Immunogenetics (2001) 53:15–21
DOI 10.1007/s002510000286

ORIGINAL PAPER

**Yuki Kohara · Kyoko Tanabe · Kunie Matsuoka
Naotoshi Kanda · Hiroshi Matsuda
Hajime Karasuyama · Hiromichi Yonekawa**

A major determinant quantitative-trait locus responsible for atopic dermatitis-like skin lesions in NC/Nga mice is located on Chromosome 9

Received: 27 September 2000 / Revised: 17 November 2000 / Published online: 31 January 2001

Abstract NC/Nga (NC) is a newly discovered model mouse for human atopic dermatitis, NC mice showing specific symptoms such as dermatitis and overproduction of IgE. To detect the loci responsible for the onset of dermatitis in the mice, backcross (N2) progeny between (NC×MSM/MS)F1 and NC were generated, where MSM/MS is an inbred strain from Japanese wild mice, *Mus musculus molossinus*. Linkage disequilibrium between dermatitis and various chromosome-specific microsatellite markers was then examined in the N2 segregants with severe dermatitis. The analysis revealed that the locus of the major determinant (designated here as *derm1*) was tightly linked to *D9Mit163*, *D9Mit72*, *D9Mit143*, *D9Mit103*, *D9Mit207*, and *D9Mit209*, because these markers showed the highest and most significant χ^2 values. Since no recombination was observed among the markers in our linkage map, a radiation hybrid (RH) panel was applied to locate the *derm1* locus more precisely. The markers were separated on the RH map, and their order was *D9Mit163–D9Mit72–D9Mit143–D9Mit103–D9Mit207–D9Mit209* from the centromere. Several functional candidate genes are located near the locus *derm1*. These candidates are *Thy1*, *Cd3d*, *Cd3e*, *Cd3g*, *Il10ra*, *Il18*, and *Csk*, all of which could be involved in allergic responses through effects on T-cell function. Of these candidates, *Csk* is the strongest for NC dermatitis, since its map position was most tightly linked to the *derm1* locus.

Keywords Atopy · Model mouse · Linkage disequilibrium · Microsatellite · RH panel

Y. Kohara · K. Tanabe · H. Yonekawa (✉)
Department of Laboratory Animal Science,
The Tokyo Metropolitan Institute of Medical Science,
3-18-22 Honkomagome, Bunkyo-ku, Tokyo, 113-8613, Japan
E-mail: yonekawa@rinshoken.or.jp
Phone: +81-3-38232101
Fax: +81-3-38247445

K. Matsuoka · H. Karasuyama
Department of Immunology,
The Tokyo Metropolitan Institute of Medical Science,
3-18-22 Honkomagome, Bunkyo-ku, Tokyo, 113-8613, Japan

Y. Kohara · N. Kanda · H. Matsuda
Department of Veterinary Medicine,
Tokyo University of Agriculture and Technology,
3-5-8 Saiwai-cho, Fuchu, Tokyo, 183-8509, Japan

Introduction

Atopic dermatitis (AD) is characterized by complex symptoms such as chronically relapsing, extreme pruritus, and eczematous skin disease, which are associated with IgE hyperresponsiveness to environmental allergens (Hanifin and Rajka 1980). The rapidly increased prevalence of AD over the past three decades has led investigators to hasten elucidation of its pathogenesis and to exploit radical treatments in this disorder (Taylor et al. 1984).

Causative factors for AD can be considered under two general categories, environmental and genetic. House dust, mites, and air pollution are among the former, and their involvement has been proven by epidemiological evidence (Hanifin 1982, 1989). On the other hand, genetic factors have been discussed. For example, several different candidate regions on chromosomes, or genes, have been reported from linkage studies on atopic and nonatopic phenotypes; these candidates include *IL4ra* (Hershey et al. 1997), 5q31–33 (Marsh et al. 1994; Ruffili and Bonini 1997), 11q13 (Cookson et al. 1989, 1992; Shirakawa et al. 1994), 6p21–23 (Young et al. 1994; Zwollo et al. 1989), and 14q12 (Moffatt et al. 1994). This situation is due to the difficulty of linkage analysis for multigenic diseases in humans, mainly caused by genetic background effects. Additionally, appropriate animal modes for human AD have been lacking.

Recently, one promising mouse model for human AD was discovered by Matsuda and co-workers (1997). This model is an inbred strain, NC/Nga (NC), which was originally established by Dr. Kondo of Nagoya University in 1957 (Festing 1996; Kondo 1984). NC mice spontaneously suffer severe dermatitis in the presence of nonspecified allergens. Morbid NC mice show symptoms such as itching, erythema, hemorrhage, edema, crust, drying, and excoriation/erosion hyperplasia of the epidermis region in the face, neck, and/or back. These symptoms are exacerbated by aging. Furthermore, NC mice display some characteristic features diagnosed by histopathological examination, e.g., macrophages and eosinophils invading the dermis, increases and activation of mast cells and lymphocytes, reduction of ceramide (Aioi et al., 2001), appearance of activated mast cells, and $CD4^+$ T cells in the lesion. These lines of evidence suggest that the symptoms shown by NC mice are similar to those of human AD from the clinical, pathological, and immunological point of view.

Mice have several advantages over humans in genetic analyses e.g., uniformity of genetic background in an inbred strain, free choice of generating genetic crosses, and ease of obtaining large numbers of F2 or N2 segregants. The discovery of conserved linkage between humans and mice facilitates identification of genes responsible for human diseases using mice models for human diseases. Therefore, we sought to identify the genetic loci responsible for human AD using the model mouse NC, and the loci identified here will be used to elucidate the genetic factors determining human AD through the conserved linkage between humans and mice.

Materials and methods

Mice

NC/Nga mice were obtained from Kanazawa University, and MSM/Ms (MSM) mice were from the National Institute of Genetics (NIG, Mishima, Japan). N2 segregants were generated by backcrossing between (NC×MSM)F1 and NC. All mice were housed under conventional conditions (25±3 °C, 50 ±5% controlled humidity, and a 14/10 h light/ dark schedule) to develop dermatitis (Matsuda et al. 1997). Regular laboratory diet (F-2; Funabashi Farm Co., Chiba, Japan) and water were supplied ad libitum. All animal experiments were approved by the committee for guidelines and regulation of animal experiments in our institute.

Dermatitis score

Onsets of dermatitis in the mice were observed until the mice reached 24 weeks of age. Each NC, MSM, (NC×MSM)F1 and [(NC×MSM)F1×NC]N2 mouse was examined every week for the clinical symptoms of dermatitis (itching, erythema/hemorrhage, edema, and excoriation/erosion) and graded according to the severity of the dermatitis: severe, mild, and no dermatitis. Severe dermatitis satisfied three conditions mentioned: (1) more than half the area of either ear lobe was torn by scratching, (2) dermatitis with more than 50 mm^2 of decalvant area was seen on either the neck or back, and (3) a more than threefold thickening and invasion of mast cells and eosinophils into the epidermis was observed in the dermatitis area by histopathological inspection (see Fig. 1). Histopathological inspection was carried out by hematoxylin and eosin staining for general histology, toluidine blue staining for mast cells, and Congo red staining for eosinophils with granules.

Genotyping of mice

Livers were removed from N2 mice with severe dermatitis and their genomic DNA samples were purified using DNAzol (Life Technologies, Gaithersburg, Md.). Genome-wide genotyping for each N2 segregant was carried out by PCR using the segregant's genomic DNA as a template. PCR primer sets specific for mouse chromosomes (Map Pairs) were purchased from Research Genetics (Huntsville, Ala.). Genomic PCR was performed in reactions (15 µl) containing genomic DNA (100 ng) and AmpliTaq Gold (PE Applied Biosystems) under standard conditions: initial denaturation for 5 min at 95 °C, 35 cycles of 94 °C for 30 s, 55 °C for 1 min, and 72 °C for 90 s, followed by a final extension for 5 min at 72 °C.

Statistical analysis

To map major quantitative trait loci (QTLs) for dermatitis, genotyping data on each marker were analyzed by the χ^2 test. χ^2 resultswere evaluated by the values for suggestive linkage ($\chi^2 \geq 8.7$) and for significant linkage ($\chi^2 \geq 15.2$) (Lander and Kruglyak 1995; Lander and Schork 1994).

Determination of locus order on chromosomes

The ordering of loci on chromosomes was determined by linkage analysis using 93 N2 segregants with severe dermatitis. To generate N2 segregants with severe dermatitis, mating groups were made by combining two (NC×MSM)F1 female mice and one male NC mouse. Finally we checked 22 mating groups for this research. When we found discordance of locus order between the maps approved by the IMGC chromosome committee and ours (shown with asterisks in Fig. 2), we verified the order by referring to our other linkage map that had been generated using 200 individuals randomly chosen from the 594 [(NC×MSM)F1×NC]N2 segregants (data not shown).

Radiation hybrid mapping

To determine the precise order of eight candidate genes, PCR primers were set on single-nucleotide polymorphism regions of each gene. The conditions of genomic PCR for each gene were the same as described above. We screened 100 cell hybrids of the Radiation Hybrid Mapping Panel (T31 Mouse/Hamster RH Panel, Research Genetics). To evaluate the order of each marker, we analyzed our radiation hybrid (RH) screening results using a software program, Auto-RHMAPPER (Whitehead Institute/MIT Center for Genome Research; http://www.genome.wi.mit.edu/cgi-bin/mouse_rh/rhmap-auto/rhmapper.cgi).

Results

Measurement of dermatitis score

We generated 43 individual (NC×MSM)F1 progeny and 594 [(NC×MSM)F1×NC]N2 segregants. Then we observed the onset of dermatitis in the parent strains,

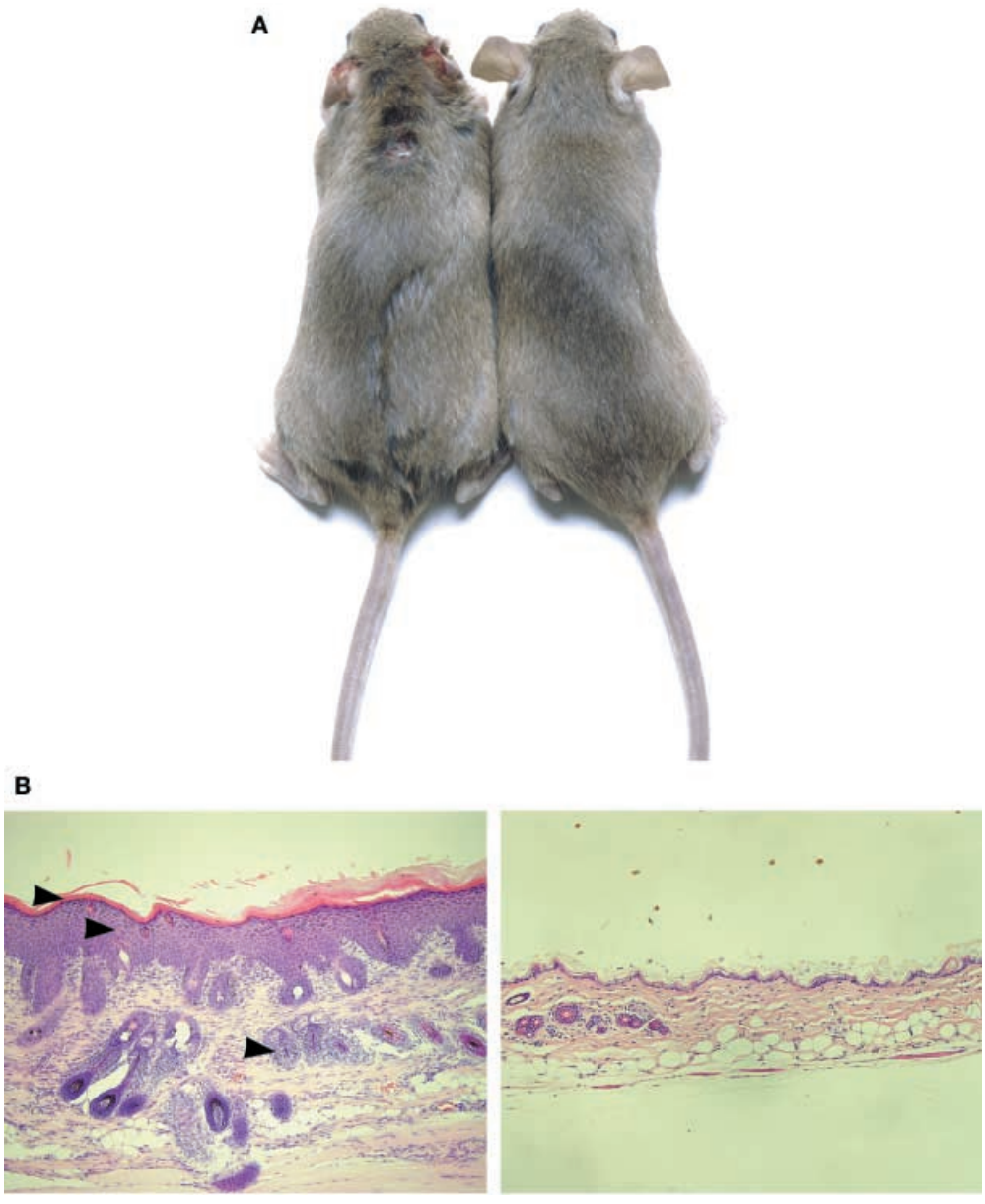

Fig. 1 **A** Clinical skin features and severity of dermatitis in [(NC×MSM)F1×NC]N2 segregants. Segregants with dermatitis (*left*), and without dermatitis (*right*). **B** Histological features of skin lesions in the segregants. Hematoxylin-and-eosin-stained sections of 17-week-old segregants without dermatitis (*right*) where there are no significant findings, and with severe dermatitis (*left*) showing marked hyperplasia and thickening of the epidermis (*arrowheads*). Infiltration of eosinophils with degranulation and dermal deposition of eosinophilic materials are seen, and lymphocytes and macrophages are also prominent (these cells are deeply stained by hematoxylin)

NC and MSM, and their F1 and N2 progeny. About 70% of NC mice were affected by dermatitis from 8 weeks of age under the conventional conditions, whereas no MSM mice showed any symptoms of dermatitis. Almost all NC mice were affected by the disease at 12 weeks of age under conventional conditions. This result is consistent with that reported previously by Matsuda and co-workers (1997). Therefore, observation was continued until the mice were over 24 weeks of age to confirm the onset of dermatitis. Of 43 (NC×MSM)F1 individuals, 4 showed weak dryness of skin over 24 weeks of age, although we could not clearly judge them as showing the onset of dermatitis (Table 1).

The 93 N2 segregant mice with severe dermatitis were subjected to histopathological examination. All of them, like authentic NC mice, showed hyperplasia of the epidermis, and increased numbers of mast cells and eosinophils with marked degranulation in their ears and necks (Fig. 1). We thus confirmed that the dermatitis observed in the N2 segregants was the same as that shown by authentic NC mice.

Of 594 N2 segregants, 93 and 190 showed severe and light dermatitis, respectively, and the rest showed no dermatitis. This suggests that a major locus is segregating for a recessive trait, which is consistent with

Table 1 Clinical skin features and severity of dermatitis in mice

Clinical score	NC	MSM	(NC×MSM)F1	(F1×NC)N2
+	37	0	0	93
+/–	5	0	4	190
–	11	19	39	311

Locus	Position (cM)	NC/NC Homo	NC/MSM Hetero	Total	χ^2
D1Mit1	8,7	23	23	46	0
D1Mit3	11.0	21	16	37	0,7
D1Mit410	17.0	38	44	82	0,4
D1Mit5	32,8	44	37	81	0,6
D1Mit19	36,9	49	37	86	1,7
D1Mit46	43,1	47	35	82	1,8
D1Mit8	52.0	50	37	87	1,9
D1Mit10	56,6	43	34	77	1,1
D1Mit311	63,1	41	33	74	0,9
D1Mit30	70.0	48	43	91	0,3
D1Mit36*	92,3	51	37	88	2,2
D1Mit14*	81,6	14	12	26	0,2
D1Mit37	101.0	17	13	30	0,5
D2Mit3	5.0	39	52	91	1,9
D2Mit464	9,5	31	41	72	1,4
D2Mit268	18.0	31	41	72	1,4
D2Mit296	18.0	38	46	84	0,8
D2Mit203	28.0	34	33	67	0
D2Mit155	30.0	29	30	59	0
D2Mit61	34.0	30	29	59	0
D2Mit56	41.0	30	29	59	0
D2Mit13	47,5	46	40	86	0,4
D2Mit259	80.0	42	44	86	0
D2Mit286	87.0	26	22	48	0,3
D2Mit263	92.0	21	19	40	0,1
D2Mit147	102.0	24	24	48	0
D3Mit60	0	48	39	87	0,9
D3Mit46	13,8	40	34	74	0,5
D3Mit4	22.0	43	41	84	0
D3Mit7	26,4	41	41	82	0
D3Mit209	33,7	45	42	87	0,1
D3Mit12	49,2	32	33	65	0
D3Nds2	64,1	42	45	87	0,1
D3Mit38	70,3	12	9	21	0,4
D3Mit31	76,2	11	8	19	0,5
D3Mit19	87,6	14	11	25	0,4
D4Mit18	5,2	53	38	91	2,5
D4Mit4	12,1	50	41	91	0,9
D4Mit53	19,8	43	38	81	0,3
D4Mit44	28,6	39	42	81	0,1
D4Mit9	44,5	40	41	81	0
D4Mit46	51.0	47	44	91	0,1
D4Mit40	59.0	39	52	91	1,9
D4Mit71	61,9	34	57	91	**5,8**
D4Mit13	71.0	35	56	91	**4,8**
D4Mit59	78,9	33	53	86	**4,7**
D5Mit146	1,0	48	43	91	0,3
D5Mit387	15.0	42	46	88	0,2
D5Mit81	28.0	40	42	82	0
D5Mit300	34.0	46	45	91	0
D5Mit239	58.0	50	41	91	0,9
D5Mit68	65.0	51	39	90	1,6
D5Mit161	70.0	50	37	87	1,9
D5Mit214	70.0	49	36	85	2
D5Mit30	72,0	49	36	85	2
D5Mit32	78.0	41	33	74	0,9
D5Mit51	81.0	44	41	85	0,1
D5Mit287	86.0	47	41	88	0,4
D6Mit46	7,3	44	46	90	0
D6Nds4	20,6	40	47	87	0,6
D6Mit94	29.0	42	49	91	0,5
D6Mit6*	35,3	42	49	91	0,5
D6Mit4*	33,5	41	50	91	0,9
D6Mit213	37.0	41	50	91	0,9
D6Mit102	38,5	44	47	91	0,1
D6Mit104	45,5	44	47	91	0,1
D6Mit61	62,3	41	50	91	0,9
D6Mit15	74.0	45	46	91	0
D7Mit21	0,5	13	5	18	**3,6**
D7Mit77	9,4	13	6	19	2,6
D7Mit156	16.0	43	41	84	0
D7Nds5	23.0	37	32	69	0,4
D7Mit230	24,5	38	37	75	0
D7Mit18	26,4	34	33	67	0
D7Mit120	26,8	34	33	67	0
D7Mit213	37.0	42	41	83	0
D7Mit185	50.0	36	33	69	0,1
D7Mit253	52,8	48	41	89	0,6
D7Mit101	60.0	40	32	72	0,9
D7Mit71	65,2	38	32	70	0,5
D7Mit14	69.0	45	38	83	0,6
D8Mit172	8.0	49	40	89	0,9
D8Mit65	22,5	43	42	85	0
D8Mit6	30.0	35	37	72	0,1
D8Mit106	38.0	33	35	68	0,1
D8Mit45	40.0	36	40	76	0,2
D8Mit11	46.0	32	38	70	0,5
D8Mit34	53.0	36	43	79	0,6
D8Mit89	59.0	13	19	32	1,1
D8Mit49	67.0	12	19	31	1,6
D8Mit93	72.0	20	24	44	0,4
D9Mit59	1.0	55	38	93	**3,1**
D9Mit2	6.0	62	31	93	**10,3**
D9Mit253	21.0	62	31	93	**10,3**
D9Mit328	23.0	62	31	93	**10,3**
D9Mit140	25.0	62	31	93	**10,3**
D9Mit23	26.0	63	30	93	**11,7**
Thy1	26.0	63	30	93	**11,7**
CD3d	26.0	63	30	93	**11,7**
CD3e	26.0	63	30	93	**11,7**
CD3g	26.0	63	30	93	**11,7**
IL10ra	26.0	64	29	93	**13,2**
D9Mit154	27.0	64	29	93	**13,2**
IL18*	29.0	64	29	93	**13,2**
D9Mit99*	29.0	64	29	93	**13,2**
D9Mit130*	27.0	65	28	93	**14,7**
D9Mit94	28.0	65	28	93	**14,7**
D9Mit4	29.0	65	28	93	**14,7**
Cyp19	31.0	65	28	93	**14,7**
D9Mit6	31.0	65	28	93	**14,7**
D9Mit48*	34.0	65	28	93	**14,7**
D9Mit163*	33.0	66	27	93	**16,4**
D9Mit72*	33.0	66	27	93	**16,4**
D9Mit143*	33.0	66	27	93	**16,4**
D9Mit103*	33.0	66	27	93	**16,4**
D9Mit207*	33.0	66	27	93	**16,4**
D9Mit209	34.0	66	27	93	**16,4**
D9Mit105	35.0	64	29	93	**13,2**
D9Mit9	48.0	63	30	93	**11,7**
D9Mit19	71.0	55	38	93	**3,1**
D9Mit82	74.0	52	41	93	1,3
D10Mit279	2.0	57	34	91	**5,8**
D10Mit4	19.0	58	33	91	**6,9**
D10Mit148	29,5	57	34	91	**5,8**
D10Mit138	30.0	57	33	90	**6,4**
D10Mit221	31.0	57	33	90	**6,4**
D10Mit15	35.0	58	32	90	**7,5**
D10Mit115	38,4	56	35	91	**4,8**
D10Nds2	58.0	47	30	77	**3,8**
D10Mit74	65.0	53	37	90	2,8
D10Mit103	70.0	59	32	91	8
D11Mit71	1,1	52	39	91	1,9
D11Mit77	2.0	50	38	88	1,6
D11Mit19	13.0	51	37	88	2,2
D11Mit53	16.0	49	35	84	2,3
D11Mit84	17.0	49	35	84	2,3
D11Mit108	18.0	49	35	84	2,3
D11Mit51	18.0	50	38	88	1,6
D11Mit20	20.0	49	37	86	1,7
D11Mit21	20.0	51	39	90	1,6
D11Mit64	28.0	54	36	90	**3,6**
D11Mit5	37.0	51	37	88	2,2
D11Mit31	40.0	53	36	89	**3,3**
D11Mit70	54.0	48	36	84	1,7
D11Mit14	57.0	46	44	90	0
D11Mit69	71.0	41	37	78	0,2
D12Mit37	1.0	51	40	91	1,3
D12Mit56	3.0	49	42	91	0,5
D12Mit58	6.0	46	45	91	0
D12Nds11	6.0	46	45	91	0
D12Mit84	11.0	45	46	91	0
D12Mit85	13.0	45	43	88	0
D12Mit59	13.0	45	46	91	0
D12Mit171	13.0	45	46	91	0
D12Mit2	19.0	46	45	91	0
D12Nds1	27.0	43	48	91	0,3
D12Mit69	28.0	45	46	91	0
D12Mit4	34.0	46	45	91	0
D12Mit157	37.0	45	46	91	0
D12Mit214	38.0	45	46	91	0
D12Mit239	44.0	47	44	91	0,1
D12Mit118	45.0	47	43	90	0,2
D12Mit179	45.0	47	44	91	0,1
D12Mit28	52.0	41	50	91	0,9
D12Mit20	58.0	44	47	91	0,1
D13Mit16	10.0	39	49	88	1,1
D13Mit15	10.0	35	46	81	1,5
D13Mit34	30.0	38	52	90	2,2
D13Mit54	35.0	31	43	74	1,9
D13Mit9	45.0	39	49	88	1,1
D13Mit37	51.0	35	44	79	1
D13Mit131	75.0	41	48	89	0,6
D14Mit2	5.0	49	42	91	0,5
D14Mit44	10.0	50	41	91	0,9
D14Mit45	12.5	50	41	91	0,9
D14Mit5	22.5	52	39	91	1,9
D14Mit216	32.5	52	39	91	1,9
D14Mit225	42.5	50	41	91	0,9
D14Mit228	46.0	44	46	90	0
D14Mit8	48.2	43	42	85	0
D14Mit36	63.0	46	45	91	0
D15Mit13	6,7	49	41	90	0,7
D15Mit6	13,7	40	39	79	0
D15Mit17	32.0	43	44	87	0
D15Mit1	46.3	34	31	65	0,1
D15Mit37	48,5	35	33	68	0,1
D15Mit41	58,8	43	40	83	0,1
D15Mit15	64,8	47	44	91	0,1
D16Mit182	3,4	9	5	14	1,1
D16Mit160*	4,6	10	9	19	0,1
D16Mit131*	4,3	54	36	90	**3,6**
D16Mit144	13,2	52	39	91	1,9
D16Mit1	14.0	52	39	91	1,9
D16Mit50	53,5	40	51	91	1,3
D16Mit94	57,6	41	50	91	0,9
D16Mit71*	70,65	37	54	91	**3,2**
D16Mit52*	66,8	39	52	91	1,9
D17Mit164	4,1	38	29	67	1,2
D17Mit133	10,4	27	20	47	1
D17Mit21	18,64	27	20	47	1
D17Mit24	20,42	28	19	47	1,7
D17Mit9	29,4	35	31	66	0,2
D17Mit20	34,3	37	33	70	0,2
D17Mit119	38,5	36	29	65	0,8
D17Mit72	47,4	37	27	64	1,6
D17Mit96	54,6	49	41	90	0,7
D17Mit123	56,7	11	13	24	0,2
D18Mit19	2.0	50	41	91	0,9
D18Mit30	4.0	36	31	67	0,4
D18Mit27	13.0	35	32	67	0,1
D18Mit14	18.0	37	31	68	0,5
D18Mit35	24.0	36	32	68	0,2
D18Mit40	37.0	31	26	57	0,4
D18Mit1	37.0	30	27	57	0,2
D18Mit130	58.0	43	44	87	0
D19Mit32	0	47	38	85	1
D19Mit16	15.0	36	39	75	0,1
D19Mit40	25.0	34	37	71	0,1
D19Mit20	26.0	33	37	70	0,2
D19Mit11	41.0	37	40	77	0,1
D19Mit67	43.0	36	38	74	0,1
D19Mit55	53.0	42	49	91	0,5
DXMit124	2,8	45	46	91	0
DXMit81	9,25	43	47	90	0,2
DXNds1	17.0	47	44	91	0,1
DXMit63	38.0	45	44	89	0
DXMit79	50,5	49	41	90	0,7
DXMit4	58.0	49	41	90	0,7
DXMit121	67.0	51	40	91	1,3
DXMit100	72,7	50	41	91	0,9

Fig. 2 Whole-genome scan of 93 segregants between (NC×MSM)F1 and NC with severe dermatitis using 225 markers on 19 mouse autosomes. The map position of each DNA marker is shown in cM. Markers with *asterisks* showed discordance between the linkage map approved by the IMGC chromosome committee and our map. The positions of *Thy1* (thymus cell antigen 1, theta), *Cd3d,e,g* (CD3 antigen delta, epsilon, and gamma polypeptide), *Il10ra* (interleukin-10 receptor, alpha), and *Il18* (interleukin-18) are also shown as candidate genes. *Homo* and *hetero* stand for homozygous and heterozygous at the DNA marker. The total number of segregants for the calculation of χ^2-values and the χ^2-values which exceed 3.0 are also presented

the results reported by Tsudzuki and co-workers (1997). We designated this locus as *derm1* following the nomenclature of Tsudzuki and co-workers (1997). However, we could not map any locus by linkage analysis for a single locus or by analysis for QTLs (by Map manager/QTL) using 200 randomly selected segregants.

Linkage disequilibrium analysis

The possibility was considered that the N2 segregants without dermatitis might be individuals that did not manifest dermatitis due to insufficient antigen challenge. We thus decided to use only the segregants with severe dermatitis. The 93 segregants with severe dermatitis were therefore subjected to linkage disequilibrium analysis. Each of the segregants was typed for 225 microsatellite markers and the ratio of homozygotes and heterozygotes for each microsatellite locus was subjected to the χ^2-test. The χ^2-score and the map positions of the 225 markers are summarized in Fig. 2. The highest χ^2-score of 16.4, which exceeded the significant level ($\chi^2 \geq 15.2$), was obtained for six markers: *D9Mit163*, *D9Mit72*, *D9Mit143*, *D9Mit103*, *D9Mit207*, and *D9Mit209*, which were located near the middle region of Chromosome (Chr) 9. This value reached the significant level of 15.4 (Lander and Kruglyak 1995). We thus judged that a major QTL that determines the onset of AD was located on this chromosomal segment and designated the QTL as *derm1*. The nomenclature *derm1* is derived from that reported by Tsuzduki and co-workers (1997).

Confirmation of locus order by linkage analysis

Since the linkage disequilibrium analysis showed a major determinant QTL for dermatitis on Chr 9, we confirmed the chromosomal order of the 225 loci. We found that the order of most loci was consistent with that approved by the IMGC chromosome committee (http://www.informatics.jax.ccr.searches/index.cgi). However, we found 17 loci showed discordance between the IMGC and our maps (the markers with asterisks in Fig. 2). To search for the genes responsible for the dermatitis in NC mice, we surveyed functional candidates on the MIT/Whitehead Institute linkage map. We found that seven candidates are located near the *derm1* locus. These candidates are thymus cell antigen 1, theta (*Thy1*), CD3 antigen delta, epsilon, and gamma polypeptide (*Cd3d,e,g*), interleukin-10 receptor, alpha (*Il10ra*), interleukin-18 (*Il18*), and C-terminal Src kinase (*Csk*), all of which are involved in T-cell functions. *Csk* could not be used in recombination analysis because of lack of polymorphism, but a location between *D9Mit163* and *D9Mit209* has been suggested. Since the *Csk* cDNA sequences did not show any polymorphisms in the coding region and exon-intron boundary between NC and MSM mice, we could not determine the position of the *Csk* locus on our linkage map. We also mapped the locus of cytochrome P450 19 aromatase (*Cyp19*) for the positional standard of the long arm of human Chr 15 (15q), because the boundary of the syntenic region between human Chr 11q and 15q is near this region of the mouse chromosome.

Analysis with an RH panel

To examine whether the six loci with no recombination shared the same position, we used a mouse-hamster RH panel. The six loci were separated on the RH map and their order was *D9Mit163*, *D9Mit72*, *D9Mit143*, *D9Mit103*, *D9Mit207*, and *D9Mit209* from the centromere (Fig. 3). We also determined the precise position and order of the candidate genes including *Csk*, which are tightly linked to *derm1*, on the RH map. *Csk* was the most closely linked to the *derm1* locus (Fig. 3). However, we could not determine the precise physical distance, because we have not yet completed physical mapping with a BAC contig, and because the distance in the RH panel (cR) does not correspond to that of the physical map.

Discussion

In this work, we demonstrated by linkage disequilibrium analysis that a major QTL that determines the AD-like skin lesions in NC mice (*derm1*) is located in the middle region of mouse Chr 9. As shown in Table 1, the ratio between segregants with dermatitis and those without dermatitis was almost 1:1 ($P<0.005$), and thus one recessive trait appears to be involved in the atopic dermatitis shown by NC mice. This seems consistent with the report by Tsudzuki and co-workers (1997). However, we failed to discover such a locus or any significant QTLs by standard linkage analysis for a single trait or by QTL analysis when we randomly chose 200 segregants with and without dermatitis. The reason for this may be as follows. The onset of atopic dermatitis is strongly influenced both by environmental factors (allergens) and by genetic factors (physical constitution or genetic background) in humans. If this is also true in mice, there are two groups with different genetic characteristics in the segregants without dermatitis: the mice in one group possessed resistant alleles for allergens and the other possessed susceptible alleles. If the latter had been exposed either very weakly or not at all to allergens, they would not have developed dermatitis. Unfortunately, we could not induce the onset of dermatitis in the NC mice by allergens, because we have not yet identified the allergens that induce the dermatitis in NC mice. Thus, we decided to examine the linkage disequilibrium

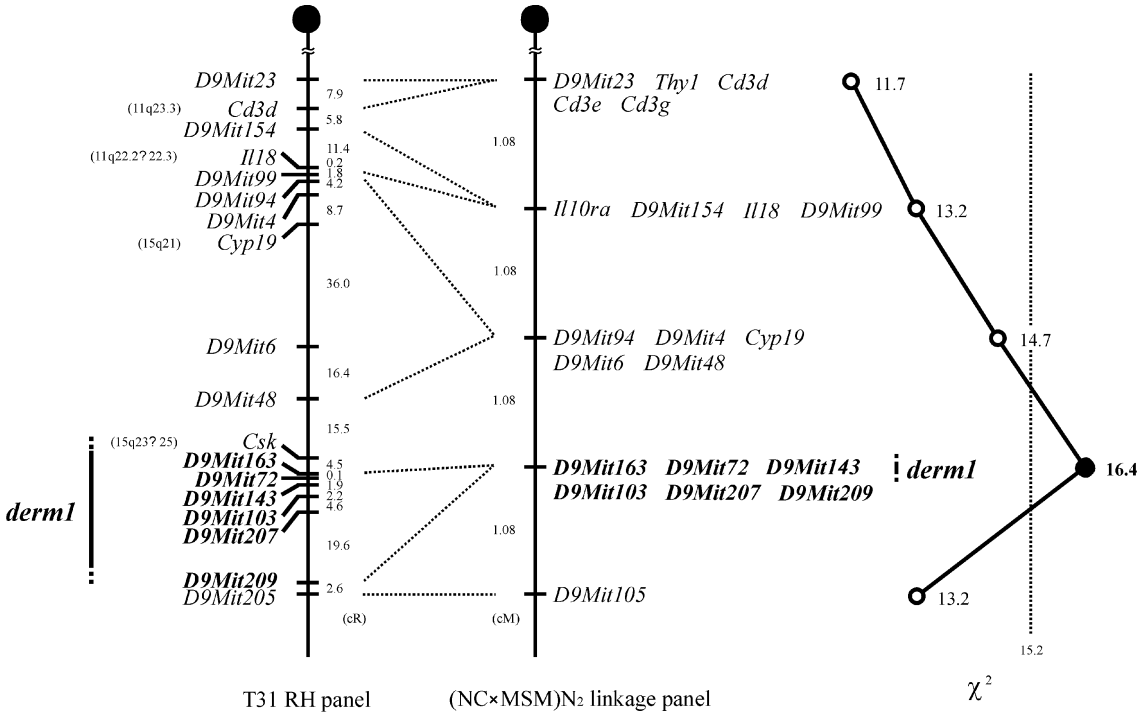

Fig. 3 A linkage map and a radiation hybrid (RH) map near the *derm1* locus on Chr 9. Locus symbols for genes are shown in Fig. 2, except *Cyp19* (cytochrome P-450 19 aromatase). Numeric designations shown to the *left* of the RH map are the homologous regions of the human chromosome at each locus

between dermatitis and various chromosome-specific microsatellite markers using only the segregants with severe dermatitis and, as a result, six significant markers were identified.

The *derm1* region on mouse Chr 9 is conserved on human Chr 11q22.2–23.3 and 15q21–25 (NCBI, Mouse To Human Homology Region Map, http://www.ncbi.nlm.nih.gov/Omim/Homology/). However, no genetic loci related to AD have been mapped to these regions in the human chromosome (Cookson et al. 1989, 1992; Hershey et al. 1997; Marsh et al. 1994; Moffatt et al. 1994; Ruffili and Bonini 1997; Shirakawa et al. 1994; Young et al. 1994; Zwollo et al. 1989). Nevertheless, many immune-competent genes have been mapped to this region in both mice and humans (Fig. 3). For example, we found that at least seven functional candidates are located near the *derm1* region, all of which are involved in T-cell functions. Although none of the candidates reached significant χ^2-scores, we could not eliminate them as AD candidates for the following reasons. Since QTL analysis, unlike linkage analysis for single loci, cannot precisely specify the map position of candidate QTLs, chromosomal regions for a QTL need to be expanded to avoid unintentional elimination of the candidate. Cytokines or receptors expressed in/on T cells and/or mast cells are suggested to be involved in human atopic disorders. For example, the CD3 delta, epsilon, and gamma chains form the T-cell receptor complex which transmits a proliferation signal through the plasma membrane (Samelson et al. 1985); thymus cell antigen 1, theta, may function as a signal transduction molecule in the cell membrane (Kroczek et al. 1986); the IL-10R alpha chain binds IL-10 with high affinity, and IL-10 induces proliferation of T cells (Ho et al. 1993); IL-18 functions in Th1-mediated immune response in collaboration with IL-12 (Akira 2000), and Csk functions as a negative regulator of Src family kinases (Bergman et al. 1992).

NC mice overproduce some Th2-specific chemokines (Vestergaad et al. 1999) and cytokines (Matsuda et al. 1997). Two of the most likely candidate genes for NC dermatitis are *Csk* and *Il18*. Since *Csk* was located much closer to the *derm1* locus in our RH map, it should be regarded as the strongest candidate. If *Csk* expression is up-regulated in NC mice, they cannot overproduce the Th2-specific cytokines (Matsuda et al. 1997) because Csk is a negative regulator of Src family kinases which activate T cells. Therefore, a lack of Csk molecules would alter Th2-type cytokine responses and accelerate the onset of NC dermatitis (Grewe et al. 1998). Alternatively, if Th2 cells are involved in NC dermatitis, the expression of IL-18 should be down-regulated in NC mice. However, the involvement of IL-18 is unlikely, given the distance of the *Il18* locus from the *derm1* locus, and that the χ^2-score of the *Il18* locus did not reach significance (Fig. 3). At any rate, the validity of these possibilities, in particular the relationship between AD and abnormal Th2 cell function, is now under investigation with measurements of the expression levels of Csk and IL-18.

Acknowledgements We thank Drs. Y. Kikkawa, M. Okamoto, and Y.Y. Maeda in our laboratory and Dr. A. Ishikawa of Nagoya University for technical help and valuable discussions. This work was supported in part by the Grant for Specially Promoted Research on Atopic Disorders from The Tokyo Metropolitan Government and by a Grant-in-Aid from the Ministry of Education, Science, Sports, and Culture, Japan.

References

Aioi A, Tonogaito H, Suto H, Hamada K, Ra C, Ogawa H, Maibach H, Matsuda H (2001) Impairment of skin barrier function in NC/NgaTnd mice as a possible murine model for atopic dermatitis. Br J Dermatol (in press)

Akira S (2000) The role of IL-18 in innate immunity. Curr Opin Immunol 12:59–63

Bergman M, Mustelin T, Oetken C, Partanen J, Flint NA, Amrein KE, Autero M, Burn P, Alitalo K (1992) The human p50csk tyrosine kinase phosphorylates p56lck at Tyr-505 and down regulates its catalytic activity. EMBO J.11:2919–2924

Cookson WO, Sharp PA, Faux JA, Hopkin JM (1989) Linkage between immunoglobulin E responses underlying asthma and rhinitis and chromosome 11q. Lancet 337:1292–1295

Cookson WO, Young RP, Sandford AJ, Moffatt MF, Shirakawa T, Sharp PA, Faux JA, Julier C, Nakumura Y, Lathrop GM, Hopkin JM (1992) Maternal inheritance of atopic IgE responsiveness on chromosome 11q. Lancet 340:381–384

Festing MFW (1996) Origins and chracteristics of inbred strains of mice. In: Lyon MF, Rastan S, Brown SDM (eds) Genetics variants and strains of the laboratory mouse. Oxford University Press, Oxford, pp 1537–1576

Grewe M, Bruijnzeel-Koomen CA, Schopf E, Thepen T, Langeveld-Wildschut AG, Ruzicka T, Krutmann J (1998) A role for Th1 and Th2 cells in the immunopathogenesis of atopic dermatitis. Immunol Today 19:359–361

Hanifin JM (1982) Atopic dermatitis. J Am Acad Dermatol 6:1–13

Hanifin JM (1989) Immunobiochemical aspects of atopic dermatitis. Acta Dermato-Venereol (Suppl) 144:45–47

Hanifin JM, Rajka G (1980) Diagnostic features of atopic dermatitis. Acta Derm Vernereol (Suppl) 92:44–47

Hershey GK, Friedrich MF, Esswein LA, Thomas ML, Chatila TA (1997) The association of atopy with a gain-of-function mutation in the alpha subunit of the interleukin-4 receptor. N Engl J Med 337:1720–1725

Ho AS, Liu Y, Khan TA, Hsu DH, Bazan JF, Moore KW (1993) A receptor for interleukin 10 is related to interferon receptors. Proc Natl Acad Sci USA 90:11267–11271

Kondo K (1984) The NC/Nga strain of mouse. In: Kyogoku M (ed) Experimental animal models of intractable diseases (in Japanese). Soft Science, Tokyo, pp 244–252

Kroczek RA, Gunter KC, Germain RN, Shevach EM (1986) Thy-1 functions as a signal transduction molecule in T lymphocytes and transfected B lymphocytes. Nature 322:10–16

Lander E, Kruglyak L (1995) Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat Genet 11:241–247

Lander ES, Schork NJ (1994) Genetic dissection of complex traits. Science 265:2037–2048

Marsh DG, Neely JD, Breazeale DR, Ghosh B, Freidhoff LR, Ehrlich-Kautzky E, Schou C, Krishnaswamy G, Beaty TH (1994) Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. Science 264:1152–1156

Matsuda H, Watanabe N, Geba GP, Sperl J, Tsudzuki M, Hiroi J, Matsumoto M, Ushio H, Saito S, Askenase PW, Ra C (1997) Development of atopic dermatitis-like skin lesion with IgE hyperproduction in NC/Nga mice. Int Immunol 9:461–466

Moffatt MF, Hill MR, Cornelis F, Schou C, Faux JA, Young RP, James AL, Ryan G, Souef P le, Musk AW, Hopkin JM, Cookson WO (1994) Genetic linkage of T-cell receptor alpha/delta complex to specific IgE responses. Lancet 343:1597–600

Ruffilli A, Bonini S (1997) Susceptibility genes for allergy and asthma. Allergy 52:256–273

Samelson LE, Harford JB, Klausner RD (1985) Identification of the components of the murine T cell antigen receptor complex. Cell 43:223–231

Shirakawa T, Hashimoto T, Furuyama J, Takeshita T, Morimoto K (1994) Linkage between severe atopy and chromosome 11q13 in Japanese families. Clin Genet 46:228–232

Taylor B, Wadsworth M, Wadsworth J, Peckham C (1984) Changes in the reported prevalence of childhood eczema since the 1939–45 war. Lancet 2:1255–1257

Tsudzuki M, Watanabe N, Wada A, Nakane Y, Hiroi J, Matsuda H (1997) Genetic analysis for dermatitis and IgE hyperproduction in the NC/Nga mouse. Immunogenetics 47:88–90

Vestergaard C, Yoneyama H, Murai M, Nakamura K, Tamaki K, Terashima Y, Imai T, Yoshie O, Irimura T, Mizutani H, Matsushima K (1999) Overproduction of Th2-specific chemokines in NC/Nga mice exhibiting atopic dermatitis-like lesions. J Clin Invest 104:1097–1105

Young RP, Dekker JW, Wordsworth BP, Schou C, Pile KD, Matthiesen F, Rosenberg WM, Bell JI, Hopkin JM, Cookson WO (1994) HLA-DR and HLA-DP genotypes and immunoglobulin E responses to common major allergens. Clin Exp Allergy 24:431–439

Zwollo P, Ansari AA, Marsh DG (1989) Association of class II DNA restriction fragments with responsiveness to *Ambrosia artemisiifolia* (short ragweed)-pollen allergen Amb a V in ragweed-allergic patients. J Allergy Clin Immunol 83:45–54