The Journal of Immunology

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

Daniel Kess, Anna-Karin B. Lindqvist, Thorsten Peters, Honglin Wang, Jan Zamek, Roswitha Nischt, Karl W. Broman, Robert Blakytny, Thomas Krieg, Rikard Holmdahl and Karin Scharffetter-Kochanek

J Immunol 2006; 177:4612-4619; ;
doi: 10.4049/jimmunol.177.7.4612
http://www.jimmunol.org/content/177/7/4612

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Identification of Susceptibility Loci for Skin Disease in a Murine Psoriasis Model[1]

Daniel Kess,[2]*‡ Anna-Karin B. Lindqvist,[2§] Thorsten Peters,*‡ Honglin Wang,* Jan Zamek,‡ Roswitha Nischt,‡ Karl W. Broman,[¶] Robert Blakytny,[†] Thomas Krieg,‡ Rikard Holmdahl,[§] and Karin Scharffetter-Kochanek[3]*‡

Psoriasis is a frequently occurring inflammatory skin disease characterized by thickened erythematous skin that is covered with silvery scales. It is a complex genetic disease with both heritable and environmental factors contributing to onset and severity. The CD18 hypomorphic PL/J mouse reveals reduced expression of the common chain of β_2 integrins (CD11/CD18) and spontaneously develops a skin disease that closely resembles human psoriasis. In contrast, CD18 hypomorphic C57BL/6J mice do not demonstrate this phenotype. In this study, we have performed a genome-wide scan to identify loci involved in psoriasiform dermatitis under the condition of low CD18 expression. Backcross analysis of a segregating cross between susceptible CD18 hypomorphic PL/J mice and the resistant CD18 hypomorphic C57BL/6J strain was performed. A genome-wide linkage analysis of 94 phenotypically extreme mice of the backcross was undertaken. Thereafter, a complementary analysis of the regions of interest from the genome-wide screen was done using higher marker density and further mice. We found two loci on chromosome 10 that were significantly linked to the disease and interacted in an additive fashion in its development. In addition, a locus on chromosome 6 that promoted earlier onset of the disease was identified in the most severely affected mice. For the first time, we have identified genetic regions associated with psoriasis in a mouse model resembling human psoriasis. The identification of gene regions associated with psoriasis in this mouse model might contribute to the understanding of genetic causes of psoriasis in patients and pathological mechanisms involved in development of disease. *The Journal of Immunology,* **2006, 177: 4612–4619.**

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)[4] loci identified so far are located on chromosome 6p (*PSORS1*; Refs. 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 (fsn; 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 β_1 integrin overexpression in murine skin (31), and transplantation of human psoriasis-affected skin onto SCID mice (32).

*Department of Dermatology and Allergic Diseases, and [†]Institute of Orthopaedic Research and Biomechanics, University of Ulm, Ulm, Germany; [‡]Department of Dermatology and Center for Molecular Medicine, University of Cologne, Cologne, Germany; [§]Department for Cell and Molecular Biology, Section for Medical Inflammation Research, University of Lund, Lund, Sweden; and [¶]Department of Biostatistics, Johns Hopkins University, Baltimore, MD 21205

Received for publication July 29, 2005. Accepted for publication July 12, 2006.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by grants to K.S.-K. and T.K. from the Center for Molecular Medicine, University of Cologne (TV60, BMBF 01 KS 9502), the individual research grant from the German Research Foundation (Deutsche Forschungsgemeinschaft) (SCHA 411/12-1, 12-2), the Swedish Strategic Foundation, and the Swedish Science Research Council. This research was further supported through a European Community Marie Curie Fellowship "Stay at Training Site" in 2000 between the University of Lund and the Commission of the European Communities.

[2] D.K. and A.-K.B.L. contributed equally to this work.

[3] Address correspondence and reprint requests to Dr. Karin Scharffetter-Kochanek, Department of Dermatology and Allergic Diseases, University of Ulm, Maienweg 12, D-89081 Ulm, Germany. E-mail address: karin.scharffetter-kochanek@uniklinik-ulm.de

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

0022-1767/06/$02.00

The mouse PL/J strain carrying the CD18 hypomorphic ($CD18^{hypo}$) mutation, with reduced expression of the common chain of β_2 integrins (CD11/CD18) to 2–16% of wild-type levels, develops 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). We earlier reported that $CD4^+$ Th1 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).

This psoriasiform skin disease is of particular interest, because it is highly dependent on the genetic background. Namely, the disease develops in $CD18^{hypo}$ PL/J mice but not when the same $CD18^{hypo}$ 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 $CD18^{hypo}$ 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 $CD18^{hypo}$ PL/J and the resistant $CD18^{hypo}$ 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)F_1 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 $CD18^{hypo}$ PL/J and the resistant $CD18^{hypo}$ 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 $CD18^{hypo}$ PL/J mice used in the present study were generated by crossing the $CD18^{hypo}$ 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 *D10mit14*. The $CD18^{hypo}$ 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 (Mb) (35 centimorgan (cM)) with the boundary markers *D10mit38* to *D10mit233*. To generate the backcross, male $CD18^{hypo}$ PL/J mice with a clinical psoriasiform phenotype were selected and crossed with female $CD18^{hypo}$ C57BL/6J mice. Female F_1 mice were backcrossed to male $CD18^{hypo}$ PL/J. Three hundred and forty-three ((PL/J × C57BL/6J)F_1 × PL/J) 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 $MgCl_2$, 0.3 μM forward and reverse primers (MWG-Biotech and Applied Biosystems), 100 μM dNTP (Amersham Biosciences), and 0.25 U of *Taq*DNA 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 $CD18^{hypo}$ PL/J and $CD18^{hypo}$ C57BL/6J strains were screened using 186 microsatellite makers across the genome. For 19 of these markers, alleles from 129/Sv were detected in the $CD18^{hypo}$ PL/J parental strain. No 129/Sv alleles were detected in the $CD18^{hypo}$ 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 $CD18^{hypo}$ 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 (PASI) score as used in assessment of the severity of human psoriasis elsewhere (41). For $CD18^{hypo}$ 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.

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 PASI 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 PASI 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 the 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 ($y = \mu + \beta q1 + \beta q2 + \beta q1xq2 + A\gamma + Z\delta q1 + Z\delta q2 + Z\delta q1xq2 + \varepsilon$) to a null model ($y = \mu + A\gamma + \varepsilon$). The epistasis, LOD support interval (LOD_{int}), compares the full model to an additive model ($y = \mu + \beta q1 + \beta q2 + A\gamma + Z\delta q1 + Z\delta q2 +$

Table 1. *Phenotypes of backcross mice*

Maxscore	Backcross Mice (*n*)	Sex		Onset[a]	AUC[b]
		Females	Males		
0	209	83	126	0	0 ($n = 197$)[c]
1	54	43	11	13.9 ± 6.2[d]	9.6 ± 3.6[d] ($n = 50$)
2	24	17	7	13.8 ± 9.3	13.8 ± 6.4 ($n = 21$)
3	45	30	15	10.7 ± 11.1	24.5 ± 10.3 ($n = 32$)
4	11	8	3	6.8 ± 4.4	33.3 ± 11.0 ($n = 11$)

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

ε). 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) (46) 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. Broman 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^{hypo} 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^{hypo}) developed a psoriasiform dermatitis strongly resembling human psoriasis, whereas CD18^{hypo} C57BL/6J mice did not show any signs of this phenotype. (PL/J × C57BL/6J)F_1 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_1 × 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% ($n = 56$) 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 ($n = 98$) 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% ($n = 126$) were males, pointing to a higher susceptibility to the psoriasiform skin disease in female CD18^{hypo} 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 with 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 II. *QTL for phenotypes of the psoriasiform skin disease identified in genome screen of CD18^{hypo} backcross mice*

Phenotype	Marker[a]	Position[b] (60)	LOD Score[c] ($n = 94$)[d]	LOD Score[c] ($n = 343$)[d]
Maxscore	*D10mit86/D10mit214*	19.0	7.7**[e]	9.7**
	D18mit194	22.0		1.8^+
	D1mit236	25.7		2.6*
Onset	*D10mit86/D10mit214*	19.0	4.9**	4.6**
	D6mit67	41.5	3.1^+	
	D1mit17	106.3		1.7^+
Susceptibility	*D10mit86/D10mit214*	19.0	6.9**	10.2**
	D18mit194	22.0		1.8^+
	D1mit236	25.7		2.3^+
AUC	*D10mit86/D10mit214*	19.0	4.4**	6.7**
	D18mit35	24.0		2.2^+
	D1mit292	107.3		2.3^+
	D4mit256	82.7		2.1^+

[a] Marker that is closest to the peak LOD score.
[b] Marker position in centimorgans according to genetic map (⟨www.informatics.jax.org/⟩).
[c] Highest LOD score (≥70% probability) of linkage on each chromosome.
[d] $n = 94$, genome-wide screen of 94 mice with most extreme phenotypes; $n = 343$, complementary screen of regions of interest, total of 343 backcrossed mice.
[e] 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.

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

Marker[a]	Affected[b] (n = 53)		Unaffected Mice (n = 41)	
	B	H	B	H
D10mit86	42	11	11	30
D10mit233/D10mit14	37/36	16/17	13	28
D6mit67	31	22	13	28
D18mit194	33	20	18	23
D1mit236/D1mcg101	34/33	19/20	19/20	22/21
D1mit111/D1mit292	30/32	23/21	18/22	23/19
D4mit170	29	24	18	23

[a] Markers showing highest LOD scores on the respective chromosome.
[b] Affected, Maxscore of 3 or 4; unaffected, maxscore of 0. B, Number of animals homozygous for PL/J alleles; H, heterozygous.

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 *D10mit86/214* (Table II). Notably, this linkage could not be due to the CD18^{hypo} mutation itself or the 129/SvEv-originating segment adjacent to the CD18 gene, because both parental 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 in 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.

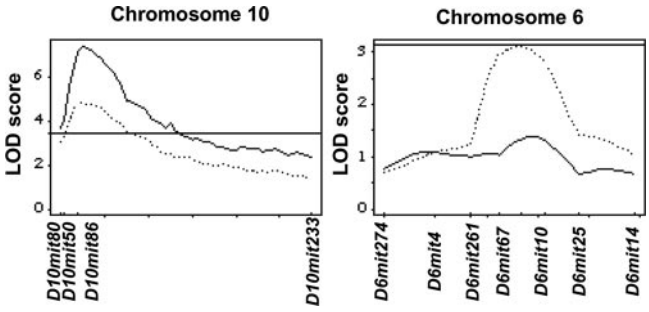

FIGURE 1. LOD score curves representing the phenotypes maxscore (solid line) and onset (dotted line) for chromosomes 6 and 10. Positions of microsatellite markers on chromosome 10 and 6, respectively, are indicated: *D10mit80*, *D10mit50/D10mit213*, *D10mit86/D10mit214*, *D10mit233/D10mit14* and *D6mit274*, *D6mit4*, *D6mit261*, *D6mit67/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.

Chromosome 10 potentially harbors two loci that affect the development of the psoriasiform skin disease in addition to the CD18^{hypo} mutation

Both the parental strains in the present backcross contain the CD18^{hypo} 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 phenotype 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*, and *D10mit214* were distributed on one chromosome, whereas markers *D10mit233* and *D10mit14* were assigned to a second chromosome. The results of this linkage analysis suggest that there are two loci on chromosome 10 contributing to the psoriasiform phenotype in addition to the CD18 locus (Table V). The locus defined by *D10mit86* and *D10mit214*, influences both the time of onset and severity, whereas the locus defined by *D10mit233* and *D10mit14* influences the susceptibility and severity of the psoriasiform skin disease. The two loci on chromosome

Table IV. *Association of single markers on chromosome 10 with the phenotype maxscore*

Marker	Genetic Position (cM)	Genotype[a]	Backcross Mice (*n*)[b]	Maxscore[c]	*p*
D10mit80	4	B	159	1.1 ± 1.3[d]	0.0002
		H	170	0.6 ± 1.0	
D10mit50	7	B	167	1.1 ± 1.3	<0.0001
		H	175	0.5 ± 1.0	
D10mit213	11	B	163	1.1 ± 1.3	<0.0001
		H	179	0.5 ± 1.0	
D10mit86	17	B	151	1.3 ± 1.4	<0.0001
		H	161	0.4 ± 0.9	
D10mit214	19	B	169	1.2 ± 1.4	<0.0001
		H	172	0.4 ± 0.9	
D10mit233	62	B	81	1.6 ± 1.5	<0.0001
		H	169	0.6 ± 1.1	
D10mit14	65	B	119	1.2 ± 1.4	0.0003
		H	172	0.6 ± 1.1	

[a] B, Number of animals homozygous for PL/J alleles; H, heterozygous.
[b] Number of backcross mice genotyped for each marker.
[c] The mean of the highest adapted PASI scores from the mice in each group.
[d] SD.

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 chromosome 1 were significantly linked to disease severity, at *D1mit236* (25 cM; 46 Mb) and at *D1mit17/D1mit292* (107 cM; 191 Mb). A locus influencing the severity of the disease was identified on chromosome 18, *D18mit194/D18mit35* (22–24 cM; 44–46 Mb) (Table II).

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 is homozygous for PL/J (LOD_{int}, 4.1) (Fig. 3). 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^{hypo} 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^{hypo} mutation perhaps is not the only disease-promoting factor in these mice, because other strains of different genetic backgrounds carrying the same CD18^{hypo} 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^{hypo} 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^{hypo} mice of the susceptible PL/J and resistant C57BL/6J strains (36).

In addition to the CD18^{hypo} 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/

Table V. *Linkage of markers on the proximal and distal part of chromosome 10 with phenotypes of the psoriasiform skin disease after dividing chromosome 10 into two chromosomes*

	Phenotype	
	D10mit86/D10mit214[a] (17–19 cM)[b]	*D10mit233/D10mit14* (62–65 cM)
Maxscore (70; 95; 99%)[d]	9.6[c]	4.6 (1.8; 2.5; 3.3)
Onset (70; 95; 99%)	4.6	1.0 (1.7; 2.5; 3.0)
Susceptibility (70; 95; 99%)	10.2	4.3 (1.8; 2.7; 3.5)
AUC (70; 95; 99%)	6.6	4.0 (1.7; 2.5; 3.1)

[a] Markers on the proximal (*D10mit86/D10mit214*) and distal part (*D10mit233/D10mit14*) of chromosome 10.
[b] Marker position on chromosome 10.
[c] LOD score.
[d] Significance levels for 70, 95, and 99% probability of linkage established by permutation tests.

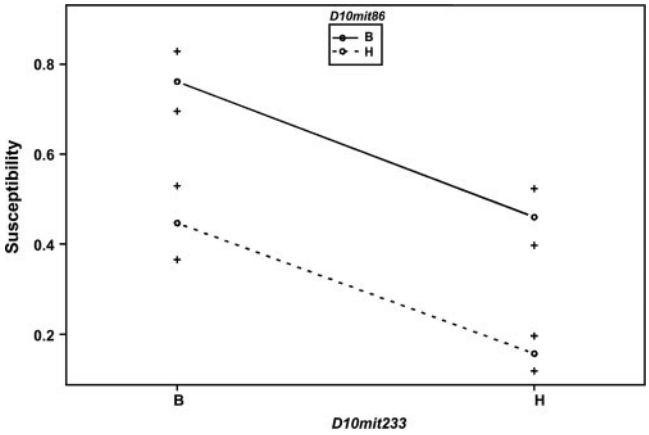

FIGURE 2. The proximal and distal loci on chromosome 10 act in an additive fashion. Mean of susceptibility for different combinations of homozygosity for PL/J (B) and heterozygosity (H) at the two chromosome 10 loci are shown. LOD scores were calculated in a two-dimensional genome scan with a two-QTL model. LOD_{jnt} of 14.2 and LOD_{int} of 0.4 revealed that the relationship between markers *D10mit86* and *D10mit233* is additive. LOD_{jnt} exceeded the threshold for 99% probability of linkage (LOD, 3.4) as calculated by permutation tests.

6J)F_1 mice suggests recessive susceptibility PL/J or dominant suppressing C57BL/6J inheritance. However, compared with 50% incidence 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 $CD18^{hypo}$ PL/J parental mice used. In the previous study, the $CD18^{hypo}$ mutation had been backcrossed for seven generations onto the PL/J strain and the incidence of psoriasiform skin disease in these mice were 100% (33). In the present study, however, after four generations of backcrossing of the $CD18^{hypo}$ mutation to PL/J background (theoretically 93.75% of the genome of PL/J origin), the incidence in the $CD18^{hypo}$ 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 impurity originated from the PL/J parentage. The impurity of the

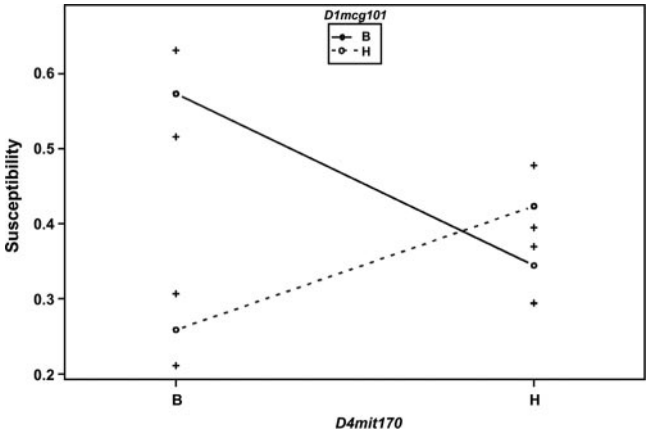

FIGURE 3. The markers *D1mcg101* on chromosome 1 and *D4mit170* on chromosome 4 show true epistatic interaction. Mean of susceptibility for different combinations of homozygosity for PL/J (B) and heterozygosity (H) for both markers. LOD scores were calculated in a two-dimensional genome scan with a two-QTL model. LOD_{jnt} of 6.5 and LOD_{int} of 4.1 revealed that the relationship between markers *D1mcg101* and *D4mit170* is mainly interactive (epistasis). LOD scores exceeded the threshold for 99% probability of linkage (LOD, 3.4) as calculated by permutation tests.

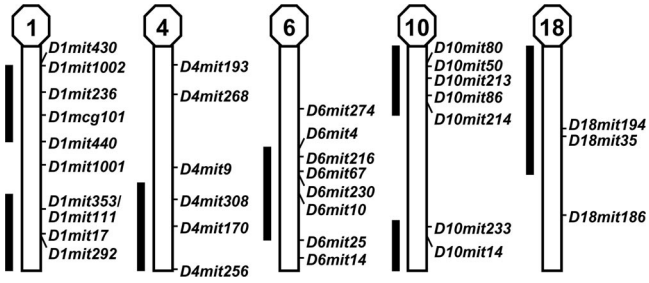

FIGURE 4. Schematic overview of gene loci showing linkage with the psoriasiform skin disease as determined by linkage analysis of a backcross between $CD18^{hypo}$ mice of the susceptible PL/J and resistant C57BL/6J strains. Positions of microsatellite markers are indicated at the *right* of each chromosome.

$CD18^{hypo}$ 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 $CD18^{hypo}$ 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 phenotypes 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 $CD18^{hypo}$ 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, because both parental strains used for generating the backcross animals carried this section of chromosome 10, all of their offspring carried a 129/SvEv-derived fragment in that region. For genetic analysis, this results in a fragment of chromosome 10 that is nonpolymorphic in the cross and hence did not contribute to any linkage 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 backcross cannot differentiate between heterozygotes and homozygotes for the C57BL/6J allele. An intercross would be more appropriate because in the F_2 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 backcross 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-α-1 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. Proximal 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 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^{hypo} 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_2 integrins. It might be that in human psoriasis not only β_2 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^{hypo} 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^{hypo} mice may significantly contribute to the elucidation of the pathogenesis of human psoriasis.

Acknowledgments

We are grateful to Samir Tawadros for his support in the mice facility with animal care, Anja Böttger for technical help, and Dr. Werner Müller, Prof. André Reis, and Prof. Thomas F. Wiencker for expertise in setting up the backcross analyses.

Disclosures

The authors have no financial conflict of interest.

References

1. Schon, M. P., and W. H. Boehncke. 2005. Psoriasis. *N. Engl. J. Med.* 352: 1899–1912.
2. Nickoloff, B. J., J. M. Schroder, P. von den Driesch, S. P. Raychaudhuri, E. M. Farber, W. H. Boehncke, V. B. Morhenn, E. W. Rosenberg, M. P. Schon, and M. F. Holick. 2000. Is psoriasis a T-cell disease? *Exp. Dermatol.* 9: 359–375.
3. Krueger, J. G. 2002. The immunologic basis for the treatment of psoriasis with new biologic agents. *J. Am. Acad. Dermatol.* 46: 1–23; quiz 23–26.
4. Breathnach, S. M., and W. G. Phillips. 1996. Psoriasis. In *Cytokines in Autoimmunity.* F. M. Brennan and M. Feldmann, eds. R. G. Landes Company, Austin, TX, pp. 175–200.
5. Prinz, J. 1997. Psoriasis vulgaris—der lange Weg zur Autoimmunerkrankung. In *Fortschritte der praktischen Dermatologie und Venerologie.* G. Plewig and B. Przybilla, eds. Springer, Berlin, pp. 21–28.
6. Valdimarsson, H., B. S. Baker, I. Jonsdottir, A. Powles, and L. Fry. 1995. Psoriasis: a T-cell-mediated autoimmune disease induced by streptococcal superantigens? *Immunol. Today* 16: 145–149.
7. Nickoloff, B. J., and F. O. Nestle. 2004. Recent insights into the immunopathogenesis of psoriasis provide new therapeutic opportunities. *J. Clin. Invest.* 113: 1664–1675.
8. Hellgren, L. 1967. *Psoriasis: The Prevalence in Sex, Age and Occupational Groups, in Total Populations in Sweden: Morphology, Inheritance and Association with Other Skin and Rheumatic Diseases.* Almqvist & Wiksell, Stockholm.
9. Henseler, T., and E. Christophers. 1985. Psoriasis of early and late onset: characterization of two types of psoriasis vulgaris. *J. Am. Acad. Dermatol.* 13: 450–456.
10. Lomholt, G. 1963. *Psoriasis: Prevalence, Spontaneous Course and Genetics.* GEC GAD, Copenhagen.
11. Ameen, M. 2003. Genetic basis of psoriasis vulgaris and its pharmacogenetic potential. *Pharmacogenomics* 4: 297–308.

12. Elder, J. T., R. P. Nair, T. Henseler, S. Jenisch, P. Stuart, N. Chia, E. Christophers, and J. J. Voorhees. 2001. The genetics of psoriasis 2001: the odyssey continues. *Arch. Dermatol.* 137: 1447–1454.
13. Nair, R. P., T. Henseler, S. Jenisch, P. Stuart, C. K. Bichakjian, W. Lenk, E. Westphal, S. W. Guo, E. Christophers, J. J. Voorhees, and J. T. Elder. 1997. Evidence for two psoriasis susceptibility loci (*HLA* and *17q*) and two novel candidate regions (*16q* and *20p*) by genome-wide scan. *Hum. Mol. Genet.* 6: 1349–1356.
14. Trembath, R. C., R. L. Clough, J. L. Rosbotham, A. B. Jones, R. D. Camp, A. Frodsham, J. Browne, R. Barber, J. Terwilliger, G. M. Lathrop, and J. N. Barker. 1997. Identification of a major susceptibility locus on chromosome 6p and evidence for further disease loci revealed by a two stage genome-wide search in psoriasis. *Hum. Mol. Genet.* 6: 813–820.
15. Tomfohrde, J., A. Silverman, R. Barnes, M. A. Fernandez-Vina, M. Young, D. Lory, L. Morris, K. D. Wuepper, P. Stastny, A. Menter, et al. 1994. Gene for familial psoriasis susceptibility mapped to the distal end of human chromosome 17q. *Science* 264: 1141–1145.
16. Matthews, D., L. Fry, A. Powles, J. Weber, M. McCarthy, E. Fisher, K. Davies, and R. Williamson. 1996. Evidence that a locus for familial psoriasis maps to chromosome 4q. *Nat. Genet.* 14: 231–233.
17. Zhang, X. J., P. P. He, Z. X. Wang, J. Zhang, Y. B. Li, H. Y. Wang, S. C. Wei, S. Y. Chen, S. J. Xu, L. Jin, et al. 2002. Evidence for a major psoriasis susceptibility locus at 6p21(*PSORS1*) and a novel candidate region at 4q31 by genome-wide scan in Chinese hans. *J. Invest. Dermatol.* 119: 1361–1366.
18. Capon, F., G. Novelli, S. Semprini, M. Clementi, M. Nudo, P. Vultaggio, C. Mazzanti, T. Gobello, A. Botta, G. Fabrizi, and B. Dallapiccola. 1999. Searching for psoriasis susceptibility genes in Italy: genome scan and evidence for a new locus on chromosome 1. *J. Invest. Dermatol.* 112: 32–35.
19. Enlund, F., L. Samuelsson, C. Enerback, A. Inerot, J. Wahlstrom, M. Yhr, A. Torinsson, J. Riley, G. Swanbeck, and T. Martinsson. 1999. Psoriasis susceptibility locus in chromosome region 3q21 identified in patients from southwest Sweden. *Eur. J. Hum. Genet.* 7: 783–790.
20. Lee, Y. A., F. Ruschendorf, C. Windemuth, M. Schmitt-Egenolf, A. Stadelmann, G. Nurnberg, M. Stander, T. F. Wienker, A. Reis, and H. Traupe. 2000. Genome-wide scan in German families reveals evidence for a novel psoriasis-susceptibility locus on chromosome 19p13. *Am. J. Hum. Genet.* 67: 1020–1024.
21. Veal, C. D., R. L. Clough, R. C. Barber, S. Mason, D. Tillman, B. Ferry, A. B. Jones, M. Ameen, N. Balendran, S. H. Powis, et al. 2001. Identification of a novel psoriasis susceptibility locus at 1p and evidence of epistasis between *PSORS1* and candidate loci. *J. Med. Genet.* 38: 7–13.
22. Bowcock, A. M., and J. N. Barker. 2003. Genetics of psoriasis: the potential impact on new therapies. *J. Am. Acad. Dermatol.* 49: S51–S56.
23. Zheng, J., S. Jin, and R. Shi. 2003. Confirmation of PSORS psoriasis susceptibility loci in a Chinese population. *Arch. Dermatol. Res.* 295: 14–18.
24. Sagoo, G. S., R. Tazi-Ahnini, J. W. Barker, J. T. Elder, R. P. Nair, L. Samuelsson, H. Traupe, R. C. Trembath, D. A. Robinson, and M. M. Iles. 2004. Meta-analysis of genome-wide studies of psoriasis susceptibility reveals linkage to chromosomes 6p21 and 4q28–q31 in Caucasian and Chinese Hans population. *J. Invest. Dermatol.* 122: 1401–1405.
25. Sundberg, J. P., D. Boggess, L. D. Shultz, and W. G. Beamer. 1994. The Flaky Skin (fsn) mutation chromosome? In *Handbook of Mouse Mutations with Skin, Hair Abnormatlities. Animal Models, Biomedical Tools.* J. P. Sundberg, ed. CRC Press, Boca Raton, FL, pp. 253–268.
26. HogenEsch, H., M. J. J. Gijbels, and C. Zurcher. 1994. The chronic proliferative dermatitis (cpd) mutation chromosome? In *Handbook of Mouse Mutations with Skin, Hair Abnormatlities. Animal Models, Biomedical Tools.* J. P. Sundberg, ed. CRC Press, Boca Raton, FL, pp. 217–220.
27. Hammer, R. E., S. D. Maika, J. A. Richardson, J. P. Tang, and J. D. Taurog. 1990. Spontaneous inflammatory disease in transgenic rats expressing HLA-B27 and human β2m: an animal model of HLA-B27-associated human disorders. *Cell* 63: 1099–1112.
28. Sano, S., K. S. Chan, S. Carbajal, J. Clifford, M. Peavey, K. Kiguchi, S. Itami, B. J. Nickoloff, and J. DiGiovanni. 2005. Stat3 links activated keratinocytes and immunocytes required for development of psoriasis in a novel transgenic mouse model. *Nat. Med.* 11: 43–49.
29. Schon, M. P., M. Detmar, and C. M. Parker. 1997. Murine psoriasis-like disorder induced by naive $CD4^+$ T cells. *Nat. Med.* 3: 183–188.
30. Pasparakis, M., G. Courtois, M. Hafner, M. Schmidt-Supprian, A. Nenci, A. Toksoy, M. Krampert, M. Goebeler, R. Gillitzer, A. Israel, et al. 2002. TNF-mediated inflammatory skin disease in mice with epidermis-specific deletion of IKK2. *Nature* 417: 861–866.
31. Carroll, J. M., M. R. Romero, and F. M. Watt. 1995. Suprabasal integrin expression in the epidermis of transgenic mice results in developmental defects and a phenotype resembling psoriasis. *Cell* 83: 957–968.
32. Boehncke, W. H., W. Sterry, A. Hainzl, W. Scheffold, and R. Kaufmann. 1994. Psoriasiform architecture of murine epidermis overlying human psoriatic dermis transplanted onto SCID mice. *Arch. Dermatol. Res.* 286: 325–330.
33. Bullard, D. C., K. Scharffetter-Kochanek, M. J. McArthur, J. G. Chosay, M. E. McBride, C. A. Montgomery, and A. L. Beaudet. 1996. A polygenic mouse model of psoriasiform skin disease in CD18-deficient mice. *Proc. Natl. Acad. Sci. USA* 93: 2116–2121.
34. Kess, D., T. Peters, J. Zamek, C. Wickenhauser, S. Tawadros, K. Loser, G. Varga, S. Grabbe, R. Nischt, C. Sunderkotter, et al. 2003. $CD4^+$ T cell-associated pathophysiology critically depends on CD18 gene dose effects in a murine model of psoriasis. *J. Immunol.* 171: 5697–5706.
35. Barlow, S. C., H. Xu, C. T. Weaver, J. R. Lindsey, T. R. Schoeb, and D. C. Bullard. 2004. Development of dermatitis in CD18-deficient PL/J mice is not dependent on bacterial flora, and requires both $CD4^+$ and $CD8^+$ T lymphocytes. *Int. Immunol.* 16: 345–351.
36. Barlow, S. C., R. G. Collins, N. J. Ball, C. T. Weaver, T. R. Schoeb, and D. C. Bullard. 2003. Psoriasiform dermatitis susceptibility in Itgb2(tm1Bay) PL/J mice requires low-level CD18 expression and at least two additional loci for progression to severe disease. *Am. J. Pathol.* 163: 197–202.
37. Wilson, R. W., W. E. O'Brien, and A. L. Beaudet. 1989. Nucleotide sequence of the cDNA from the mouse leukocyte adhesion protein CD18. *Nucleic Acids Res.* 17: 5397.
38. Wilson, R. W., C. M. Ballantyne, C. W. Smith, C. Montgomery, A. Bradley, W. E. O'Brien, and A. L. Beaudet. 1993. Gene targeting yields a CD18-mutant mouse for study of inflammation. *J. Immunol.* 151: 1571–1578.
39. Jirholt, J., A. K. Lindqvist, J. Karlsson, A. Andersson, and R. Holmdahl. 2002. Identification of susceptibility genes for experimental autoimmune encephalomyelitis that overcome the effect of protective alleles at the eae2 locus. *Int. Immunol.* 14: 79–85.
40. Truett, G. E., P. Heeger, R. L. Mynatt, A. A. Truett, J. A. Walker, and M. L. Warman. 2000. Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and tris (HotSHOT). *BioTechniques* 29: 52, 54.
41. Jemec, G. B., and H. C. Wulf. 1997. The applicability of clinical scoring systems: SCORAD and PASI in psoriasis and atopic dermatitis. *Acta Derm. Venereol.* 77: 392–393.
42. Waterston, R. H., K. Lindblad-Toh, E. Birney, J. Rogers, J. F. Abril, P. Agarwal, R. Agarwala, R. Ainscough, M. Alexandersson, P. An, et al. 2002. Initial sequencing and comparative analysis of the mouse genome. *Nature* 420: 520–562.
43. R Development Core Team. 2004. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
44. Broman, K. W., H. Wu, S. Sen, and G. A. Churchill. 2003. R/qtl: QTL mapping in experimental crosses. *Bioinformatics* 19: 889–890.
45. Sen, S., and G. A. Churchill. 2001. A statistical framework for quantitative trait mapping. *Genetics* 159: 371–387.
46. Churchill, G. A., and R. W. Doerge. 1994. Empirical threshold values for quantitative trait mapping. *Genetics* 138: 963–971.
47. Karlsson, J., X. Zhao, I. Lonskaya, M. Neptin, R. Holmdahl, and A. Andersson. 2003. Novel quantitative trait loci controlling development of experimental autoimmune encephalomyelitis and proportion of lymphocyte subpopulations. *J. Immunol.* 170: 1019–1026.
48. Gelfand, J. M., R. Weinstein, S. B. Porter, A. L. Neimann, J. A. Berlin, and D. J. Margolis. 2005. Prevalence and treatment of psoriasis in the United Kingdom: a population-based study. *Arch. Dermatol.* 141: 1537–1541.
49. Madland, T. M., E. M. Apalset, A. E. Johannessen, B. Rossebo, and J. G. Brun. 2005. Prevalence, disease manifestations, and treatment of psoriatic arthritis in Western Norway. *J. Rheumatol.* 32: 1918–1922.
50. Kawada, A., T. Tezuka, Y. Nakamizo, H. Kimura, H. Nakagawa, M. Ohkido, A. Ozawa, A. Ohkawara, H. Kobayashi, S. Harada, and A. Igarashi. 2003. A survey of psoriasis patients in Japan from 1982 to 2001. *J. Dermatol. Sci.* 31: 59–64.
51. Ahmed, S. A., B. D. Hissong, D. Verthelyi, K. Donner, K. Becker, and E. Karpuzoglu-Sahin. 1999. Gender and risk of autoimmune diseases: possible role of estrogenic compounds. *Environ. Health Perspect.* 107(Suppl. 5): 681–686.
52. Fillmore, P. D., E. P. Blankenhorn, J. F. Zachary, and C. Teuscher. 2004. Adult gonadal hormones selectively regulate sexually dimorphic quantitative traits observed in experimental allergic encephalomyelitis. *Am. J. Pathol.* 164: 167–175.
53. Lahita, R. G. 1999. The role of sex hormones in systemic lupus erythematosus. *Curr. Opin. Rheumatol.* 11: 352–356.
54. Verthelyi, D. 2001. Sex hormones as immunomodulators in health and disease. *Int. Immunopharmacol.* 1: 983–993.
55. Hewett, D., L. Samuelsson, J. Polding, F. Enlund, D. Smart, K. Cantone, C. G. See, S. Chadha, A. Inerot, C. Enerback, et al. 2002. Identification of a psoriasis susceptibility candidate gene by linkage disequilibrium mapping with a localized single nucleotide polymorphism map. *Genomics* 79: 305–314.
56. Samuelsson, L., C. Stiller, C. Friberg, C. Nilsson, A. Inerot, and J. Wahlstrom. 2004. Association analysis of cystatin A and zinc finger protein 148, two genes located at the psoriasis susceptibility locus *PSORS5*. *J. Invest. Dermatol.* 122: 1399–1400.
57. Johansson, A. C., M. Sundler, P. Kjellen, M. Johannesson, A. Cook, A. K. Lindqvist, B. Nakken, A. I. Bolstad, R. Jonsson, M. Alarcon-Riquelme, and R. Holmdahl. 2001. Genetic control of collagen-induced arthritis in a cross with NOD and C57BL/10 mice is dependent on gene regions encoding complement factor 5 and FcγRIIb and is not associated with loci controlling diabetes. *Eur. J. Immunol.* 31: 1847–1856.
58. Wakeland, E., L. Morel, K. Achey, M. Yui, and J. Longmate. 1997. Speed congenics: a classic technique in the fast lane (relatively speaking). *Immunol. Today* 18: 472–477.
59. Olofsson, P., J. Holmberg, J. Tordsson, S. Lu, B. Akerstrom, and R. Holmdahl. 2003. Positional identification of *Ncf1* as a gene that regulates arthritis severity in rats. *Nat. Genet.* 33: 25–32.
60. Lander, E. S., L. M. Linton, B. Birren, C. Nusbaum, M. C. Zody, J. Baldwin, K. Devon, K. Dewar, M. Doyle, W. FitzHugh, et al. 2001. Initial sequencing and analysis of the human genome. *Nature* 409: 860–921.