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A 9-Centimorgan Interval of Chromosome 10 Controls the T Cell-Dependent Psoriasiform Skin Disease and Arthritis in a Murine Psoriasis Model

Honglin Wang,* Daniel Kess,* Anna-Karin B. Lindqvist,† Thorsten Peters,* Anca Sindrilaru,* Meinhard Wlaschek,* Robert Blakytny,‡ Rikard Holmdahl,† and Karin Scharffetter-Kochanek2*

Psoriasis is a chronic genetic disease of unresolved pathogenesis affecting 3% of the general population of which 10–40% also develop arthritis (1). It presents with erythematous, scaling skin lesions (2) and can manifest as psoriatic arthritis (PsA),3 an inflammatory joint disease resulting in extensive bone resorption and joint destruction (3). Genome-wide linkage studies have noted overlapping regions of significance for human skin psoriasis and PsA within and outside of the MHC region (4). Different cell types have been proposed to be the primary triggers in the pathogenesis of psoriasis (2), with T cells being essential for its initiation (5–7), whereas macrophages maintain its inflammatory state (8, 9). Both genetic and environmental factors contribute to its manifestation. Psoriasis is frequently inherited between successive generations, but it does not follow a classical autosomal Mendelian profile. Type I psoriasis has an early onset, before the age of 40 years, and is suggested to be heritable (10). Type II psoriasis is characterized by a late onset and its heritability is not entirely clear.

To identify genetic factors underlying this skin disorder, several linkage analysis studies have been performed in humans. Although at least six different psoriasis susceptibility loci, designated PSORS1–PSORS6, have been mapped by using genome-wide scans, the cause of human psoriasis remains unknown (11–19).

Research into the pathogenesis of human psoriasis has profited from suitable animal models. Most of these, however, display only a single or a few aspects resembling human psoriasis (20–27). In contrast, the mouse PL/J strain carrying a CD18 hypomorphic (CD18hyp) mutation, with reduced expression of the common chain of β2 integrins (CD11/CD18) spontaneously develops a skin disease that closely resembles human psoriasis. In contrast, the same mutation on C57BL/6J background did not demonstrate this phenotype. By a genome-wide linkage analysis, two major loci were identified as contributing to the development of psoriasiform dermatitis under the condition of low CD18 expression. Using a congenic approach, we now demonstrate that the introduction of a 9-centimorgan fragment of chromosome 10 derived from the PL/J strain into the disease-resistant CD18 hypomorphic C57BL/6J was promoting the development of psoriasiform skin disease and notably also arthritis. We therefore designated this locus psoriasiform skin disease-associated locus 1 (PSD1). High numbers of CD4+ T cells and TNF-α producing macrophages were detected both in inflamed skin and joints in these congenic mice, with a complete resolution upon TNF-α inhibitor therapy or depletion of CD4+ T cells. For the first time, we have identified a distinct genetic element that contributes to the T cell-dependent development of both psoriasiform skin disease and associated arthritis. This congenic model will be suitable to further investigations of genetic and molecular pathways that cause psoriasiform dermatitis and arthritis, and it may also be relevant for other autoimmune diseases. The Journal of Immunology, 2008, 180: 5520–5529.
region on chromosome 17 that includes the ICAM-2 locus, an important ligand of the CD11/CD18 heterodimers (13).

The CD18<sup>hyp</sup> PL/J model is of particular interest, because the development of psoriasiform skin disease is highly dependent on the genetic background; that is, the psoriasiform dermatitis develops only in CD18<sup>hyp</sup> PL/J mice, but not in C57BL/6J or 129/SvEv the genetic background; that is, the psoriasiform dermatitis developmental process is highly dependent on the genetic background. Performing a genome-wide linkage analysis, we identified two quantitative trait loci (QTL) on chromosome 10 with significant linkage to the development of psoriasiform skin disease and one QTL on chromosome 6 with linkage to its early onset (34). To further investigate whether loci on chromosome 10 (PSD1, or psoriasiform skin disease-associated locus 1) and/or chromosome 6 are causal for the development of psoriasiform skin disease, we have developed speed congenic strains by means of microsatellite-assisted selection (35).

We demonstrate herein that the CD18 hypomorphic C57BL/6J mice, resistant to development of psoriasiform skin disease, could be made susceptible to the disease by introducing a 9-centi-morgan (cM) fragment of chromosome 10 derived from the PL/J strain. This chromosome 10 congenic strain developed both the psoriasiform skin disease and severe arthritis, which strongly resembled human psoriasis and associated PsA. The congenic strain for the chromosome 6 locus did not develop skin and/or joint disease.

These data identify the PSD1 locus on chromosome 10 as a verified genetic element contributing to both the inflammatory skin disease and arthritis in the CD18<sup>hyp</sup> murine model. Our model is highly suitable to further dissect genes and molecular pathways underlying inflammatory skin and joint diseases.

Materials and Methods

Genotyping

DNA was prepared from tail biopsies by a quick alkaline lysis protocol (36). PCR was performed with 10 ng of tail DNA according to published procedures (34). PCR products were analyzed on a MegaBACE 1000 (Amersham Biosciences) according to the manufacturer’s protocol.

Selection of markers for congenic screening

Eighty-six microsatellite markers, previously identified as being polymorphic between the PL/J and 129/SvEv strains (34), were genotyped in the PL/J CD18<sup>hyp</sup> parental strains listed in bold.

Table I. Location of an insert originating from the 129/SvEv strain in both PL/J CD18<sup>hyp</sup> and C57BL/6J CD18<sup>hyp</sup> parental strains for congenic breeding

<table>
<thead>
<tr>
<th>Marker&lt;sup&gt;a&lt;/sup&gt;</th>
<th>cM&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Mb&lt;sup&gt;c&lt;/sup&gt;</th>
<th>C57BL/6J</th>
<th>PL/J</th>
<th>129/SvEv&lt;sup&gt;d&lt;/sup&gt;</th>
<th>C57BL/6J&lt;sup&gt;e&lt;/sup&gt;</th>
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<td>101</td>
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</table>

<sup>a</sup> Markers used for identification of an insert originating from the 129/SvEv strain on chromosome 10.

<sup>b</sup> Genetic position according to Mouse Genome Informatics (http://www.informatics.jax.org).

<sup>c</sup> Physical position according to Ensembl Mouse Genome Server; the physical position of D10mit66 is not available (http://www.ensembl.org/Mus_musculus).

<sup>d</sup> Genotypes of wild-type mice for C57BL/6J and PL/J strains given as size of PCR products in base pairs (bp).

<sup>e</sup> Genotypes of parental CD18<sup>hyp</sup> strain for congenic breeding and 129/SvEv strain used as embryonic stem cells for the generation of CD18<sup>hyp</sup> mutation, all given as size of PCR products in base pairs (bp). PCR products for inserts originating from the 129/SvEv strain in both PL/J CD18<sup>hyp</sup> and C57BL/6J CD18<sup>hyp</sup> parental strains are listed in bold.
CD18<sup>hypo</sup> PL/J (N5) and CD18<sup>hypo</sup> C57BL/6J (N10) (The Jackson Laboratory) strains, which have been used as parental strains for the construction of the congenics. As screening markers for the speed congenic procedure, an additional 91 microsatellite markers were selected for backcrossing. The 91 markers, with an average marker distance of 15.5 cM, covered all chromosomes and could identify contaminating PL/J-derived fragments present in the CD18<sup>hypo</sup> PL/J (N5) donor strain to enable elimination of these fragments in recipient mice during the congenic breeding.

**Generation of congenic mouse strains**

Congenic mice for the regions of interest on chromosomes 10 and 6 were produced by breeding the PL/J genomic fragments onto C57BL/6J background. Speed congenic procedure was used as previously described (35). The donor strain was a CD18<sup>hypo</sup> PL/J (N5) strain originating from the CD18<sup>hypo</sup> PL/J (N4) mice previously described (37), but with an additional generation of backcrossing onto the PL/J inbred strain. Theoretically, ~3.125% of the genome in this CD18<sup>hypo</sup> PL/J (N5) strain still originates from 129/SvEv (38). For the selection of one donor mouse, male PL/J CD18<sup>hypo</sup> (N5) mice with severe psoriasis phenotype were genotyped with 86 microsatellite markers polymorphic between the PL/J and 129/SvEv inbred strains. The PL/J CD18<sup>hypo</sup> (N5) male with the least contamination of 129/SvEv alleles (3 out of 86 microsatellite markers, beside the 129/SvEv-derived CD18 insert on chromosome 10) was selected as the donor for construction of the congenics (Table I).

The selected CD18<sup>hypo</sup> PL/J (N5) male was mated with two females of the CD18<sup>hypo</sup> C57BL/6J inbred strain. The CD18<sup>hypo</sup> C57BL/6J inbred strain is available at The Jackson Laboratory (www.jax.org) as well as results from 10 generations of backcrossing. The CD18<sup>hypo</sup> C57BL/6J (N10) mice were genotyped and no 129-derived fragments were identified using the 86 microsatellite markers. Although we cannot exclude the presence of 129-derived fragments outside of the PSD1 region in the genome of N10 CD18<sup>hypo</sup> C57BL/6J mice, this did not affect the findings of the PSD1 involvement in the development of the psoriasiform skin disease and arthritis. Six generations of backcrossing were performed onto CD18<sup>hypo</sup> C57BL/6J (N10) background, where the donor for each generation was selected based on its genotype according to the speed congenic procedure (35) to keep the donor fragment in the region of interest and to eliminate donor fragments such as those derived from like PL/J, as well as from 129/SvEv, in the rest of the genome. N6 mice harboring PL/J fragments of interest on N10 CD18<sup>hypo</sup> C57BL/6J background were intercrossed to generate the homozygous congenic strains for each congenic fragment, respectively. To monitor the psoriasiform skin disease clinically and histologically, we used littermate mice from the intercross of N6 or from the N6 backcross generation were used as controls. All control mice were littermates to the different congenic mice from the intercrossing of the PL/J and 129/SvEv, in the rest of the genome. N6 mice harboring PL/J fragments of interest on N10 CD18<sup>hypo</sup> C57BL/6J background were intercrossed to generate the homozygous congenic strains for each congenic fragment, respectively.

The severity of skin disease and arthritis was assessed twice a week for an observation period of up to 30 wk using an adapted psoriasis area and severity index (PASI) (34) and an adapted arthritis score (39). For CD18<sup>hypo</sup> mice, the PASI 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. The clinical arthritis score was adapted from a described scoring system (39) as follows: 0, no symptoms; 1, slight swelling and/or erythema; 2, pronounced edematous swelling; 3, deformed paw or joint with ankylosis and joint rigidity. The sum of all four paws was scored twice a week with a maximal score of 12 per mouse.

The skin disease and arthritis were assessed in homozygous congenic mice. As clinical appearance and histology of the skin and joints are identical in PSD1 congenic and chromosome 10C congenic mice containing the PSD1 region, we used the PSD1 congenic strain for further analysis. When we investigated the incidence of psoriasisform skin disease and arthritis, mice heterozygous to the different congenic mice from the intercrossing of the N6 backcross generation were used as controls. All control mice were heterozygous PL/J and C57BL/6J in the regions of interest. We assumed that heterozygous mice were suitable as controls in evaluation of the incidence of the phenotypes since our previous data have shown that mice heterozygous for the PSD1 region do not develop psoriasisform skin disease (34). These control mice were specified in Table II. Additionally, CD18<sup>hypo</sup> PL/J mice at N5 generation were used for phenotyping to assess the time course of the disease. All experiments were done in compliance with the German Law for Welfare of Laboratory Animals.

**Assessment of the psoriasiform skin disease and arthritis**

The severity of skin disease and arthritis were assessed twice a week for an observation period of up to 30 wk using an adapted psoriasis area and severity index (PASI) (34) and an adapted arthritis score (39). For CD18<sup>hypo</sup> mice, the PASI 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. The clinical arthritis score was adapted from a described scoring system (39) as follows: 0, no symptoms; 1, slight swelling and/or erythema; 2, pronounced edematous swelling; 3, deformed paw or joint with ankylosis and joint rigidity. The sum of all four paws was scored twice a week with a maximal score of 12 per mouse.

**Immunofluorescence staining**

Frozen cyrosctions of skin or joints from both male and female mice at 5 mo of age with the indicated phenotype were fixed in ice-cold acetone for 10 min before staining. To stain keratinocytes, CD4 T cells, macrophages, and TNF-α, we used rabbit anti-K14 (Covance), anti-CD4, anti-CD8, and anti-mouse TNF-α (BD Pharmingen, clone MP6-XT22) in conjunction with streptavidin Cy3 conjugate (Dianova) mAb. DAPI (4’6-diamidino-2-phenylindole) (Fluka) was used for staining of nuclei. All Abs were diluted in an Ab diluent (Dako).

**Histologic processing**

Skin and paw joints from the PSD1 congenic and control mice were fixed in 4% paraformaldehyde solution for 24 h, and in the case of joints these were decalcified for 3 wk in 15% EDTA solution, dehydrated, and embedded in paraffin. Paraffin-embedded 5-μm sections of skin and joints were stained with H&E as described (37).

**Administration of etanercept**

Etanercept (100 μg per mouse) was administrated i.p. every day for a period of 30 days to neutralize TNF-α. In parallel, injection of 0.9% NaCl was used as control. Effects of etanercept on the severity of arthritis and the psoriasiform dermatitis were assessed by the adapted arthritis score and the
congenic strains. In the present study we continued to use the CD18<sup>hypo</sup> strains previously reported (34) for the generation of congenic strains. In this study the PL/J CD18<sup>hypo</sup> strain has been further backcrossed. The CD18<sup>hypo</sup> mutation on chromosome 10 originates from 129/SvEv (34), and thus PL/J CD18<sup>hypo</sup> and C57BL/6J CD18<sup>hypo</sup> still contain genomic DNA fragments from the 129/SvEv strain surrounding the CD18 gene on chromosome 10. To determine the exact position of 129/SvEv inserting in the genome of PL/J CD18<sup>hypo</sup> and C57BL/6J CD18<sup>hypo</sup>, respectively, a set of microsatellite markers for chromosome 10 was used for genotyping. In the PL/J CD18<sup>hypo</sup> (N5) mice, the 129/SvEv genomic DNA fragments on chromosome 10 surrounding the CD18<sup>hypo</sup> mutation were determined to be 27 cM long, between markers D10mit299 and D10mit162 (Table I). For the C57BL/6J CD18<sup>hypo</sup> inbred strain (N10, The Jackson Laboratory), the 129/SvEv genomic fragment was determined to be 30 cM long, between markers D10mit194 and D10mit162 (Table I).

**Construction of congenic strains for PL/J alleles by marker-assisted selection**

We have previously identified two loci on chromosome 10 and one locus on chromosome 6 as being significantly linked to the psoriasiform skin disease and earlier onset of disease in the most severely affected mice, respectively (34). These loci were identified in a cross where both parental strains carried a mutation resulting in reduced expression of the β<sub>2</sub> integrin chain CD18, the CD18<sup>hypo</sup> mutation. The two loci on chromosome 10 were shown to interact in an additive fashion in disease development. To confirm these loci and further investigate their role in the development of psoriasiform skin disease, each locus, derived from the disease-susceptible CD18<sup>hypo</sup> PL/J (N5) strain, was backcrossed onto the resistant CD18<sup>hypo</sup> C57BL/6J inbred strain to produce congenic strains. Consequently, the congenic mice produced in the present study all carry the CD18<sup>hypo</sup> mutation, and the effect of the congenic fragment on disease development was evaluated under the condition of low expression of CD18. This is relevant since the loci were identified under the same conditions (34).

Ninety-one informative markers covering the genome with an average marker distance of 15.5 cM were genotyped in each successive generation to define the introgressed regions of interest derived from CD18<sup>hypo</sup> PL/J and to enable C57BL/6J purity in the

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### Results

**Identification of 129/SvEv alleles in PL/J CD18<sup>hypo</sup> and C57BL/6J CD18<sup>hypo</sup> parental strains**

In the present study we continued to use the CD18<sup>hypo</sup> strains previously reported (34) for the generation of congenic strains. In

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### Statistical analysis

Quantitative results are presented as mean values ± SD. Mean values were tested by means of a two-tailed heteroscedastic Student’s t test.

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### Table II. Summary of clinical outcome in PSD1 congenic mice

<table>
<thead>
<tr>
<th>Strain†</th>
<th>Psoriasiform Skin Disease Incidence (%)&lt;sup&gt;‡&lt;/sup&gt;</th>
<th>PASI Score (mean ± SD)&lt;sup&gt;§&lt;/sup&gt;</th>
<th>Arthritis Incidence (%)&lt;sup&gt;‡&lt;/sup&gt;</th>
<th>Arthritis Score (mean ± SD)&lt;sup&gt;‡&lt;/sup&gt;</th>
<th>p&lt;sup&gt;‡&lt;/sup&gt;</th>
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<td>0</td>
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<td>*a&lt;sup&gt;e3&lt;/sup&gt;</td>
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<td>3.43 ± 2.34</td>
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<td>0</td>
<td>—f4</td>
<td>0/10 (0)</td>
<td>0</td>
</tr>
<tr>
<td>Chromosome 6</td>
<td>0/9 (0)</td>
<td>0</td>
<td>—f4</td>
<td>0/9 (0)</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>0/32 (0)</td>
<td>0</td>
<td>—f4</td>
<td>0/32 (0)</td>
<td>0</td>
</tr>
<tr>
<td>PL/J</td>
<td>9/10 (90%)</td>
<td>3.30 ± 1.19</td>
<td>—f5</td>
<td>0/10 (0)</td>
<td>0</td>
</tr>
<tr>
<td>CD18&lt;sup&gt;hypo&lt;/sup&gt;</td>
<td></td>
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</tbody>
</table>

<sup>†</sup>N6 congenic mice were genotyped by microsatellite markers, which can be retrieved from http://www.ensembl.org. Thirty-two heterozygote littermates at different target regions (8 mice per group) served as controls. The data from these 32 control mice were pooled together since none of control mice developed the psoriasiform skin disease and/or arthritis.

†Onset and severity of the psoriasiform skin disease were estimated by an adapted PASI score every 2 wk for 30 wk in each group.

‡Adapted PASI scores were calculated for affected and unaffected mice at 5 mo of age and are presented as group means with standard errors. Mice that had to be sacrificed due to severe disease were given a score of 7.

§The p values calculated with the Newman-Keuls test were considered significant at p < 0.01 (**), 0.001 (***), 0.05 (*) or 0.01 (+). The p value was calculated from the comparison of adapted PASI score between control mice and chromosome 10A or chromosome 10B or chromosome 10C or chromosome 10D congenic mice; f1–f4, the p value was calculated from the comparison of adapted PASI score between PL/J CD18<sup>hypo</sup> mice and chromosome 10B or chromosome 10C or chromosome 10D congenic mice; f7–f10, the p value was calculated from the comparison of arthritis score between PL/J CD18<sup>hypo</sup> mice and chromosome 10B or chromosome 10C or chromosome 10D congenic mice. The p value for chromosome 6 congenic mice was not listed. —, no significance.

Arthritis developed in 6 out of 8 chromosome10B mice that showed severe psoriasiform skin disease at 5 mo of age.

Adapted clinical arthritis scores were calculated for affected and unaffected mice at 5 mo of age and all are presented as group means with standard errors.

NS PL/J CD18<sup>hypo</sup> mice were typed by Southern blot for assessment of the psoriasiform skin phenotype and arthritis.

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**FIGURE 2.** Frequency of the psoriasiform skin disease in chromosome 10B and chromosome 10C congenic strains and N5 CD18<sup>hypo</sup> PL/J mice. To assess the time course for the development of psoriasiform skin disease in PL/J CD18<sup>hypo</sup> mice and chromosome 10B and chromosome 10C congenic mice, the onset of psoriasiform skin disease was routinely monitored using an adapted PASI score every 2 wk for 18 wk in each group. Statistical significant differences in the time of onset of the psoriasiform skin disease were found between CD18<sup>hypo</sup> PL/J mice and chromosome 10C congenic mice at 6 wk, and between CD18<sup>hypo</sup> PL/J mice and chromosome 10B congenic mice or chromosome 10C congenic mice at 8 wk. p values calculated with the Newman-Keuls test were considered significant for p < 0.05 (++) or p < 0.01 (+).
Six generations of marker-assisted backcrossing were performed using the speed congenic approach (35). Congenic mice were established for the chromosome 10 regions (Fig. 1) and the chromosome 6 regions (data not shown).

In total, five congenic strains were generated. The congenic mouse strains were named according to the congenic intervals on chromosomes 6 and 10: chromosome 6 (D6mit274–D10mit14, 50 cM) (data not shown), chromosome 10A (D10mit75–D10mit126,
FIGURE 4. The congenic interval PSD1 on chromosome 10 is responsible for the development of arthritis in PSD1 congenic mice. A, A representative female chromosome 10B congenic mouse carrying PSD1 locus derived from CD18<sup>hyp</sup> PL/J parents is shown with psoriasiform skin disease, as well as reddened swollen ankle and paw joints indicative of arthritis (white arrows) (left mouse) compared with normal ankle and paw joints (white arrows) of the littermate control wild-type C57BL/6J in the PSD1 region derived from the intercross of the N6 generation (right mouse). B, Representative H&E staining of sections from metatarsals of the arthritic paw of a PSD1 congenic mouse and (C) from a normal paw of a littermate control mouse. D, Higher magnification of the boxed area in B shows bone erosion (black arrows) with numerous osteoclasts. E, Higher magnification of the area indicated by the asterisk in B reveals pannus growing over the cartilage with cartilage damage (arrows). F, A marked macrophage (F4/80) infiltrate was found both in the dermis, in the joint capsule, and in the synovia of PSD1 congenic mice, compared with almost no staining in control mice (G). H, Abundant staining for TNF-α-producing cells was found in the dermis as well as the joint capsule, synovia, and adjacent bone of PSD1 congenic mice, compared with almost no staining in the dermis and the joint capsule of a littermate control mouse without the PSD1 fragment (H). I, Double staining for F4/80 and TNF-α of inflamed joints derived from PSD1 congenic mice reveals macrophages to be a major source for TNF-α. Arrows indicate the position of joints with the capsule and bone. Cell nuclei were counterstained with DAPI (original magnification of C and D, ×10; E and F, ×40; G–J × 20). e, Epidermis; b, bone marrow; c, capsule with synovia; d, dermis. Dotted lines indicate the border between epidermis and dermis. One representative experiment out of four is shown.

19 cM), chromosome 10B (D10mit126–D10mit38, 6 cM), chromosome 10C (D10mit75–D10mit271, 70 cM) and chromosome 10D (D10mit233–D10mit271, 8 cM) (Fig. 1).

The PSD1 locus, a 9-cM genetic element of chromosome 10, controls the susceptibility for the psoriasiform skin disease and arthritis

Phenotypic evaluation of the psoriasiform skin disease was performed in homozygous congenic mice. Littermate mice heterozygous at the PL/J-originated congenic fragment for each locus were used for controls, which were generated as littermates to the homozygous at the PL/J-originated congenic fragment for each locus were formed in homozygous congenic mice. Littermate mice heterozygous at the target regions developed any signs of psoriasiform skin disorder or arthritis (Table II).

Since the presence of the congenic fragment in the chromosome 10B mice is enough to make the otherwise resistant C57BL/6J CD18<sup>hyp</sup> susceptible for both the psoriasiform skin disease and arthritis, these data clearly indicate that a susceptibility locus common for psoriasiform skin disease and arthritis is located within the 19.8-megabase-pair (9-cM) interval (D10mit126–D10mit194) on chromosome 10 (Fig. 1). We therefore designated this locus psoriasiform skin disease-associated locus 1 (PSD1). The chromosome 10B mice are referred to as the PSD1 congenic mice. Interestingly, 9 out of 10 PL/J CD18<sup>hyp</sup> mice (NS) developed the severe psoriasiform skin disease; however, none developed arthritis at 5 mo of age (Table II).

Interestingly, and in line with our previous observation that the locus on chromosome 6 promotes earlier onset of the psoriasiform skin disease (34), the onset of disease in chromosome 10B and chromosome 10C congenic strains, which did not carry chromosome 6 region, was later than the onset in CD18<sup>hyp</sup> PL/J (NS) mice (Fig. 2). No signs of psoriasiform skin disorder and/or arthritis were observed in the congenic mice carrying the chromosome 6 fragment alone (Table II).
Characterization of the psoriasiform skin disease in congenic mice carrying the PSD1 susceptibility locus

All chromosome 10B and chromosome 10C congenic mice carrying the PSD1 locus developed a progressive psoriasiform skin disease, characterized by severe erythema and scale and crust formation on the back (Fig. 3, A and B). In contrast, control mice did not show any pathological skin phenotype (Fig. 3C). The psoriasiform skin disease in mice carrying the PSD1 locus showed an identical clinical picture as observed in CD18<sub>yypo</sub> PL/J mice (8, 28). Histological analysis of skin sections from PSD1 congenic mice revealed hyperplasia of the epidermis (Fig. 3D), hyperkeratosis (Fig. 3, D and E), subcorneal microabscesses (Fig. 3E), and a diffuse inflammatory cell infiltrate in the dermis (Fig. 3F), as compared with normal epidermis and dermis in control mice (Fig. 3G). Keratin 14 staining confirmed epidermal hyperplasia in the lesional skin from PSD1 congenic mice (Fig. 3H) compared with control mice (Fig. 3I). As the psoriasiform skin disease critically depends on the presence of CD4<sup>+</sup> T cells and activated TNF-α-releasing macrophages (8, 37), cryosections derived from skin of affected PSD1 congenic and control mice were stained with anti-mouse CD4, anti-mouse F4/80, and anti-TNF-α mAb. Increased numbers of CD4<sup>+</sup> cells, macrophages, and strong staining for TNF-α were observed in the dermis of PSD1 congenic mice (Fig. 3, J, L, and N), but not in sections derived from control mice (Fig. 3, K, M, and O).

Characterization of psoriatic arthritis in mice carrying the PSD1 congenic locus

Similar to human psoriasis patients, the chromosome 10B and chromosome 10C mice carrying the PSD1 locus developed both the psoriasiform skin disease and an arthritis with swelling and erythema of ankle joints as well as the tarsometatarsal and metatarsophalangeal paw joints (Fig. 4, A, left mouse, indicated by white arrows), compared with a littermate control (Fig. 4, A, right mouse). H&E staining revealed marked arthritic changes in the joints of PSD1 congenic mice (Fig. 4B), compared with normal paw joints of the littermate control mice (Fig. 4C). Higher magnification of Fig. 4B showed infiltration of bones by numerous osteoclasts (Fig. 4D) and pannus formation with cartilage damage (Fig. 4E).

We earlier reported an increase in macrophage numbers with increased TNF-α release in the psoriasisform skin of CD18<sub>yypo</sub> PL/J mice (8). Notably, in the present study we observed significantly increased numbers of macrophages not only in the dermis, but also in the joint capsule and synovia of the joints in PSD1 carrying mice (Fig. 4F) compared with a virtual absence of macrophages in control mice (Fig. 4G). A striking number of inflammatory cells revealed strong staining for TNF-α in the skin and in the joint capsule, synovia, and bone of the joints in PSD1 carrying mice (Fig. 4H) with almost no TNF-α staining in control mice (Fig. 4I). Double staining confirmed that virtually all F4/80<sup>+</sup> macrophages in the inflamed joints, including the capsule and synovia of PSD1-carrying mice, produced TNF-α (Fig. 4J). These data provide strong evidence that C57BL/6J CD18<sub>yypo</sub> mice congenic for a 9-cM genetic element of chromosome 10 from the PL/J strain (PSD1 locus) develop not only the psoriasiform skin disease but also a severe psoriatic arthritis.

TNF-α inhibitor treatment and CD4-depleting Abs result in the resolution of psoriasiform dermatitis and arthritis in PSD1 congenic mice

To confirm that the PSD1 locus is responsible for the T cell-dependent and TNF-α-mediated inflammatory skin as well as joint disease, the chromosome 10B congenic (PSD1 congenic) mice were treated with a TNF-α inhibitor (etanercept), consisting of a recombinant soluble TNF-α receptor fusion protein, or with anti-CD4 depletion Abs, as described previously for CD18<sub>yypo</sub> PL/J mice (8, 37). The clinical arthritis score before anti-TNF-α treatment (5.5 ± 1.73) improved substantially in the PSD1 congenic mice after 4 wk of TNF-α inhibitor treatment (2.25 ± 0.5, p = 0.044) (Fig. 5A), while no improvement of the arthritis scores was observed in PSD1 congenic mice treated with 0.9% NaCl (p = 0.1) (Fig. 5B). The adapted PASI scores increased before TNF-α inhibitor treatment (3.75 ± 0.5), and they revealed a substantial improvement after the TNF-α inhibitor treatment with etanercept (1.5 ± 0.58, p = 0.04) (Fig. 5C). In contrast, no significant changes of the adapted PASI score were observed in PSD1 mice treated with 0.9% NaCl (p = 0.65) (Fig. 5D). These data unequivocally show that the PSD1 interval controls TNF-α-dependent skin and joint inflammation. Similar data were obtained after 4 wk of CD4 T cell depletion (data not shown), further suggesting that both the psoriasiform skin disease and the associated arthritis share a CD4<sup>+</sup> T cell-dependent pathogenesis.

Discussion

In the present study we confirmed that a locus on chromosome 10, previously identified by us (34), drives the development of psoriasiform skin disease as well as arthritis in the presence of low expression of the common chain of β<sub>2</sub> integrins (CD18). We show that a congenic strain containing the 9-cM PL/J element on chromosome 10 (D10mit126 to D10mit194), designated as chromosome 10B PSD1 congenic strain, on the resistant C57BL/6J CD18<sub>yypo</sub> background developed psoriasiform skin disease, and most notably also a severe arthritis of joints. The PL/J background in the presence of low CD18 expression drives the development of psoriasiform skin disease but rarely in combination with arthritis. In contrast, the two produced congenic strains harboring fragments...
telomeric and centromeric of the 9-cM PSD1 fragment (chromosomes 10A and 10D) did not develop the psoriasiform skin disease and/or arthritis within an observation period of 10 mo.

These data indicate that a gene (or genes) within the PSD1 locus contribute(s) to pathogenic events common to the psoriasiform skin disease and arthritis in this context under the condition of low expression of CD18.

Since the chromosome 10C mice, congenic for a larger fragment of the chromosome 10 including the PSD1 locus, developed a phenotype identical to the PSD1 congenic (chromosome 10B), we conclude that the PSD1 region is the major locus within that region. In our previous study, we found another locus on the lower part of chromosome 10 that was also linked to the psoriasiform skin disease and that interacted with PSD1 locus in an additive fashion in the disease development (34). Herein, our data demonstrate that chromosome 10C congenic mice containing two susceptible loci reveal a slightly earlier onset of disease when compared with chromosome 10B congenic only carrying PSD1 region on chromosome 10. However, the difference is not statistically significant, and further dissection of potentially interacting genes is required in future analyses.

Based on many observations, including the fact that human psoriasis is inducible in SCID mouse xenograft models by the injection of T cells from human skin lesions, it is largely accepted that psoriasis is a T cell-dependent disease (40, 41). Also, CD4⁺ T cells are crucial for the induction and development of the psoriasiform skin disease in the CD18⁺ PL/J psoriasis model (37, 42). The fact that depletion of CD4⁺ T cells in the PSD1 congenic strain resulted in the resolution of both the psoriasiform skin disease and the arthritis verifies the PSD1 element as causally contributing to the manifestation of a T cell-dependent psoriasiform inflammation in the two organs (data not shown).

These data unequivocally indicate that the PSD1 genetic element harbors important modifier genes that are causally involved in a shared T cell-dependent pathogenesis of skin and joint inflammation. CD18⁺ PL/J mice rarely reveal the combined clinical
picture of psoriasiform skin disease and arthritis in a backcross to the PL/J background for six generations. This discrepancy indicates that either other PL/J loci are resistance loci for arthritis, or that the C57BL/6J genome harbors susceptibility loci for arthritis apart from the PSD1 region and the CD18^{hypo} mutation. Crossing the PSD1 congenics to the parental CD18^{hypo} C57BL/6J mice to observe whether an F1 at the PSD1 locus with homozygous C57BL/6J background would be sufficient for arthritis, or creating an F1 with the N5 CD18^{hypo} PL/J donor to study whether the homozygosity across the PSD1 interval in setting of background heterozygosity would be permissive for arthritis can be further ways to dissect the genetic modifier(s) of the arthritis.

In addition to chromosome 10 regions, a locus on chromosome 6 was identified to predominantly influence the time point of disease development (34). In the present study, congenic strain carrying the PL/J element on chromosome 6 did not develop the psoriasiform skin disorder, however, chromosome 10B and chromosome 10C congenic strains lacking the PL/J region on chromosome 6 had delayed onset of the skin disease when compared with CD18^{hypo} PL/J (N5) mice, which have both chromosome 10 and chromosome 6 genetic context.

Human psoriasis and psoriatic arthritis are interrelated disorders, as up to 40% of psoriasis patients also suffer from psoriatic arthritis (43–47).

The PSD1 on murine chromosome 10 is syntenic to human chromosome 6q16 and 6q21–q24, as shown in Fig. 6. There are no reports in the literature associating 6q with human psoriasis. All genes identified to date with a known function in the reports in the literature associating 6q with human psoriasis. All chromosome 6q16 and 6q21–q24, as shown in Fig. 6. There are no increased in numbers, and that the resolution of both psoriasiform skin disease and arthritis occurred upon treatment with TNF-α inhibitor (etanercept) in PSD1 congenic mice strongly suggest that the PSD1 locus is important for a T-cell induced, and macrophage-sustained, pathogenesis of both the psoriasiform skin disease and arthritis. This is most interesting, as neutralization of TNF-α in human psoriasis successfully treats both skin and joint inflammation (57).

In the present study we identified the 9-cM PSD1 genetic element on chromosome 10 that distinctly contributes to the susceptibility to psoriasiform skin disease and arthritis in an otherwise resistant strain. The pathogenic pathways controlled by the gene(s) in this locus need further elucidation. The PSD1 locus harbors 46 published genes. Continuous backcrossing will be performed to further dissect the PSD1 region into smaller genetic intervals for further identification of modifier genes, as well as their function and interaction with the β2 integrins in psoriasiform skin and joint disease. The PSD1 congenic mouse strain qualifies as a valuable tool for preclinical studies and the identification of novel treatment strategies.

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References


