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A Genome Scan Using a Novel Genetic Cross Identifies New Susceptibility Loci and Traits in a Mouse Model of Rheumatoid Arthritis

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Proteoglycan-induced arthritis (PGIA) is a murine model for rheumatoid arthritis (RA) both in terms of its pathology and its genetics. PGIA can only be induced in susceptible mouse strains and their F2 progeny. Using the F2 hybrids resulting from an F1 intercross of a newly identified susceptible (C3H/HeJCr) and an established resistant (C57BL/6) strain of mouse, our goals were to: 1) identify the strain-specific loci that confer PGIA susceptibility, 2) determine whether any pathophysiological parameters could be used as markers that distinguish between nonarthritic and arthritic mice, and 3) analyze the effect of the MHC haplotype on quantitative trait loci (QTL) detection. To identify QTLs, we performed a genome scan on the F2 hybrids. For pathophysiological analyses, we measured pro- and antiinflammatory cytokines such as IL-1, IL-6, IFN-γ, IL-4, IL-10, IL-12, Ag-specific T cell proliferation and IL-2 production, serum IgG1 and IgG2 levels of both auto- and heteroantibodies, and soluble CD44. We have identified four new PGIA-linked QTLs (Pgia13 through Pgia16) and confirmed two (Pgia5, Pgia10) from our previous study. All new MHC-independent QTLs were associated with either disease onset or severity. Comprehensive statistical analysis demonstrated that while soluble CD44, IL-6, and IgG1 vs IgG2 heteroantibody levels differed significantly between the arthritic and nonarthritic groups, only Ab-related parameters colocalized with the QTLs. Importantly, the mixed haplotype (H-2\(d\) and H-2\(k\)) of the C3H × C57BL/6 F2 intercross reduced the detection of several previously identified QTLs to suggestive levels, indicating a masking effect of unmatched MHCs. 

Rheumatoid arthritis (RA) is a complex disease that affects approximately 1% of the human population. A strong association with the MHC is the most important known genetic predisposition factor for RA. However, the MHC alone is insufficient for disease induction. While linkage of RA to HLA has been repeatedly confirmed, several possible linkages outside the MHC were noted in affected sib pair studies (1–4). The complex and polygenic nature of autoimmune diseases makes genetic studies extremely difficult, especially in a genetically heterogeneous human population. Consequently, few studies have targeted the human genome for exploration of non-MHC-linked genes in RA (1–4) or ankylosing spondylitis (5, 6).

To investigate loci associated with RA, several studies have employed animal models, which have the advantage of a controlled environment and known genetic background. To date, disease-associated loci were identified in animal models for arthritis induced by adjuvant (7) or oil (8), pristane (9), type II collagen (10–14), and proteoglycan (15). Together, these studies have identified a large number of loci associated with clinical symptoms of arthritis, thus illustrating the underlying complexity of autoimmune diseases. Many of these quantitative trait loci (QTL) colocalize to homologous chromosomal regions, suggesting common genetic components (16–19). Presumably, certain genes associated with these loci will correspond to genes involved in RA susceptibility (1–4). While these studies have helped define the genetic relatedness and similarities of the available models of RA, none have successfully narrowed the genetic interval of any QTL to the point in which positional cloning can be employed. Thus, the central problem of the identification of the disease-responsible genes remains. The use of different genetic crosses, increasingly dense genetic maps, and congenic strains as well as the completion of the human and mouse genome projects will most likely make these goals a reality.

Proteoglycan (aggrecan)-induced arthritis (PGIA) is an autoimmune murine model with 100% incidence in the BALB/c strain (20–23). To date, several inbred mouse strains have been tested, but only BALB/c mice were susceptible to PGIA. Recently, we reported that F2 hybrids of BALB/c and DBA/2 mice (both contain the H-2\(d\) haplotype) are resistant to PGIA, while 15% of the F2 hybrids of this intercross were susceptible (15). We sought to identify non-MHC-related loci linked to PGIA through the use of an exhaustive genome-wide scan of BALB/c × DBA/2 F2 hybrids and identified 12 QTLs linked to PGIA. While there was homology between many of the QTLs when compared with other studies (4, 6, 10, 24–28), some seemed unique to PGIA (15).

Our aims in this study were multifold. First, we wanted to evaluate PGIA in different genetic backgrounds. To this end, we initiated a pilot study using MHC-unmatched F2 hybrids of BALB/c × C57BL/6 (H-2\(a\) and H-2\(b\), respectively) and BALB/c × C3H.
(H-2\(^k\)) intercrosses (29). When we found an inordinate number of arthritic F\(_2\) individuals in the BALB/c × C3H cross, we retrospectively tested the parental C3H line and found it to be susceptible to PGIA (65), thus indicating a second PGIA-susceptible MHC haplotype: H-2\(^d\). This finding compelled us to perform a large scale set of experiments using MHC-unmatched C3H × C57BL/6 F\(_2\) hybrids, thus permitting for the first time analysis of PGIA in a background completely devoid of both BALB/c content and the H-2\(^d\) haplotype. We hypothesized that genetic analysis of a second susceptible mouse strain of the same autoimmune model might be helpful for the identification of both strain-specific and shared susceptibility loci. Such loci may correspond with loci common to multiple autoimmune models.

Secondly, since C3H and C57BL/6 mice have different MHC haplotypes (H-2\(^k\) and H-2\(^b\), respectively), we could evaluate the effect of unmatched MHC haplotypes on both disease susceptibility and the identification of non-MHC loci. We hypothesized that in unmatched studies, the MHC from the susceptible background may have a masking influence on other loci, since the MHC typically follows an additive inheritance pattern with a large individual effect, while other QTLs more typically follow recessive inheritance patterns with minimal individual effects. This hypothesis is supported by a general observation that in various autoimmune models in which the MHC was unmatched, only a few individual QTLs have been identified. Taken in total, however, these independent studies have reported a large number of loci (7–14, 24–28). In contrast, in our previous study, which made use of the matched H-2\(^d\) haplotype, 12 PGIA-linked non-MHC loci were identified (15). This observation is further supported by analysis of human patients with RA (1–4), ankylosing spondylitis (5, 6), insulin-dependent diabetes mellitus (30–42), or systemic lupus erythematosus (43–47) in human populations, in which few loci other than the MHC were found to demonstrate significant linkage to the disease traits.

Finally, we recently demonstrated that while BALB/c mice are predisposed to a Th2-type immune response, PGIA is associated with a shift toward Th1 dominance (48, 49). To investigate the immunological pathways more thoroughly, we measured various inflammatory and immunological parameters related to immune responses and/or arthritis in C3H × C57BL/6 F\(_2\) hybrids in PGIA. If any statistically significant linkage to the disease state could be established, it would provide us both additional pathophysiological markers and more information on the immunological pathways involved in arthritis development.

Materials and Methods

Animals, Ag, immunization, and assessment of arthritis

BALB/c female mice (K51; Charles River Laboratories, Kingston, NY) were mated with C57BL/6 (National Cancer Institute, Raleigh colony) or C3H (National Cancer Institute, C3H/HeJCr Kingston colony) males, and the resulting F\(_1\) offspring were intercrossed to generate F\(_2\) hybrids. Parent BALB/c mice from the Kingston colony were selected to achieve 100% incidence in the parental line (23). Alternatively, C3H/HeJCr female mice were mated with C57BL/6 males, and the resulting F\(_1\) offspring were intercrossed to generate F\(_2\) hybrids. Parent C57BL/6 male mice were obtained from Jackson Laboratory (Bar Harbor, ME), whereas the second and third boosters contained Ag in IFA. Arthritic mice were sacrificed within 1 wk of arthritis onset. Those mice that did not develop arthritis within 5 wk after the fourth injection were boost once more between days 84–90 and sacrificed 4 wk later. Arthritis was assessed daily, and the maximum paw score (0–4) of each animal was used to generate a severity (0–16) arthritis score (22, 23). In addition, a special onset score (0–5) has been established for this study. A maximum score of 5 was given for earliest onset (day 28 or earlier). On each subsequent day, as animals developed arthritis, scores were reduced by a value of 0.1. For example, while an animal that developed arthritis on day 28 would have an onset score of 5, an animal that developed arthritis on day 38 would have a score of 4. In addition, all clinically questionable joints/paws (score <2) were scored by histology. The total score was calculated by multiplying the severity score by the onset score.

Measurement of Abs and T cell response

Abs to the immunizing human and mouse (self) cartilage proteoglycans were determined by ELISA (20, 22). Maximum 96-well plates (Nunc, Hanover Park, IL) were coated with either chondroitinase ABC-digested human (for heteroantibodies) or mouse (for autoantibodies) cartilage proteoglycans (0.1 μg Ag protein per well of each). Proteoglycan-specific Abs were determined in serial dilutions of immune sera (1:500–1:2,500) using peroxidase-conjugated goat anti-mouse IgGs, IgG1, and IgG2a (Zymed Laboratories, San Francisco, CA) second Abs, and then expressed in arbitrary units. These units were calculated in each case as a ratio of the serum dilution of the experimental sample relative to the dilution of the standard (pooled arthritic serum; n = 62) at the median of the maximum and minimum absorbance levels measured on the same plate.

Ag-specific T cell responses (IL-2 production) were measured in quadruplicate samples of spleen cells (3 × 10\(^5\) cells/well) cultured in the presence of 100 μg PG protein/ml. IL-2 was measured in supernatants harvested on day 2 by the proliferation of the IL-2-dependent cell line, cytotoxic T lymphocyte assay (CTL). Ag-specific T cell proliferation was assessed on day 5 by the incorporation of \(^3\)H[thymidine (22, 50). In both cases, the Ag-specific response was expressed as a stimulation index, which is a ratio of incorporated \(^3\)H[thymidine (cpm) in Ag-stimulated cultures relative to cpm in nonstimulated cultures (22, 23). Proteoglycan-specific IFN-γ and IL-4 production by T cells in identical culture conditions, as described for CTL assay, was determined in 4-day-old conditioned media (2.5 × 10\(^5\) mononuclear cells/ml) using capture ELISAs from R&D Systems (Minneapolis, MN). Serum IL-1 was determined by bioassay using D10S cells, as described (51). Soluble CD44 was determined by a capture ELISA developed in our laboratory (52). Serum IL-6, IL-10, and IL-12 levels were determined by capture ELISAs (R&D Systems, or Pharmingen, San Diego, CA).

Genome screening

Genomic DNA was isolated from 48 BALB/c × C57BL/6, 48 BALB/c × C3H, and 190 C3H × C57BL/6 F\(_2\) hybrids and subjected to an exhaustive genome-wide screen with an average of 139 simple sequence-length polymorphic (SSLP) markers (SSLP) markers (MWG Biotech, High Point, NC), as described previously (15). The average spacing of the markers was 14 cM, with 91% of the genome covered within 20 cM of a marker. The list of markers used is available upon request. Genetic linkage maps of the SSLP markers were constructed with MapMaker/EXP v3.0b (53) using error detection. SSLP markers identified to contain unlikely recombination events were reanalyzed in the laboratory. The linkage maps and marker order were then confirmed using the Jackson web resource: http://www.informatics.jax.org/searches/marker_form.shtml. Linkage of potential QTLs to SSLP markers was determined both with MapMarker/QTL v1.9b (54) and QTIL Cartographer v1.13 (55, 56). Logarithm of the odds (LOD) scores of 3.9 or greater was considered significant, as suggested (57).

Statistical analysis

Statistical analysis was performed using SPSS v7.5 (SPSS, Chicago, IL). The Mann-Whitney and Wilcoxon tests were used for intergroup comparisons. For determination of correlation coefficients, Spearman’s p test was used. To determine statistically significant linkage to PGIA, the immunological parameters were compared in the arthritic and nonarthritic groups. Significance was set at p < 0.05. For comparisons between MHC haplotypes and disease incidence, the χ\(^2\) test was used.

Results

PGIA susceptibility in different genetic backgrounds

To confirm PGIA susceptibility loci from our previous study, identify new loci, and assess the effect of different genetic backgrounds on PGIA, we initiated a small pilot study using two separate intercrosses (n = 48 for each) consisting of F\(_2\) hybrids from BALB/c × C57BL/6 and BALB/c × C3H intercrosses. As we were interested in monitoring the effect of the MHC locus, we selected the resistant C57BL/6 and the C3H strains (15, 21) specifically because they differed from BALB/c mice (H-2\(^d\) haplotype) at the
Characterization of PGIA in C3H × C57BL/6 F2 hybrids
To further investigate the genetics of PGIA in C3H/HeJCr (henceforth C3H) mice, we initiated a set of experiments using C3H × C57BL/6 F2 hybrids (n = 190). Since the C3H and C57BL/6 strains differ in haplotype, we expected to confirm MHC involvement. As we were interested in monitoring the disease-related activation of the immune system in PGIA, we assayed both general inflammatory and Ag-specific immune responses. All intercross mice were immunized by the same standard protocol as described above and scored from week 12 for clinical appearance of arthritis. Of the 190 mice, 77 (41%) developed arthritis after an average of 2.8 (SD) wk with an average severity score of 4.9. In contrast, the BALB/c and C3H strains (48, 49) none of the Th1/Th2-specific cytokines were significantly different between the arthritic and nonarthritic groups, whereas serum levels of IL-1 were highly correlated between arthritic and nonarthritic mice (Table I and Fig. 1). Surprisingly, some general markers of inflammation, such as IL-6 and soluble CD44 levels, were significantly lower in arthritic than in nonarthritic groups, whereas serum levels of IL-1 were highly comparable. Another unexpected result was that in contrast to the Th1 dominance found in arthritic individuals of the parental BALB/c and C3H strains (48, 49) none of the Th1/Th2-specific cytokines were significantly different between the arthritic and nonarthritic groups of any of the F2 hybrid crosses. While there was no difference in autoantibody levels (in any combination) between the two groups, heteroantibody levels were significantly reduced and showed a relative IgG1 isotype dominance in nonarthritic mice (Table I and Fig. 1).

To investigate linkage between any of the measured parameters (Table I), we determined their correlation coefficients in all possible combinations. Those parameters exhibiting significant correlations (p < 0.05 and |r| > 0.3) are shown in Fig. 2. As expected, there was a tight relationship between auto- and heteroantibody production in both the nonarthritic and arthritic groups from all crosses. None of the other parameters showed any correlation in either the BALB/c × C57BL/6) or BALB/c × C3H F2 hybrids. Statistically significant linkage was established in the C3H × C57BL/6 F2 hybrids among several of the different parameters. However, none of the correlations was as striking as the linkage nonarthritic groups.

Table I. Statistical comparisons of pathophysiological markersa

<table>
<thead>
<tr>
<th>Parameters Assayed</th>
<th>Nonarthritic (n = 113)</th>
<th>Arthritic (n = 77)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1</td>
<td>8.7198 ± 0.30</td>
<td>9.1332 ± 0.37</td>
<td>NS</td>
</tr>
<tr>
<td>IL-6</td>
<td>19.614 ± 4.84</td>
<td>7.8545 ± 1.85</td>
<td>&lt;0.0003</td>
</tr>
<tr>
<td>sCD44</td>
<td>6.3569 ± 0.19</td>
<td>5.7938 ± 0.16</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>IL-4</td>
<td>102.85 ± 14.9</td>
<td>64.389 ± 13.9</td>
<td>&lt;0.07</td>
</tr>
<tr>
<td>IL-12</td>
<td>56.851 ± 3.37</td>
<td>47.167 ± 20.9</td>
<td>NS</td>
</tr>
<tr>
<td>Hetero-IgG1/IgG2a</td>
<td>9.5195 ± 13.5</td>
<td>188.89 ± 81.0</td>
<td>NS</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>96.798 ± 15.2</td>
<td>120.43 ± 30.0</td>
<td>NS</td>
</tr>
<tr>
<td>CTLL (IL-2)</td>
<td>2.2713 ± 0.15</td>
<td>2.4442 ± 0.20</td>
<td>NS</td>
</tr>
<tr>
<td>Proliferation</td>
<td>1.5097 ± 0.08</td>
<td>1.6327 ± 0.14</td>
<td>NS</td>
</tr>
<tr>
<td>Autoantibodies</td>
<td>0.5617 ± 0.07</td>
<td>0.6722 ± 0.11</td>
<td>NS</td>
</tr>
<tr>
<td>Heteroantibodies</td>
<td>0.3413 ± 0.05</td>
<td>0.5594 ± 0.10</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Auto-IgG1/IgG2a</td>
<td>5.9066 ± 0.31</td>
<td>5.2842 ± 0.41</td>
<td>NS</td>
</tr>
<tr>
<td>Hetero-IgG1/IgG2a</td>
<td>9.6586 ± 0.64</td>
<td>7.6993 ± 0.64</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>IgG1 hetero/auto</td>
<td>2.9335 ± 0.35</td>
<td>2.1795 ± 0.13</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>IgG2a hetero/auto</td>
<td>1.5097 ± 0.08</td>
<td>1.5942 ± 0.12</td>
<td>NS</td>
</tr>
</tbody>
</table>

a Values of IL-1, IL-4, IL-6, IL-10, and IL-12 are in picograms per milliliter sera or pg/2.5 × 108 nucleated spleen cells (for IFN-γ and IL-4). CTLL (IL-2 production) and proliferation are expressed as stimulation indices. sCD44 values are in micrograms per milliliter. Autoantibodies for native mouse proteoglycan and heteroantibodies for immunizing human cartilage proteoglycan are expressed as arbitrary units (see Materials and Methods). IgG1 and IgG2a refer to the ratios of proteoglycan specific Ab isotypes measured in sera of immunized animals. Values of p < 0.05 (in bold) are considered significant.
between serum IL-1 levels and in vitro Ag-specific IL-2 production by spleen cells measured by the CTLL assay. In this study, the arthritic group showed a significant ($p < 0.001, \sigma = 0.4169$) correlation between serum IL-1 and T cell IL-2 production; no significant relationship was detected in the nonarthritic group (Fig. 2).

**PGIA QTL analysis in C3H × C57BL/6 F$_2$ hybrids**

To identify QTLs genetically linked to PGIA, a genome scan of the 19 autosomes using polymorphic SSLP markers was performed on F$_2$ hybrids from all three crosses. Since PGIA has a nonparametric distribution in F$_2$ hybrids, we used the penetrance (PEN) command from MapMaker/QTL, which assumes 1) a nonparametric distribution of the trait and 2) a binary affected or nonaffected status of the traits tested, as described in our first study (15). The initial scan of the two pilot crosses (BALB/c × C57BL/6 and BALB/c × C3H F$_2$ hybrids) recovered loci from our first study (15), and identified several new potential QTLs (Table II). However, the genome scan of the C3H × C57BL/6 F$_2$ hybrids demonstrated linkage only at the MHC locus. Interestingly, numerous other loci that were significant and named in our previous study (15) were suggestive of linkage here (Table II). To determine the strength of the MHC effect exerted on arthritis in these F$_2$ hybrids, we compared arthritis incidence, onset, severity, and total arthritis score with the number of H-2$^k$ alleles present at the MHC locus (Fig. 3). These data demonstrate that while the H-2$^k$ allele exerts a strong additive

**FIGURE 2.** Correlations of different pathophysiological parameters in arthritic and nonarthritic mice. All laboratory measurements were compared and potential linkage in any combination analyzed. The significance ($p$) and the correlation coefficient ($\sigma$) are given on the right-hand corner of each panel. Arb., Arbitrary.
influence on arthritis in the C3H × C57BL/6 F2 intercross, the H-2d allele exerts a dominant protective effect on disease incidence. In the BALB/c × C57BL/6 pilot study, we found that the H-2d had an additive effect on disease severity, but not on incidence, while in contrast, in the BALB/c × C3H cross, we found no statistically significant difference between the H-2d and H-2k haplotypes on either disease severity or incidence (data not shown).

When the loci recovered in the crosses reported in this work were compared with the loci recovered from the BALB/c × C57BL/6 intercross reported in the original study (15) (Table II), we made an important observation. In F2 intercrosses that involved either matched MHC haplotypes (BALB/c × DBA/2, both H-2d) or two susceptible MHC haplotypes (BALB/c × C3H, H-2d and H-2k), more loci with higher LOD values were recovered than in crosses involving the unmatched H-2k haplotype BALB/c × C57BL/6 and C3H × C57BL/6 F2 intercrosses). This led us to the hypothesis that the MHC may mask the detection of disease affecting loci in crosses involving unmatched MHC haplotypes.

To find the best genetic model for identification of QTLs in an MHC-unmatched cross, we analyzed the C3H × C57BL/6 F2 intercross experiment originated from the C3H background. The genetics of the individual QTLs were all recessive, whereas the MHC (Pgia17) demonstrated additive inheritance. Subsequently, we tried to dissect the arthritis trait by separating it into the subtraits of onset and severity. While the MHC locus showed linkage to both subtraits, Pgia5 and Pgia13 were linked to disease onset, and Pgia10, Pgia14, and Pgia15 were linked to disease severity (Fig. 5). To determine whether any of the pathophysiological parameters (Table I) could be genetically linked to any of the QTLs, we performed a genome scan using that trait information. While the scan identified areas suggestive of linkage for many of the traits (data not shown), only the Ab-related traits showed significant associations with different chromosomal regions. Interestingly, in each case, the QTL associated with PG-specific Ab production demonstrated colocalization with a QTL linked to arthritis (either onset or severity; Fig. 5). That two Ab-related traits were linked to the MHC was not unexpected, as the immune response to Ag is dependent on the MHC. Subsequent analysis demonstrated that these two traits were linked to the H-2d haplotype (data not shown).

**Discussion**

We report in this work a genetic analysis of PGIA focusing on the newly identified susceptible strain C3H/HeJCr, in conjunction with resistant strain C57BL/6. Our goals for this study were to confirm...
previously described PGIA QTLs, identify new QTLs, investigate the effect of genetic background on PGIA, and identify other possible pathophysiological markers characteristic of arthritis. Dating back to 1987, other groups and ourselves have reported that only BALB/c mice are susceptible to PGIA (20, 21, 23, 58–60). However, in the present study, we unexpectedly found greater than 50% incidence of PGIA in BALB/c × C3H F₂ intercross mice. Subsequent analysis confirmed that C3H/HeJCr was indeed a second PGIA-susceptible strain, compelling us to focus on the C3H × C57BL/6 F₂ intercross. It is important to note that while the BALB/c and C3H strains differ at the MHC locus (H-2 d and H-2 k, respectively), they do share a common heritage. The C3H strain originated from a cross between a female Bagg albino (BALB/c strain founder in 1913) and a male DBA in 1920 (61). This common heritage suggests that important non-MHC susceptibility loci were already present in the Bagg albino and some arthritis susceptibility genes were subsequently transmitted to both the BALB/c and C3H strains over 280 generations ago. A dedicated study of PGIA in various C3H colonies using proteoglycan Ags is reported elsewhere.4

The most important goal of this study was to confirm QTLs from our first study using a BALB/c-independent system. Consid-}


gering the use of the C3H mouse as a susceptible strain, it was perhaps not surprising that only two of the five non-MHC loci identified corresponded to previously identified loci (15). How-


ever, we believe that more loci would have been identified if not for the masking influence of the MHC. Many of the loci that were suggestive of linkage (LOD scores between 2 and 3.9) corre-


cponded to loci identified as definitive QTLs in our previous report (Table II) (15). Considering the potential masking influence of the MHC complex, additional studies are needed with MHC-matched susceptibility and resistant strains, as the contribution of the MHC in unmatched studies appears to make identification of other loci difficult.

While none of the predicted immunological parameters tested to date showed statistically significant correlations with PGIA, the finding that the proteoglycan-specific heteroantibody production correlated very well with proteoglycan-specific autoantibody production was expected. We have long known that the presence of autoantibodies was a good predictor of which animals would develop arthritis (20, 21, 58). Despite this observation, we would always find some arthritic individuals with no detectable autoan-


tibodies as well as nonarthritic animals with autoantibodies. PGIA is a T cell-mediated autoimmune disease (22, 23, 58, 62, 63), and proteoglycan-specific Abs alone were unable to transfer the disease to naive syngenic recipients (58). The apparent correlations
between 1) autoantibody production and disease susceptibility and 2) arthritis onset and autoantibody level (21–23) suggest, however, that while autoantibodies per se cannot account for arthritis severity, they can be used as susceptibility markers as they reflect the degree of B cell self-tolerance.

The other statistical differences that we found may provide important starting points for further studies. Perhaps most interesting in the C3H × C57BL/6 F₂ intercross are the drastic differences in IL-6 levels between the arthritic and nonarthritic groups (Fig. 1) and the positive correlation between serum IL-1 levels and in vitro IL-2 production in the arthritic, but not in the nonarthritic groups (Fig. 2). This is different from what we found in another study of ours, in which both IL-1 and IL-6 serum levels of arthritic C3H mice from 10 colonies were significantly higher than in nonarthritic mice. Furthermore, while it has been documented that the onset of PGIA is associated with a shift toward a Th1-type response in BALB/c (48) and C3H/HeJcr parent strains, this observation (based on either Ag-specific IL-4 vs IFN-γ production or Th2-supported IgG1 and Th1-supported IgG2a ratios) was not confirmed in arthritic C3H × C57BL/6 F₂ intercross mice.

In an effort to identify those loci that may be the most important in determining arthritis susceptibility, we searched for homologous regions that were identified in other models or human studies to date. While we have found overlap between some of our QTLs (15) and those reported for other RA model systems, it is important to point out dissimilarities with other model systems. In contrast with collagen-induced arthritis (7, 10–12), adjuvant-induced arthritis (7), and pristane-induced arthritis (9), which all report arthritic individuals in the F₂ generation, PGIA follows a different mode of inheritance, with arthritic individuals recovered only in the F₂ generation of crosses involving susceptible and resistant strains. Despite the contrast in the inheritance pattern of the different models, all of the new non-MHC QTLs identified in this study did show homology with QTLs from other studies. Pgia13 on chromosome 4 showed linkage with Lmb1 in the mouse model for lupus (26). Pgia14 on chromosome 12 demonstrated homology with both Pia3 in pristane-induced arthritis (9) and a QTL at 14q13 linked with RA (4). Pgia15, on chromosome 13, showed linkage with Mica3, a locus affecting collagen-induced arthritis in mice (12). Recently, a study was published on a genome scan in C3H × C57BL/6 F₂ hybrids for QTLs associated with Lyme disease (64). We were excited to find that in addition to the MHC locus, there was a close colocalization of Pgia14 with Bb6, which was associated with IgG and IgM production, and the suggestive locus we found on chromosome 5 showed colocalization with Bb2, which was associated with ankle swelling in the Lyme disease model (64). Pgia5, on chromosome 9, which was identified in our first study (15), was again recovered in this study, and showed colocalization with Bb9, which was associated with IgG production (64). That these three PGIA-linked QTLs colocalized with QTLs identified in a mouse model for Lyme disease suggests that at least in part, common genetic pathways may play roles in RA and Lyme disease. While it is premature to attempt to assign candidate genes to either the QTLs identified in this study or in our previous study, it seems reasonable to focus on those loci that seem to be involved in multiple autoimmune disorders and especially on those linked to RA. Identification of these genes will most likely provide important insights into the genetics of many different autoimmune diseases.

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