, proteoglycan; QTL, quantitative trait locus; SPL, spondylitis index; SPLρ, late-onset SPL; LRS, likelihood ratio statistic; PGIS, PG-induced spondylitis; SAA, serum amyloid A; rρ, Spearman’s correlation coefficient; PGIA, PG-induced arthritis.

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maintained in a pathogen-free environment. At 12 wk of age, mice were immunized i.p. with 100 μg of PG (measured as protein) emulsified with 2 mg of dimethyl dioctyl sodium sulfate (DDA) adjuvant in 100 μl of PBS (0.15 M NaCl, 0.01 M potassium phosphate, pH 7.4) as described (21, 22). Mice received total of four injections every 3 wk (days 0, 21, 42, and 63) and were sacrificed 3–4 wk after the fourth PG injection, i.e., on days 84–91 of the experiment.

Clinical and immunological phenotypes

The axial skeleton spanning the spine from the midcervical to distal lumbar region was dissected, fixed in neutral buffered 10% formalin, decalcified, and embedded in paraffin. Spine sections were stained with H&E and individual IVDs scored as described earlier (15). In brief, enthesitis, inflammatory cell accumulation around the IVD (discitis), and/or infiltration of the annulus fibrosus with or without evident tissue damage was recorded as severity score 1; massive but <50% resorption/erosion of the IVD received a score of 2; nearly complete resorption (>50%) of the IVD was recorded as score 3; and cartilaginous/bony ankylosis was given a score of 4. An average of 18 IVDs per mouse was scored. Finally, a spondylitis index (SPI) for each animal was calculated by dividing the sum of scores (all IVD scores) with the number of IVDs examined histologically. In addition, a specific index for mice exhibiting late onset of spondylitis (SPILS) was also calculated. A mouse was assigned SPILS”1” if at least one of the IVDs had a mild severity score of 1. Otherwise a mouse with progressive spondylitis was assigned SPILS”0”, even if IVDs had a more advanced form of the disease indicated by severity scores 2, 3, or 4.

Ag (PG)-specific T cell proliferation was measured by [3H]thymidine incorporation in response to in vitro PG treatment as described (22). Ag-induced cytokine production (IL-2, IFN-γ, IL-4, and TNF-α), and serum levels of Abs, amyloid A, and cytokines (IL-1, IL-4, IL-6, and TNF-α) were determined using ELISA as described previously (21–23).

Genomic markers

Markers were selected for detectable simple sequence length polymorphisms between the parent BALB/c and DBA/2 strains from the mouse genome database (www.informatics.jax.org) or alternatively were designed using primers flanking regions of short, usually <100 bp, tandem repeats in the mouse genome. Differences in length between PCR fragments of BALB/c and DBA/2 alleles were >3%. Polymorphism between the strains was detected in 3.5% high resolution Agapone agarose gel (National Diagnostics) upon staining with ethidium bromide and UV illumination. All 20 mouse chromosomes, except chromosome Y, were covered with a total of 224 polymorphic markers at an average spacing of 6.2 cM (10.8 Mbp).

Results

Clinical and immunological parameters of spondylitis in parental BALB/c and DBA/2 strains

Upon systemic immunization with cartilage Pg, BALB/c mice develop spondylitis with an incidence of 61.5% (Table I and Fig. 1). Using the same immunization protocol, DBA/2 mice demonstrated

| Table I. Comparison of clinical and immunological parameters between BALB/c and DBA/2 progenitor mouse strains upon systemic immunization with PG for induction of PGIS* |
|---------------------------------|-----------------|-----------------|-----------------|
| **Clinical traits**             | Units           | BALB/c          | DBA/2           | p Value        |
| PgIS incidence                  | %               | 61.5            | 4.0             | 0.00000****    |
| SPI                             | arb             | 0.364 ± 0.196   | 0.016 ± 0.012   | 0.0071**       |
| SPI*                           | arb             | 0.429 ± 0.067   | 0.220 ± 0.057   | 0.0120*        |
| Pg-specific cytokine production in vitro | pg/ml | 756.0 ± 104.8   | 228.4 ± 46.3    | 0.0002***      |
| IL-4                            | pg/ml           | 3891.5 ± 726.7  | 524.8 ± 224.0   | 0.0003***      |
| IFN-γ                           | pg/ml           | 256.2 ± 40.0    | 230.0 ± 54.9    | 0.7045         |
| TNF-α                           | pg/ml           | 1290.7 ± 251.7  | 681.3 ± 217.0   | 0.0489*        |
| Serum cytokines                 | pg/ml           | 9.0 ± 0.7       | 10.4 ± 3.2      | 0.6650         |
| IL-1β                            | pg/ml           | 57.8 ± 12.1     | 50.1 ± 11.9     | 0.0130*        |
| IL-4                            | pg/ml           | 1585.1 ± 345.1  | 1278.0 ± 783.2  | 0.7257         |
| TNF-α                           | pg/ml           | 66.0 ± 13.6     | 289.4 ± 71.9    | 0.0127*        |
| Hetero-IgG1                      | pg/ml           | 6.3 ± 1.0       | 5.6 ± 2.3       | 0.7919         |
| Hetero-IgG2a                     | pg/ml           | 6.1 ± 1.1       | 17.4 ± 3.4      | 0.00086***     |
| Auto-IgG1                       | pg/ml           | 326.0 ± 71.8    | 100.3 ± 40.6    | 0.0100**       |

*Data represent mean values ± SEM. Nonpaired two-sample t test for equality of means was applied for immunological traits; Mann-Whitney U test was used to estimate the differences between BALB/c and DBA/2 in clinical traits. Spondylitis indices SPI and SPI* were measured in arbitrary units (arb). Statistically significant differences are indicated by asterisks: *p < 0.05; **p < 0.01; ***p < 0.0003; ****, p < 8.7 × 10^-16. Detailed description of clinical and immunological parameters is in Materials and Methods.

Genome screening and statistical analysis

Genomic DNA was isolated from mouse kidney using protease K and sodium laurel sulfate (23, 24). DNA was genotyped with simple sequence length polymorphic markers (MWG Biotech) using conventional PCR and gel-electrophoresis as described previously (23, 24). Initial linkage map was generated with Map Manager QTX (25) using the Kosambi mapping function. The order of markers was further adjusted using the “ripple” command and then confirmed according to physical positions of oligonucleotide primers in the National Center for Biotechnology Information mouse genome assembly (http://www.ensembl.org/Mus_musculus/) and the Celera Discovery System genome database (www.celeradiscoverysystem.com/). Single marker effect was estimated using marker regression in Map Manager QTX. For traits that demonstrated association with genomic markers stronger than χ² > 10 (p < 0.01), both simple and complex interval mappings were performed on the entire genome using Windows QTL Cartographer (26). Experiment-specific empirical thresholds for likelihood ratio statistic (LRS) were established for each trait with a permutation test (n = 2000, 1 cM walk speed) according to an algorithm proposed by Churchill and Doerge (27) and implemented in Map Manager QTX and Windows QTL Cartographer (25, 26). Levels for genome-wide highly significant (α < 0.001) and significant (α < 0.05) linkage were used (28). For a suggestive linkage, we used a p < 0.05 chromosome-wise significance level (α < 0.63), which corresponds to one false-positive QTL for the entire genome (28).

Statistical analysis was performed using the SPSS statistical software package. As spondylitis indices demonstrated nonparametric distribution in the F2 hybrid population, we used the Mann-Whitney U test to examine differences between groups and the Spearman’s correlation coefficient (r,) to evaluate biases between traits. χ² statistics and Kruskal-Wallis H tests were used to determine the difference between distributions of traits in the genotypes. The two-sample Student’s t test was used for comparison of means of two groups, when data showed normal distribution. The significance level was set at p < 0.05.
much lower susceptibility (4%) with notably less severe IVD damage than BALB/c mice, and spine ankylosis was never detected in the DBA/2 strain (19).

The major immunological characteristics were measured in progenitor mice and correlated with disease susceptibility (Table I). The DBA/2 strain had almost three times higher serum levels of IgS as compared with BALB/c mice (17.8 vs 6.5 mg total Ab per milliliter of serum with IgG1 being the major Ig isotype in both strains). The serum level of IgG2a isotype showed an opposite distribution between the strains: it was three times more abundant in BALB/c. Based on the IgG1/IgG2a Ig isotype ratio, both BALB/c and DBA/2 strains demonstrated a Th2-type immune response; however, in PG-immunized DBA/2 mice, the Th2-type response was more prominent. Interstrain differences found in histological scores were highly correlated with the elevated concentration of proinflammatory cytokines IL-1β and IL-6 in sera of spondylitis-susceptible BALB/c mice, although serum levels of IL-4 and TNF-α were comparable.

Ag (PG)-stimulated lymphocytes of BALB/c mice demonstrated significantly higher production of IFN-γ and IL-4 when compared with lymphocytes from DBA/2, and the IFN-γ/IL-4 ratio was 2.2-times higher in BALB/c (5.1) than in DBA/2 mice (2.3). This observation also indicates that BALB/c mice with PG-induced spondylitis (PGIS) exhibit a more significant shift to Th1 dominance, whereas the virtually resistant DBA/2 strain does not.

Correlations between clinical and immunological traits in F₁ and F₂ hybrid populations

The incidence of spondylitis in F₁ hybrids of BALB/c and DBA/2 mice was 35.5%, approximately the median value between the progenitor strains, although the severity of the disease was relatively low (SPI 0.16) (Fig. 1). Unexpectedly, the incidence of spondylitis in F₂ hybrid mice was the same as in the susceptible parent BALB/c strain with a severity score nearly twice higher than in BALB/c mice (Fig. 1).

Because mice of the F₁ generation are genetically homogeneous, only nongenetic environmental factors might be responsible for trait variance. In contrast, F₂ hybrid mice are genetically heterogeneous and both genetic and environmental factors might be in action. Thus, the correlation between spondylitis and immunological parameters in F₁ and F₂ populations must come from different sources. One of the best examples is the SPI, which very tightly correlated with SPI₂ in the F₁ population (r = 0.97), but this correlation was notably weaker in F₂ hybrid mice (r = 0.54) because the heterozygous combination of genes differently affected the SPI and SPI₂ in (BALB/c × DBA/2) F₂ hybrids (Table II).

To determine the most important immunological parameters that might be associated with spondylitis in F₁ and F₂ hybrid populations, we calculated correlation coefficient r (Table II). The strongest positive correlation of PGIS was found with serum concentrations of amyloid A (SAA; an acute phase protein in mice) and IL-6, r = 0.28 and 0.43, respectively (Table II), which is consistent with an even stronger correlation between SAA and IL-6 (r = 0.74). Spondylitis correlated positively with Ab production (both for IgG1 and IgG2a isotypes) in the F₂ hybrid population. Surprisingly, spondylitis correlated negatively with all measured T cell responses (T cell proliferation, IFN-γ, IL-4, and TNF-α production) but only in the F₂ mice (Table II). There was no correlation between spondylitis and Ag-specific T cell responses (or the serum levels of most Abs) in homozygous F₁ hybrid mice (Table II). In contrast, these correlations are strong in the segregating F₂ population, suggesting that spondylitis is controlled by immune response-related genes and/or allele combinations in this animal model. In summary, enhanced serum levels of IL-6, SAA, and Ab, together with decreased production of IFN-γ, IL-4, and TNF-α by PG-stimulated T cells, could be considered the strongest predictors of spondylitis in this MHC-matched cross of PGIS-susceptible and resistant mice.

**Linkage analysis for spondylitis in (BALB/c × DBA/2) F₂ hybrids**

To map non-MHC spondylitis-susceptibility genes, we used a BALB/c × DBA/2 cross, where both progenitor strains carry the same H-2d haplotype, thus deliberately excluding the effects of the MHC genes from linkage analysis. To perform an effective scan for murine genes regulating SPI and SPI₂ clinical traits, F₂ hybrid male and female mice were immunized with PG, scored for spondylitis, and a set of immune-related parameters was measured. Accordingly, all mice were genotyped for the entire mouse genome (20 chromosomes) with 224 markers. Interval mapping and single marker effect analysis indicated the presence of a very limited number of chromosome loci controlling spondylitis (SPI, SPI₂), serum level of Ab (IgG2a isotype to mouse PG), and IL-6 in the sera of F₂ mice, whereas other traits did not show significant linkage (Fig. 2).

The major genetic locus controlling spondylitis was identified on the telomeric part of chromosome 18 (Pgis1). The LRS reached a value of 31, which exceeds the highly significant cut-off level introduced by Lander and Kruglyak (28) and empirically established a highly significant threshold at α < 0.001 (Fig. 3A). Composite interval mapping (Windows QTL Cartographer, standard model 6 with control of five markers inside of a 10 cM window) confirmed the QTL peak position for markers D18Mit51 and D18Mit142 (Fig. 3A).

The second major spondylitis QTL was identified on chromosome 2 (Pgis2). This locus, however, was fairly vague, when simple interval mapping was used, and occupied up to one-third of the chromosome (Fig. 3B). Using composite interval mapping, we found the peak for this QTL (LRS 16.9, α < 0.05) located near marker D2Mit241 (Fig. 3B). When the integrated effects of each chromosome upon spondylitis susceptibility was calculated, chromosomes 2 and 18 appeared to jointly control 40.5% of the entire SPI trait variance in the F₂ population (Fig. 2).
The additional spondylitis phenotype index, SPI$_{LS}$, was introduced in this study. This is a binary index with score “1” for mice with late onset of spondylitis, and it was tightly correlated with PGIS severity. Despite the high correlation between SPI and the incidence rate of spondylitis, and it was tightly correlated with low Pgis2 expression in the F2 population, which might come either from the parental BALB/c strain. For both BALB/c and DBA/2 strains, we calculated the average SPI separately for BALB/c homozygous (PgisB$^H$), DBA/2 homozygous (PgisB$^D$), and BALB/c-DBA/2 heterozygous mice (PgisB$^H^D$), and for each QTL (Fig. 3F). Unexpectedly, we have found that F2 mice, which were homozygous for the DBA/2 allele of Pgis1 (Pgis1D), developed spondylitis at a significantly higher incidence and severity rate than BALB/c homozygous animals (PgisB$^H$ vs PgisB$^D$, $p < 0.000003$), which suggested that the major disease susceptibility allele was derived from a resistant DBA/2 strain. Another QTL, Pgis2 on chromosome 2, showed “normal” phenotype-genotype relationship, and mice with BALB/c homozygosity for this region were significantly more spondylitis-susceptible than F2 hybrid mice with DBA/2 homozygosity (Pgis2$^H$ vs Pgis2$^D$, $p < 0.0005$), thus indicating that the disease allele was derived from the spondylitis-susceptible BALB/c strain. For both Pgis1 and Pgis2 QTLs, the BALB/c alleles were dominant over the DBA/2 allele, since BALB/c-DBA/2 heterozygous mice had as high SPI as BALB/c homozygous animals (Fig. 3F).

Thus, in F2 hybrid mice the highest susceptibility to spondylitis was observed in DBA/2 homozygous animals (Pgis1D$^D$); that seems to contradict the virtual resistance of the parental DBA/2 mice having the same genotype of the Pgis1 locus (Table I and Fig. 1). As we found only two major loci controlling spondylitis in the BALB/c × DBA/2 cross, to explain the silence of the Pgis1D$^D$ allele in the parental DBA/2 strain, we examined the influence of one locus to the other. The SPI and incidence of the disease were calculated based on the hypothesis of cooperation between Pgis1 and Pgis2 loci; average values for phenotypes were calculated separately for each of the nine allele combinations (Figs. 4). When the effects of Pgis1 genotypes upon clinical traits were analyzed independently of the Pgis2 locus, Pgis1D$^D$ homozygous mice were most spondylitis-susceptible, PgisB$^H$ homozygous mice demonstrated the lowest susceptibility to the disease, and PgisB$^H$ heterozygous animals were in-between (Fig. 3F). Surprisingly, the relationship between Pgis1 alleles was found critically dependent upon the genetic composition of the Pgis2 locus. It seemed that at

### Table II. Cross-correlation of clinical and immunological traits in the genetically homogenous F$_1$ and the segregating F$_2$ hybrid populations of the BALB/c × DBA/2 cross upon immunization with PG$^a$

<table>
<thead>
<tr>
<th>Serum Ab</th>
<th>SPI</th>
<th>Auto-IgG1</th>
<th>Auto-IgG2a</th>
<th>Hetero-IgG1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hetero-IgG2a</td>
<td>0.49/0.17</td>
<td>0.37/0.26</td>
<td>0.50/0.50</td>
<td>(0.35)/0.27</td>
</tr>
<tr>
<td>Hetero-IgG1</td>
<td>(0.35)/0.18</td>
<td>0.87/0.59</td>
<td>(0.05)/0.16</td>
<td></td>
</tr>
<tr>
<td>Auto-IgG2a</td>
<td>(0.09)/(0.09)</td>
<td>(0.28)/0.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Auto-IgG1</td>
<td>0.37/0.23</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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*a* Spearmans’s correlation coefficient ($r_s$) for F$_2$ hybrids is shown first, and $r_s$ for F$_2$ hybrids is presented after the backslash. Insignificant correlations are placed in parentheses, whereas significant correlations ($p < 0.05$) are shown in bold. Estimations for cross-correlations between clinical and immunological traits are based on the entire $F_1$ ($n = 31$) and $F_2$ ($n = 223$) populations. nd, No data available. See Table I and Materials and Methods for the description of immunological traits.
At least one copy of the \( Pgis2 \) of BALB/c origin (\( Pgis2^B \)) was necessary for the high penetrance of the \( Pgis1^D \) allele and its function as a spondylitis-permissive gene (Fig. 4). Thus, mice bearing the \( Pgis1^D \) allele were the most spondylitic (average SPI/H11005 0.96) and mice with the \( Pgis1^B \) allele were the least affected (SPI/H11005 0.13) (Fig. 4, block A). However, the phenotypic difference between \( Pgis1^D \) and \( Pgis1^B \) allele-carrying mice vanished on a pure \( Pgis2^D \) genetic background (Fig. 4, block C). Thus, the spondylitis-promoting \( Pgis1^D \) allele needs the \( Pgis2^B \) allele for the complete trait penetrance and full action. F2 hybrid mice that carry the most malicious \( Pgis1^D-Pgis2^B \) allele combination (Fig. 4, the bar marked with black arrow) demonstrated the highest spondylitis severity and 100% incidence of the disease, further confirming the importance of both loci for disease development.

**Discussion**

In this study, we present for the first time a complete genome scan for genetic loci controlling spondylitis susceptibility in mice. We determined the genetic basis of trait variance in a complex phenotype that developed from natural genetic variation instead of artificial modification, i.e., gene deficiency. Despite a number of murine models for spondyloarthropathy established and studied earlier, the genomic basis of disease susceptibility is not known, except for the contribution of \( HLA-B27 \) and a few other genes to disease pathology (11–14, 29, 30).

Earlier we demonstrated that spondylitis susceptibility of mice of different parental strains and genetic crosses was associated with certain H-2-permissive haplotypes, which indicates the leading role of the MHC in murine arthritis and spondylitis (19). The major goal of this study was finding non-MHC genes. We have found only two major genetic loci regulating spondylitis in the BALB/c × DBA/2 cross: the \( Pgis1 \) disease-controlling allele originating from the DBA/2 strain (although the gene remained silent in this strain), and the \( Pgis2 \) allele which is derived from the BALB/c strain. Obviously, the combined effects of these two loci/genes resulted in high disease incidence in BALB/c × DBA/2 F1 hybrids (35.5%), and even higher susceptibility in F2 hybrid mice (61.7%) when two spondylitis-promoting alleles of two genes supplemented each other. Similarly, disease severity, which was defined only in SPI-positive mice, was even higher in F2 hybrids than in BALB/c progenitor mice (Fig. 1) due to a cumulative effect of two permissive genes in the F2 population.

Despite the differences between spondylitis-susceptible and resistant mice in immune function-related traits (T and B cell responses, and serum cytokines), and the known pattern of interaction among SPI-linked genes inside the \( Pgis1 \) and \( Pgis2 \) loci, at this stage of investigation it is not possible to identify spondylitis-contributing primary causative genes within these loci. Comparison of genomic maps of disease-controlling loci, as shown in Fig.
5, in human patients and in mice and syntenic mapping, might aid in identification of gene candidates in future studies.

The major SPI locus in the BALB/c × DBA/2 cross was found on the telomeric region of mouse chromosome 18 (Figs. 2 and 3A). The Pgis1 locus is flanked by D18Mit55 and D18Mit80 markers and occupies the region from 54 Mbp to the chromosome telomere. The Pgis1 locus overlaps with murine QTLs for a number of autoimmune diseases such as murine lupus (Lbw6), Pgia11 and Cia18, experimental allergic encephalomyelitis (Eae25), wound healing (Heal9), and chronic multifocal osteomyelitis (Cmo) (Fig. 5).

The murine Pgis1 locus (54–81 Mbp) is homologous with human chromosomes 5q (segments 110–129 Mbp and 137–150 Mbp) and 18q (segments 17–52 Mbp and 64–76 Mbp) (www.ncbi.nlm.nih.gov/mapview/). These human chromosome segments contain two loci for AS; one on chromosome 18q was found in Oxford pedigree AS(Ox) (9), and the AS(Eu) locus was found in the kindred of European origin on chromosome 5q (10) (Fig. 5).

The list of gene candidates in the locus includes metalloproteinase Adamts19, IL17b, MHC class II-associated invariant chain Cd74, macrophage CSF I receptor Csf1r, and others. The region also contains the Nfatc1 gene encoding calcineurin-dependent NF1 of activated T cells. This gene regulates IL-2 and IL-4 gene transcription, differentiation, proliferation, and activation-induced cell death in T lymphocytes (http://harvester.embl.de/harvester/).

Because we have found a significant correlation between SPI and T cell responses, this gene inside the Pgis1 locus is a plausible candidate as a primary causative factor.

FIGURE 3. Simple and composite interval mapping for all genome-wide significant QTLs (α < 0.05) shown in Fig. 2. LRS thresholds were suggestive (long-dashed line), significant (short-dashed line), or highly significant (dotted line). Composite interval mapping (thick solid curves) confirms and narrows the QTL position given by simple interval mapping (light solid curves). Chromosomes 2 and 18 carry the major QTLs for spondylitis (A–C). Chromosome 11 carries a locus controlling the production of IgG2a to mouse Pg (D), while chromosome 14 contains a locus controlling serum IL-6 level (E). Association of spondylitis with genotypes of QTL peak markers is shown on F. Genotypes: B, BALB/c homozygous, □; D, DBA/2 homozygous, ■; H, heterozygous, □. Student’s t test p values for the difference in SPI between BALB/c- and DBA/2-homozygous mice are presented for each QTL. The dominant spondylitis-susceptibility Pgis2 allele of chromosome 2 was originated from the BALB/c strain, and a recessive spondylitis-susceptible allele, located inside the Pgis1 locus of chromosome 18, was from the DBA/2 strain.
The size of the second major locus, Pgis2, is larger than Pgis1. The Pgis2 locus is flanked by the D2Mit293 and D2Mit156 markers located at 26.4 and 57.0 Mbp, respectively. Simple interval mapping suggested slightly different positions for SPI and SPI_{1,8} on mouse chromosome 2 (Fig. 3, B and C). However, we maintain the hypothesis that a single gene/locus in this region controls disease susceptibility based on the following considerations. First, the mode of inheritance for SPI and SPI_{1,8} peak position markers is identical, and demonstrated a dominant action of the BALB/c allele. Second, we did not succeed in splitting Pgis2 into two loci by varying the number of markers in composite interval mapping. Third, both SPI and SPI_{1,8} clinical traits were closely correlated both in the F1 and F2 hybrids of the BALB/c × DBA/2 cross.

The murine Pgis2 locus (24–57 Mbp) contains numerous autoimmune loci linked to Ag-induced bronchial hyperresponsiveness (Abhr1 and Abhr2), experimental autoimmune gastritis (Aig), collagen-induced arthritis (Cia2 and Cia4), serum transfer-induced arthritis (Stia2), and experimental allergic encephalomyelitis (Eae21) (Fig. 5). Interestingly, Pgis2 also overlaps with QTLs controlling the anatomical development of the skeleton such as femoral cross-sectional area (Fcsa5) and periosteal circumference and femur length (Pcfm1).

Combining data from publicly available online genome resources and analysis of published literature (http://www.ncbi.nlm.nih.gov/mapview/), we found that the Pgis2 locus is syntenic with two segments in the human genome: one located on chromosome 2q between 143 and 161 Mbp, and the second on chromosome 9 between 120 and 137 Mbp. Both intervals on chromosomes 2 and 9 contain QTLs for AS identified in British pedigree (6–9) (Fig. 5).

![FIGURE 4. The effect of the Pgis1 locus upon spondylitis depends on the allelic composition of Pgis2. Pairwise Mann-Whitney U test p values indicate difference in spondylitis severity (SPI) and incidence (%) between mice carrying different combinations of Pgis1 and Pgis2 alleles. Genotype description is the same as in Fig. 3. Table presents mean SPI, SEM, and Pgis1B and Pgis2B backgrounds (blocks A and B).](http://www.jimmunol.org/)

![FIGURE 5. Murine spondylitis loci overlap with chromosome loci controlling different autoimmune diseases in mice and humans. Black vertical boxes show murine spondylitis-linked chromosome segments, where numbers on the right represent the exact QTL location at a distance of million base pairs from the chromosome centromere. Murine QTLs are shown on the left in italics. Abb, bronchial hyperresponsiveness; Pcfm, periosteal circumference and femur length; Fcsa, femoral cross-sectional area; Sbmd, spinial bone mineral density; Stia, serum transfer-induced arthritis; Aig, autoimmune gastritis; Cia, collagen-induced arthritis QTLs 2 and 4; Eae, experimental allergic encephalomyelitis; Heal, wound healing; Lbw, murine lupus; Cmo, chronic multifocal osteomyelitis; Pgia11, Pgi-induced arthritis QTL 11. Gene candidates within these loci include IL-1m, IL-1R antagonist inhibitor; IL-1f5, IL-1f6, IL-1f7, IL-1f8, IL-1 family members 5, 6, 7, and 8; C8g, complement component 8; C5, hemolytic complement 5; Adamts9, a metalloproteinase 19 of the ADAMTS family; IL17b, IL 17 β-chain; Cd74, MHC class II associated invariant chain; Csf1r, macrophage CSF 1 receptor; and Nfatc1, NF 1 of activated T cells. Syntenic with mouse, human chromosome loci are presented as gray bars on the right. The murine Pgis1 locus is homologous to loci in human chromosomes 5 and 18; the Pgis2 locus is syntenic with human chromosomes 2 and 9. Base pair positions are indicated on the right of the human chromosome regions. Gray arrows point to identified human ankylosing spondylitis loci; AS(Ox), loci found in the Oxford pedigree (9); AS(Eu), loci in pedigree of European origin (10).](http://www.jimmunol.org/)
Besides this, linkage with C5 deficiency monogenic syndrome resulting in recurrent local and systemic infections and systemic lupus erythematosus (Mendelian inheritance in man 120900) was also found in the syntenic human loci.

Gene candidates in the locus include complement component 8 \( C8 \) and an IL-1 gene cluster, namely IL-1R antagonist and five members of the IL-1 family. The latter gene cluster is considered the major gene candidate for AS on human chromosome 2 in the Oxford study (8). After initial genome scan and identification of positive linkage on chromosome 2 (7, 9, 31), the Oxford group found significant association between single polymorphisms and IL-1-related haplotypes (8). In our study, IL-1 itself did not show any significant linkage, probably because the difference in serum concentrations of IL-1 was not significantly different in BALB/c and DBA/2 mice (Table I). Any of the gene candidates located between the telomeric end of \( Pgis2 \) locus and the IL-1 cluster might also be functionally associated with the spondylitis phenotype.

The best-known gene proposed to have a crucial role in ankylosing spondylitis in mice is the \( Ank \) gene. Spontaneous mutation(s) on chromosome 15, resulting in progressive ankylosis, was described in 1981 (29) and 1988 (32, 33). Later, the mutation was mapped inside the \( Ank \) gene (30). At present it is believed that mutation of this gene is responsible for cranioetaphyseal dysplasia (34) and crystal deposition arthropathy (Mendelian inheritance in man 118600). In our genome scan we found only a suggestive QTL on mouse chromosome 15, on which the \( Ank \) gene is located (Fig. 2). However, this locus was linked with \( PgIa \) in our earlier studies (35, 36), suggesting that chromosome 15 loci might be partially involved in spondylitis in this murine model.

In addition to the two major SPI loci on chromosomes 2 and 18, and a suggestive locus on chromosome 15, four more loci demonstrated suggestive linkage (Fig. 2). We found QTLs on chromosomes 11, 12, and 19 (LRS 11.0, 12.5 and 12.1, respectively). Suggestive spondylitis QTL on the telomeric part of chromosome 12 contains a cluster of Ig H chain genes (www.ensembl.org/Mus_musculus/). This locus has been found in several crosses produced in our laboratory to control \( PgIa \) or serum concentration of \( IgG2a \), or both traits, although this QTL reached a significant level of linkage with arthritis only in the \( C3H \times C57BL/6 \) cross (37).

Major QTLs for \( IgG2a \) and IL-6 did not fall into either \( Pgis1 \) or \( Pgis2 \) regions on chromosomes 2 and 18. However, there is an overlap between spondylitis and immunological QTLs when a suggestive threshold for linkage is considered. The telomere part of chromosome 11 carries suggestive a spondylitis-susceptibility locus, which coincides with the major peak for \( IgG2a \) (Fig. 2). Additionally, our earlier studies showed two arthritis-controlling QTLs on chromosome 11 (\( Pgis7 \) and \( Pgis28 \)) (35–37), which occupy the same regions as newly discovered QTLs for \( IgG2a \) in this murine cross. Chromosome 14 carried the arthritis \( Pgis29 \) locus which shares a position with IL-6 QTL.

The pivotal role of both T and B cell populations in the induction of murine arthritis and spondylitis was shown in the experiments with adoptive disease transfer (38). Depletion of donor lymphocytes with Abs specific to Th and T suppressor populations, similar to depletion of B cells, prevented the successful disease transfer. Neither anti-Pg Abs nor Pg-specific B cells alone were able to transfer disease, but the cooperation between T and B cell subpopulations is necessary (38–40). Progenitor BALB/c mice upon immunization with Pg produced twice more IL-6 than DBA/2 mice (57.8 pg/ml vs 30.1 pg/ml, \( p < 0.013 \)); and the \( IgG2a \) Ig isotype concentration was three times higher whereas \( IgG1 \) concentration was three times lower in BALB/c than in DBA/2 mice (Table I). The correlations between disease severity and Th2-supported Ag-specific Ig levels were significant in (BALB/c \( \times \) DBA/2) \( F_2 \) hybrid mice as well. The strongest correlations with spondylitis were found for the IL-6 and \( IgG1 \) Ab isotype: the SPI vs IL-6 coefficient of correlation \( r \) was 0.43, and for SPI vs \( IgG1 \) was 0.23. Th1-type associated IFN-\( \gamma \) and TNF-\( \alpha \) production by Pg-stimulated lymphocytes in vitro were found to be negatively correlated with spondylitis in the \( F_2 \) hybrid mice further supporting the leading role of Th2-type cells in disease pathogenesis. Therefore, we conclude that a strong genetic predisposition toward a Th2 response and susceptibility to arthritis and spondylitis is similar in progenitor strains and in genetically mixed \( F_2 \) hybrid mice, and the \( F_2 \) population is modeling similar pathology as seen in the parental BALB/c strain.

Colocalization of QTLs for clinical and immunological traits, as was demonstrated for chromosomes 11 and 12, is a powerful approach to the effective mapping of spondylitis-susceptibility genes and better understanding of the involvement of these loci, thus shedding light upon the mechanisms of spondylitis.

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References


