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Identification of Susceptibility Loci for Skin Disease in a Murine Psoriasis Model


Psoriasis (OMIM 177900) is a universal inflammatory skin disease whose cause is not entirely clear (1). Different cell types have been suspected to be the primary triggers in the pathogenesis of psoriasis (2). Increasing evidence has led to the current view that psoriasis is a T cell-mediated inflammatory autoimmune disease (3–6). With a population prevalence of ~2%, psoriasis vulgaris is one of the most frequent skin disorders, clinically presenting with thickened erythematous skin covered with silvery scales (7). It appears to result from a combination of genetic and environmental factors. It is frequently inherited between successive generations, but does not follow a classical autosomal mendelian profile. Type I psoriasis has an early onset, before the age of 40, and is suggested to be heritable (8–10). Type II psoriasis is characterized by a late onset and its heritability is not entirely clear. Environmental factors that are likely to influence psoriasis development are streptococcal infections and stress or trauma (Koebner reaction to injury) (11, 12).

To identify genetic factors underlying this skin disease, several linkage analysis studies have been performed. Psoriasis susceptibility (PSORS) loci identified so far are located on chromosomes 6p (PSORS1; Ref. 13 and 14), chromosome 17q (PSORS2; Ref. 15), on 4q (PSORS3 and PSORS9; Refs. 16 and 17), 1q (PSORS4; Ref. 18), 3q (PSORS5; Ref. 19), 19p (PSORS6; Ref. 20), 1p (PSORS7; Ref. 21), and 16q (PSORS8; Ref. 13). Efforts have been made to identify the gene underlying the PSORS1 locus, because this locus has repeatedly been shown to have the highest linkage in several studies. However, the susceptibility gene has not yet been discovered (11). In addition, the PSORS2 and PSORS9 loci have been confirmed in independent studies (22–24). The remaining PSORS loci were found only in single studies and could not be reproduced with other patient groups. Several animal models show a skin phenotype resembling human psoriasis both in terms of the clinical and histological features as well as various aspects of its pathogenesis. These models include the mouse mutations flaky skin (fns; Ref. 25), chronic proliferative dermatitis (cpd; Ref. 26), transgenic HLA-B27 rats (27), mice constitutively expressing stat3 (28), graft-vs-host disease due to differences in the minor histocompatibility Ags (29), epidermal dysregulation of NF-κB-mediated signaling (30), transgenic β1 integrin overexpression in murine skin (31), and transplantation of human psoriasis-affected skin onto SCID mice (32).

Abbreviations used in this paper: PSORS, psoriasis susceptibility; PASI, psoriasis area and severity index; QTL, quantitative trait locus; LOD, logarithm of odds; CD18\(^{88\text{th}}\); CD18 hypomorphic; LODint, LOD support interval; CM, centimorgan; Mb, megabase; AUC, area under the curve.

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CD18hypo mutation is present in C57BL/6J or 129/Sv mice (33), the F1 generation did not develop the psoriasiform skin disease. We earlier reported that CD4+ T cells play a key role in the pathogenesis of the psoriasiform skin disease in this mouse, which was also demonstrated to be dependent on a reduced CD18 gene expression (34). Therefore, this CD18-dependent psoriasis model mimics the autoreactive nature of T cells in psoriasis (34, 35), as do some other models (29, 32). The CD18-dependent psoriasis model mimics the autoreactive nature of T cells in psoriasis (34, 35), as do some other models (29, 32).

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This disease is characterized by erythema, alopecia, crusts, and scaling as well as abnormal keratinocyte proliferation/differentiation, subcorneal microabscesses, and an increased inflammatory infiltrate in the dermis (33). We earlier reported that CD4+ Th1 cells develop a skin disease that closely resembles human psoriasis. This disease is characterized by erythema, alopecia, crusts, and scaling as well as abnormal keratinocyte proliferation/differentiation, subcorneal microabscesses, and an increased inflammatory infiltrate in the dermis (33).

The disease develops in CD18hypo PL/J mice but not when the same CD18hypo mutation is present in C57BL/6J or 129/Sv mice (33, 36). There are no differences in CD18 expression in the three mutant inbred strains carrying the CD18hypo mutation (33). This offers the unique possibility to identify genes increasing susceptibility to disease. The polygenic influence on disease development was previously demonstrated in a backcross between the susceptible CD18hypo PL/J and the resistant CD18hypo C57BL/6J strains ((PL/J × C57BL/6J) × PL/J), where 50% of the mice showed signs of the psoriasiform dermatitis, whereas mice of the (PL/J × C57BL/6J)F1 generation did not develop the psoriasiform skin disease signs (33).

In the present study, we have identified loci determining psoriasis development under the conditions of reduced CD18 expression, using genome-wide linkage analysis of a backcross between the susceptible CD18hypo PL/J and the resistant CD18hypo C57BL/6J strains. A major locus on chromosome 10, distinct from the CD18 gene, was identified. An additional locus telomeric to the CD18 gene was found on chromosome 10. Furthermore, we identified a locus on chromosome 6 contributing to the most severe form of the psoriasiform dermatitis in this cross. An additional locus was located on chromosome 18. We were also able to demonstrate true epistatic interactions between two distinct loci on chromosomes 1 and 4, respectively. The chromosome 4 locus was detected only after allowing for genetic interaction, demonstrating the complex etiology of this psoriasiform skin disease. In conclusion, we have identified, for the first time, loci playing a role in a murine psoriasis model that strongly resembles human psoriasis.

Materials and Methods

Nature of the targeting mutation

Previously, a construct for homologous recombination in the CD18 gene was prepared by screening a murine genomic DNA library using the mouse cDNA clone p17.4 as a probe for CD18 gene (37). A single λ clone containing exons 1–3 was isolated and a construct for homologous recombination was prepared as published earlier (38). The construct was used with the intent to produce an insertion mutation duplicating exons 2 and 3 with interruption of one copy of exon 3 with the neomycin resistance cassette. The insertion mutation resulted in a hypomorphic rather than in a null allele due to low expression of a cryptic promoter in the plasmid construct. Homozygous mutant mice have a 2 or 16% of normal CD18 expression on leukocytes in the resting or activated state, respectively.

Mice

The CD18hypo PL/J mice used in the present study were generated by crossing the CD18hypo mutation, derived from 129/SvEv background (34), to the PL/J inbred strain, followed by four generations of backcrossing to PL/J (N4). Consequently, ~6.25% of the genome still originates from 129/Sv (39). The 129/SvEv-derived fragment surrounding the CD18 gene comprises 71 Mb (36 cM) with the boundary markers D10mit194 to D10mit114. The CD18hypo C57BL/6J mice had been backcrossed to C57BL/6J for 10 generations (The Jackson Laboratory), and we therefore considered them to be of a homogeneous C57BL/6J background (39). The 129/SvEv-derived fragment surrounding the CD18 gene was 70 megabase (Mbp) (35 centimorgan (cM)) with the boundary markers D10mit138 to D10mit233. To generate the backcross, male CD18hypo PL/J mice with a clinical psoriatic phenotype were selected and crossed with female CD18hypo C57BL/6J mice. Female F1 mice were backcrossed to male CD18hypo PL/J. Three hundred and forty-three (PL/J × C57BL/6J)F1 backcross mice were generated. All mice were kept under specific pathogen-free conditions in compliance with the German Law for Welfare of Laboratory Animals.

Genotyping

DNA was prepared from tail biopsies by a alkaline lysis protocol (40). Briefly, tail biopsies were incubated in 50 mM NaOH for 2 h at 95°C, vortexed, and neutralized in 1 M Tris-HCl, pH 8.0. After centrifugation the supernatant was used for PCR. This was performed with 10 ng of DNA in a reaction volume of 10 μl containing the following: 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM MgCl2, 0.3 μM forward and reverse primers (MWG-Biotech and Applied Biosystems), 100 μM dNTPs (Amersham Biosciences), and 0.25 U of TaqDNA polymerase (Amersham Biosciences). Forward primers were labeled with fluorescent dyes.

PCR was performed in a thermal cycler (MJ Research) under the following amplification conditions: denaturation at 94°C for 2.5 min, annealing at 56°C for 45 s, polymerization at 72°C for 1 min, followed by 30 cycles of 94°C for 30 s, 56°C for 45 s, and 72°C for 1 min. The final cycle ended by elongation at 72°C for 6 min. PCR products were analyzed on a MegaBACE 1000 (Amersham Biosciences), according to the manufacturer’s protocol.

Selection of markers for genome screen

The parental CD18hypo PL/J and CD18hypo C57BL/6J strains were screened using 186 microsatellite markers across the genome. For 19 of these markers, alleles from 129/Sv were detected in the CD18hypo PL/J parental strain. No 129/Sv alleles were detected in the CD18hypo C57BL/6J parental strain. Eighty-three markers with an average marker distance of 17 cm were selected for genome-wide screen (data not shown). Of these markers, 16 identified 129/Sv fragments in the CD18hypo PL/J parental strains. The genome-wide screen was performed on 53 mice with the most severe disease and 41 unaffected mice.

For the complementary screen of loci identified in the genome-wide screen, an additional 12 markers were genotyped (data not shown). In total, 343 backcross mice were genotyped in the complementary screen.

Evaluation of the psoriasiform skin disease

The severity of clinical signs in backcross mice was evaluated every 2 wk for up to 20 mo using an adapted psoriasis area and severity index (PSAI) score as used in assessment of the severity of human psoriasis elsewhere (41). For CD18hypo mice, the PSAI score was modified accordingly: 0, no signs; 1, erythema of the ears, scaling of the tail; 2, hair loss in addition to the signs for 1; 3, hair loss, isolated or widespread slight scaling; 4, moderate scaling on a large area of the body or strong scaling at a few, small or large regions.

Because distinct components of the disease are influenced by different gene loci, for each backcross mouse the following phenotypes were determined: 1) maxscore (maximal degree of disease severity) is the highest adapted PASI score observed for each individual mouse; 2) onset is the week when first signs of the disease appeared; 3) susceptibility for the disease was considered as positive, if the adapted PSAI score was 1 or higher; 4) area under the curve (AUC) (42), as a measurement of the overall severity of the disease, is the accumulated sum of the adapted PSAI scores determined every 2 wk for 34 wk after weaning.

Statistical and linkage analysis

Quantitative trait loci (QTL) linkage analysis was conducted using the R (43) and the R/qtl software (44). Results were obtained under the imputation model (45). The susceptibility, onset, maxscore, and AUC phenotypes were analyzed separately under the assumption of sex as an interactive covariate. For the X chromosome, default procedure in R/qtl is to keep four genotype groups (males are hemizygous A or B, and females are AB or BB) separate: The “null model” for the X chromosome includes sex as a covariate. To investigate genetic interactions a two-dimensional genome scan with a two-QTL model was performed. The two-QTL model compares a full model in the presence of covariates (y = μ + βy1 + βy2 + βy1y2 + Ay + Zy1 + Zy2 + Zy1y2 + e) to a null model (y = μ + Ay + e). The epistasis, LOD support interval (LODint), compares the full model to an additive model (y = μ + βy1 + βy2 + Ay + Zy1 + Zy2 + Zy1y2 + e).
e), q1 and q2 are unknown genotypes at two different locations, A, a matrix of covariates, and Z, a matrix of QTL interacting covariates. Empirical significance levels (70, 95, 99%) were established by permutation tests (1000 permutations) for each phenotype. For the genome-wide screen, genome-wide significance was applied, and for the second analysis of only the loci of interest, locus-wide significance was applied. Separated significant levels were established, by permutation, for the X chromosome (K.W. Bromman et al., unpublished observations).

Other statistical analyses were performed with the nonparametric Mann-Whitney U or Kruskal-Wallis tests comparing two or more groups, respectively.

Results

In a backcross between CD18<sup>hypo</sup> PL/J and C57BL/6J mice, 16% of offspring develop a severe psoriasiform phenotype.

PL/J mice carrying a hypomorphic mutation in the CD18 gene (CD18<sup>hypo</sup>) developed a psoriasiform dermatitis strongly resembling human psoriasis, whereas CD18<sup>hypo</sup> C57BL/6J mice did not show any signs of this phenotype. (PL/J × C57BL/6J)F<sub>1</sub> mice are not susceptible to this disease, indicating the presence of recessive susceptibility genes. To identify chromosomal regions containing genes involved in the development of the psoriasiform skin disease, (PL/J × C57BL/6J)F<sub>1</sub> × PL/J backcross mice were observed for their psoriasiform phenotype using an adapted PASI score for clinical assessment.

Two hundred and nine of 343 offspring of the backcross generation (61%) did not show any signs of the psoriasiform dermatitis (Table I, maxscore of 0), whereas for 16% (<em>n</em> = 56) a severe phenotype (maxscore of 3 or 4) was observed. The first signs of the psoriasiform skin disease (onset) developed earlier in mice reaching high adapted PASI scores compared with less affected animals. We observed a difference in the susceptibility to the psoriasiform skin disease between males and females. Seventy-three percent of susceptible mice were females (<em>n</em> = 98) with at least twice as many as males for all severity degrees observed in this psoriasiform skin disease of this population. In contrast, within unaffected mice, 60% (<em>n</em> = 126) were males, pointing to a higher susceptibility to the psoriasiform skin disease in female CD18<sup>hypo</sup> mice compared with their male littermates.

Additional genes on chromosome 10 influence the development of the psoriasiform skin disease.

To perform a cost-efficient but still powerful genetic mapping of susceptibility loci for the psoriasiform skin disease, we used a two-step mapping approach. First, a genome-wide linkage analysis was performed on 94 mice from the backcross with the most extreme phenotypes, 53 with the most severe disease (maxscore of 3 or 4) and 41 unaffected mice using 83 microsatellite markers covering the genome with an average distance of 17 cm. The proportion of female and male mice was identical in both groups. Second, marker density was increased in regions of interest determined in the genome-wide screen, and the complete backcross of 343 mice was subsequently genotyped.

### Table I. Phenotypes of backcross mice

<table>
<thead>
<tr>
<th>Maxscore</th>
<th>Backcross Mice (n)</th>
<th>Sex</th>
<th>Onset&lt;sup&gt;a&lt;/sup&gt;</th>
<th>AUC&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Females</td>
<td>Males</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>209</td>
<td>83</td>
<td>126</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>54</td>
<td>43</td>
<td>11</td>
<td>13.9 ± 6.2&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>17</td>
<td>7</td>
<td>13.8 ± 9.3</td>
</tr>
<tr>
<td>3</td>
<td>38</td>
<td>15</td>
<td>7</td>
<td>10.7 ± 11.1</td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>8</td>
<td>3</td>
<td>6.8 ± 4.4</td>
</tr>
</tbody>
</table>

<sup>a</sup>Week of onset; mean of onset from mice in each group.
<sup>b</sup>Sum of adapted PASI scores up to 34 wk; mean accumulated score (42) from mice in each group.
<sup>c</sup>Because 32 animals died earlier than 34 wk after weaning, the actual number of mice for calculation of AUC is given in parentheses.
<sup>d</sup>SD.

### Table II. QTL for phenotypes of the psoriasiform skin disease identified in genome screen of CD18<sup>hypo</sup> backcross mice

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Marker&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Position&lt;sup&gt;b&lt;/sup&gt; (60)</th>
<th>LOD Score&lt;sup&gt;c&lt;/sup&gt; (&lt;em&gt;n&lt;/em&gt; = 94)&lt;sup&gt;d&lt;/sup&gt;</th>
<th>LOD Score&lt;sup&gt;c&lt;/sup&gt; (&lt;em&gt;n&lt;/em&gt; = 343)&lt;sup&gt;e&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maxscore</td>
<td>D10mit186/D10mit214</td>
<td>19.0</td>
<td>7.7**</td>
<td>9.7**</td>
</tr>
<tr>
<td></td>
<td>D18mit194</td>
<td>22.0</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D1mit236</td>
<td>25.7</td>
<td>2.6*</td>
<td></td>
</tr>
<tr>
<td>Onset</td>
<td>D10mit186/D10mit214</td>
<td>19.0</td>
<td>4.9**</td>
<td>4.6**</td>
</tr>
<tr>
<td></td>
<td>D6mit67</td>
<td>41.5</td>
<td>3.1*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D1mit17</td>
<td>106.3</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>Susceptibility</td>
<td>D10mit186/D10mit214</td>
<td>19.0</td>
<td>6.9**</td>
<td>10.2**</td>
</tr>
<tr>
<td></td>
<td>D18mit194</td>
<td>22.0</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D1mit236</td>
<td>25.7</td>
<td>2.3*</td>
<td></td>
</tr>
<tr>
<td>AUC</td>
<td>D10mit186/D10mit214</td>
<td>19.0</td>
<td>4.4**</td>
<td>6.7**</td>
</tr>
<tr>
<td></td>
<td>D18mit55</td>
<td>24.0</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D1mit292</td>
<td>107.3</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D4mit256</td>
<td>82.7</td>
<td>2.1</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Marker that is closest to the peak LOD score.
<sup>b</sup>Marked position in centimorgans according to genetic map (www.informatics.jax.org).
<sup>c</sup>Highest LOD score (≥70% probability) of linkage on each chromosome.
<sup>d</sup><em>n</em> = 94, genome-wide screen of 94 mice with most extreme phenotypes; <em>n</em> = 343, complementary screen of regions of interest, total of 343 backcrossed mice.
<sup>e</sup>Significance levels: *70%, **95%, and ***99% probability of linkage established by permutation tests. Genome-wide significance applied for the genome screen. Locus-wide significance applied for complementary screen of loci of interest.
The QTLs identified in the genome-wide screen of the first 94 mice are shown in Table II. Highly significant linkage (with a probability of >99%) was found for a locus on chromosome 10 close to the marker D10mit86/D10mit14 (Table II). Notably, this linkage could not be due to the CD18<sub>hypo</sub> mutation itself or the 129/SvEv-originating segment adjacent to the CD18 gene, because both paternal strains of the cross carry this region, and thus all mice of the backcross. However, these results distinctly indicate that additional gene(s) on chromosome 10 influence the development of the psoriasiform disease in this cross. The psoriasiform skin disease-promoting alleles in this locus originate from PL/J (Table III).

We identified a locus on chromosome 6 influencing the time point of onset of the disease (Table II and Fig. 1). The peak is located between markers D6mit4 and D6mit25 with the highest logarithm of odds (LOD) score close to marker D6mit67. The disease-promoting allele originates from the PL/J parental strain (Table III). Linkage was indicated for the phenotypes severity (expressed as maxscore) and susceptibility at the same region on chromosome 6 (LOD, 1.4 and 1.6, respectively). In addition, indication of linkage was detected for markers on chromosomes 1, 4, and 18 (Table II) in which the disease-promoting alleles again originate from PL/J (Table III).

**Linkage analysis of all 343 mice strengthens support for the chromosome 10 locus**

As a second step in our mapping approach, we increased the marker density of the chromosome 1, 4, 6, 10, and 18 regions identified in the genome-wide screen and an additional 249 mice were included in the analysis. Linkage analysis was performed on all 343 mice (Table II). The locus on chromosome 10 clearly contributed to the disease in the additional 249 mice as well as the LOD score being increased to a maximum of LOD of 10.2 (Table II). In contrast, the locus on chromosome 6 that was linked to the time point of onset of the analysis of the phenotypic extreme individuals was no longer associated with susceptibility phenotypes. This indicates that the susceptibility allele of the locus on chromosome 6 is present in the most severely affected mice and contributes to that phenotype. However, when adding the rest of the backcross mice in the analysis, this effect was diluted and no longer detectable.

**Chromosome 10 potentially harbors two loci that affect the development of the psoriasiform skin disease in addition to the CD18<sub>hypo</sub> mutation**

Both the parental strains in the present backcross contain the CD18<sub>hypo</sub> mutation on chromosome 10, originally generated in 129/SvEv embryonic stem cells. Therefore, in the present study, all offspring of the backcross mice carry genomic DNA fragments from the 129/SvEv strain that surround the CD18 gene on chromosome 10. For this reason, this part of the genome does not segregate as does the rest of the genome in this backcross, but rather appears as nonpolymorphic in the linkage analysis. Because no recombinations in this fragment were detectable, the genetic distance is estimated to be very short, although the physical distance is 71 Mb. To test whether this may skew the linkage analysis results, a separate analysis was performed to determine the association of individual markers on chromosome 10 with the phenotypic maxscore (Table IV). For all seven markers mice with homozygosity for PL/J alleles (B) show a significantly higher maxscore than heterozygous mice (H) with the most prominent difference being for markers D10mit86 and D10mit214 (p < 0.0001).

To circumvent the nonpolymorphic 129/SvEv region and to dissect whether more than one additional locus besides CD18 is present on chromosome 10, linkage analysis was performed after treating it as two chromosomes. Markers D10mit80, D10mit50, D10mit213, D10mit86/D10mit214, D10mit233/D10mit14 and D6mit274, D6mit4, D6mit261, D6mit67/D6mit14, D6mit230, D6mit10, D6mit25, and D6mit14. For markers mentioned at the same genetic position, no recombination was detected between these markers in the cross. Horizontal lines indicate significance levels for linkage with a probability of 95%: maxscore, LOD (0.95) = 3.3; onset, LOD (0.95) = 3.2.

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**Table III. Dominance relationship between PL/J and C57BL/6J alleles at the loci associated with the psoriasiform skin disease**

<table>
<thead>
<tr>
<th>Marker&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Affected&lt;sup&gt;b&lt;/sup&gt; (n = 53)</th>
<th>Unaffected (n = 41)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>H</td>
</tr>
<tr>
<td>D10mit86</td>
<td>42</td>
<td>11</td>
</tr>
<tr>
<td>D10mit233/D10mit14</td>
<td>37/36</td>
<td>16/17</td>
</tr>
<tr>
<td>D6mit47</td>
<td>31</td>
<td>22</td>
</tr>
<tr>
<td>D18mit194</td>
<td>33</td>
<td>20</td>
</tr>
<tr>
<td>D1mit236/D1uncg101</td>
<td>34/33</td>
<td>19/20</td>
</tr>
<tr>
<td>D1mit111/D1mit292</td>
<td>30/32</td>
<td>23/21</td>
</tr>
<tr>
<td>D4mit170</td>
<td>29</td>
<td>24</td>
</tr>
</tbody>
</table>

<sup>a</sup> Markers showing highest LOD scores on the respective chromosome.

<sup>b</sup> Affected, Maxscore of 3 or 4; unaffected, maxscore of 0. B, Number of animals homozygous for PL/J alleles; H, heterozygous.
10 worked independently of each other in an additive fashion (Fig. 2) with the D10mit86/D10mit214 locus having the stronger impact.

Susceptibility loci for the psoriasiform skin disease on chromosomes 1 and 18

Three additional loci were found to be of significance as indicated in the genome-wide screen of all 343 mice: Two locations on chromosomes 1 and 18 were also shown to play a role in the severity of the psoriasiform skin disease. The chromosome 18 locus, D18mit35, showed a suggestive LOD score (LOD, 1.7) for onset and was confirmed at a higher significance level, 99% probability of linkage (LOD, 3.0) (Fig. 3). Consequently, heterozygosity at the chromosome 18 locus overcame the effect of the chromosome 4 locus, which is the definition of an epistatic interaction.

Genetic interaction of the locus on chromosome 1 determines the involvement of the chromosome 4 locus in disease severity

The most centromeric of the two loci on chromosome 1 acts in a true epistatic fashion with a locus on chromosome 4, D4mit170 (67 cM) (Fig. 3). The locus on chromosome 4, D4mit308 (57.4 cM), most likely representing the same linked region as D4mit170, was only detected from a suggestive LOD score (LOD, 1.7) for onset in the initial genome-wide screen (Table II).

When applying a two-dimensional screen searching for any pairs of loci affecting the disease in an interactive fashion, we were able to show that the effect of the chromosome 4 locus was only penetrant if the genotype at the centromeric chromosome 1 locus was homozygous for PL/J (LOD, 4.2) (Table II). Consequently, heterozygosity at the chromosome 1 locus overcame the effect of the chromosome 4 locus, which is the definition of an epistatic interaction.

Discussion

CD18<sup>hypo</sup> PL/J mice show a psoriasiform skin disease strongly resembling human psoriasis in many aspects of its pathogenesis, thus representing an animal model that may contribute to a better understanding of this condition or other inflammatory skin diseases. The CD18<sup>hypo</sup> mutation perhaps is not the only disease-promoting factor in these mice, because other strains of different genetic backgrounds carrying the same CD18<sup>hypo</sup> mutation do not reveal any signs of this skin disease. Therefore, this polygenic mouse model is particularly suitable for the identification of genes involved in the development of the psoriasiform phenotype, with an impact also for the human disease.

In this study, we performed the first genome-wide linkage analysis in an animal model for psoriasis to search for loci that control clinical manifestations of this inflammatory skin disease. In the CD18<sup>hypo</sup> psoriasiform mouse model, the mutation leading to reduced CD18 expression is known (33). Important modifier loci with dominant protective genes from the C57BL/6J mouse were identified in a backcross between CD18<sup>hypo</sup> mice of the susceptible PL/J and resistant C57BL/6J strains (36).

In addition to the CD18<sup>hypo</sup> mutation, we found that chromosome 10 harbors two loci contributing to the disease (Fig. 4). A locus on chromosome 6 was identified to predominantly influence the time point when the first signs appear, potentially leading to a more severe condition. We also demonstrated an epistatic interaction between a locus on chromosome 1 and one on chromosome 4. The effect of the chromosome 4 locus was only penetrant if the genotype at the chromosome 1 locus was homozygous for PL/J. Consequently, heterozygosity at the chromosome 1 locus overcame the effect of the chromosome 4 locus, which is the definition of an epistatic interaction.

An additional locus on chromosome 1 and one on chromosome 18 were also shown to play a role in the severity of the psoriasiform skin disease. As observed in an earlier backcross experiment (33), the fact that no psoriasiform phenotype developed in (PL/J × C57BL/
used. In the previous study, the CD18hypo mutation had been back-
pressed C57BL/6J inheritance. However, compared with 50% in-
cidence of psoriasiform skin disease in these mice were 100% (33).

We could detect the impurity of 129/Sv alleles when genotyping 
backcross mice. However, it was determined in the screen of the 
parental strains, before the genome screen, that all the 129/Sv im-
purity originated from the PL/J parentage. The impurity of the 
6JF1 mice suggests recessive susceptibility PL/J or dominant 
suppressing C57BL/6J inheritance. However, compared with 50% in-
cidence in the backcross mice of the previous study (33), the 
incidence in the present backcross was lower. This bias could be 
due to the genetic impurity of the CD18hypo PL/J parental mice 
used. In the previous study, the CD18hypo mutation had been back-
crossed for seven generations onto the PL/J strain and the inci-
dence of psoriasiform skin disease in these mice were 100% (33).

In the present study, however, after four generations of backcross-
ing of the CD18hypo mutation to PL/J background (theoretically 
93.75% of the genome of PL/J origin), the incidence in the CD18hypo PL/J parental mice was 91%.

We could detect the impurity of 129/Sv alleles when genotyping 
backcross mice. However, it was determined in the screen of the 
parental strains, before the genome screen, that all the 129/Sv im-
purity originated from the PL/J parentage. The impurity of the 
CD18hypo PL/J, the most likely explanation for the lower incidence of psoriasiform skin disease compared with the previous study (33), points toward recessive PL/J disease alleles. Consequently, using the impure CD18hypo PL/J strain in the present cross most likely reduced the power of localizing the recessive PL/J disease-promoting genes. The detected 129/Sv alleles most likely did not influence disease development, because no differences in pheno-
types were seen between mice carrying the 129/Sv fragment and 
those homozygous for PL/J in the same fragment.

The genomic DNA fragments on chromosome 10 surrounding the 
CD18hypo mutation still originating from 129/SvEv in the 
present cross was 71 Mb (36 cM) long between markers 
D10mit194 (29 cM) and D10mit14 (65 cM). Consequently, be-
cause both parental strains used for generating the backcross ani-
mals carried this section of chromosome 10, all of their offspring 
carried a 129/SvEv-derived fragment in that region. For genet-
ic analysis, this results in a fragment of chromosome 10 that is non-
polyomorphic in the cross and hence did not contribute to any link-
age information. However, this did not skew the linkage analyses 
results, because LOD scores for chromosomal regions on both 
sides of the inserted region could be confirmed, when determining 
association of individual markers or performing linkage analysis 
after division of chromosome 10 into two chromosomes. These 
results suggest that, in addition to the region around marker 
D10mit214, which shows the highest LOD scores, a second gene 
locus on chromosome 10 around markers D10mit233 and 
D10mit14 might be linked with the psoriasiform skin disease.

The observation that the PL/J susceptible genetic influence is 
mainly recessive (33) suggested that, in the present cross, loci 
could be expected with a dominant protective effect inherited from 
C57BL/6J. In fact, all loci with linkage or suggestive linkage to the 
psoriasiform skin disease confirmed this expectation. However, 
it is not possible to conclude whether the PL/J alleles are recessive or 
codominant compared with the C57BL/6J alleles, because a back-
cross cannot differentiate between heterozygotes and homozygotes 
for the C57BL/6J allele. An intercross would be more appropriate 
because in the F2 generation homozygosity for PL/J origin could 
be expected with a dominant protective effect inherited from 
C57BL/6J. In fact, all loci with linkage or suggestive linkage to the 
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cross cannot differentiate between heterozygotes and homozygotes 
for the C57BL/6J allele. An intercross would be more appropriate 
because in the F2 generation homozygosity for the C57BL/6J allele 
also appears and, furthermore, could provide additional linkage 
data because in complex disorders the genetic context is decisive 
for the effect of single genes (47).

We observed a higher incidence of skin disease in female back-
cross mice (54%) than in male backcross mice (22%). This is in 
agreement with some reports on human psoriasis where it appears 
to be slightly more prevalent in women than in men (www. 
emedicine.com/oph/topic483.htm). A recent study found that the 
prevalence of psoriasis in the United Kingdom is greater in young 
female patients compared with young male patients and declines
significantly in patients 70 years and older, regardless of sex (48). In contrast, a recent survey of psoriasis in 694 patients from Norway did not show any significant sex difference for both sexes (49). Also, in a survey of psoriasis patients in Japan, evidence points to a paternal overtransmission of psoriasis to males being more frequently affected (65.8%) compared with females (34.2%) (50). Thus, ethnic factors also appear to influence the sex prevalence of psoriasis. Similar to our observation, the difference in susceptibility between sexes is a common feature of most models for autoimmune diseases, like systemic lupus erythematosus, rheumatoid arthritis, and experimental autoimmune encephalomyelitis, as well as of the human diseases (51), even though the sex effect in some animal models is reversed compared with the human disease. The sexual dimorphism may be due to sex hormones, which can influence the immune system (51–54).

Due to the current view that psoriasis is a T cell-mediated immunological disease (3), genes affecting immunological functions, especially that of T cells, are most promising candidates. The current mouse map (Mouse Genome Database) contains a number of such candidate genes for the regions identified in the herein performed linkage analysis (42). These include the IFN-γR, TNF-α-induced protein, IL-22 binding protein, leukocyte Ig-like receptor 5, and gap junction protein-a1 on chromosome 10, as well as CD8, CD207 (langerin), receptors for IL-5, -12, and -23, and CXCL12 on chromosome 6. However, the regions identified in this genome-wide linkage analysis are large and contain a considerable number of genes. Therefore, the size of the support interval should be reduced before performing candidate gene analyses.

The murine localizations of candidate regions can be used to predict the positions of human candidate susceptibility loci. Primal mouse chromosome 10 exhibits extensive homology with human chromosome 6q16–25, whereas the central part of mouse chromosome 6 shows homology with regions on different human chromosomes, namely 2p13–11, 3p26–25, 3q21, and 4q22–27 (Mouse Genome Database). One of these regions (3q21) has already been described to be linked with psoriasis vulgaris (PSORS5) (19). However, the underlying gene in this region has not yet been identified. SLC12A8 coding for a member of the solute carrier family 12 proteins has been suggested as candidate gene (55). Two other candidate genes from this region, the zinc finger protein 148 and cystatin A, could not be shown to be associated with psoriasis vulgaris in a large family set showing linkage to the PSORS5 locus (56). The corresponding human regions to the chromosome 1 and 4 loci identified in the present study are 1q23-44 (PSORS4) and 1p36-22 (PSORS7), respectively, which have also shown linkage with psoriasis vulgaris (18, 21). Therefore, it may be relevant for human psoriasis to unravel the underlying genes involved in the skin disease of the CD18<sup>−/−</sup> mouse model. However, strongest linkage was observed for a region on the proximal part of chromosome 10. The corresponding region in human located on 6q16-25 has not yet been identified in linkage analyses as being of importance in psoriasis patients. The same is true for regions corresponding to the proximal end of chromosome 18, which are distributed on different human chromosomes on 5q22-33, 10p12-11, and 18q11-12.

The CD18 gene is located in a region on human chromosome 21q, for which no linkage has so far been observed in studies with psoriasis families. However, PSORS2 on 17q with ICAM-2 (15) and PSORS6 on 19p with ICAM-1 (20) contain two genes coding for the most important ligands of the β<sub>2</sub> integrins. It might be that in human psoriasis not only β<sub>2</sub> integrins themselves but also components essential for their signaling and/or effects could be altered.

The gene locus showing strongest linkage to psoriasis vulgaris so far is PSORS1 on 6p21 (13, 14) containing, among others, the MHC gene HLA-C. In the present backcross, the MHC/H2 region was not demonstrated to be associated with skin disease development. This is in accordance with observations in an earlier backcross between CD18<sup>−/−</sup> PL/J and C57BL/6J strains, which revealed no correlation between the H2 region and the psoriasiform skin disease (33). Even though we cannot fully exclude the possibility that the susceptible strain had lost the variation in the H2 locus during inbreeding, we now have preliminary data indicating that congenic mice carrying PL/J alleles on chromosome 10 developed the psoriasiform skin phenotype (our unpublished data). These data indicate that the H2 locus is not necessarily required for the manifestation of the phenotype in this mouse model. However, our finding that the H2 locus does not represent a major manifestation in the mouse model does not argue against the involvement of MHC in human psoriasis development. It is worth noting that some of the chromosomal regions identified in the present study have previously been shown to be susceptibility loci in mouse models of other complex inflammatory diseases. The region on the distal end of chromosome 1 was mapped in several diseases (47). This region is supposed to contain genes important in a number of processes leading to the development of autoimmune diseases (57). Furthermore, the loci on chromosomes 6, 10, and 18 have also been identified in studies of experimental autoimmune encephalomyelitis (39). We have started to produce speed congenic strains (58) for chromosome 10 and 6 candidate regions which will allow exclusion of certain candidate genes listed above and reduce the support interval to one that is amenable to physical mapping. Indeed, this approach has earlier been successful in identifying disease-causing genes (59). Because these genes identified in the mouse model might also play a role in the human disease, genetic analysis of CD18<sup>−/−</sup> mice may significantly contribute to the elucidation of the pathogenesis of human psoriasis.

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**Disclosures**

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