Genetic Polymorphisms in the Promoter of the Interferon Gamma Receptor 1 Gene Are Associated with Atopic Cataracts

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Purpose. Previous reports have shown genetic predisposition for atopic dermatitis (AD). Some of the severe complications of AD manifest in the eye, such as cataract, retinal detachment, and keratoconjunctivitis. This study was conducted to examine the genetic association between the atopy-related genes and patients with ocular complications (ocular AD).

Methods. Seventy-eighty patients with ocular AD and 282 healthy control subjects were enrolled in an investigation of the association between the atopy-related genes (*FCERB*, *IL13*, and *IFNGR1*) and ocular AD. Genetic association studies and functional analysis of single nucleotide polymorphisms (SNPs) were performed.

Results. The −56TT genotype in the *IFNGR1* promoter region was significantly associated with an increased risk of ocular AD under recessive models (χ^2 test, raw $P = 0.0004$, odds ratio 2.57). The −56TT genotype was more common in atopic cataracts. A reporter gene assay showed that, after stimulation with IFN-γ, the *IFNGR1* gene promoter construct that contained the −56T allele, a common allele in ocular AD patients, manifested higher transcriptional activity in lens epithelial cells (LECs) than did the construct with the −56C allele. Real-time PCR analysis demonstrated higher *IFNGR1* mRNA expression in the LECs in atopic than in senile cataracts. iNOS expression by *IFNGR1*-overexpressing LECs was enhanced on stimulation with IFN-γ and LPS.

Conclusions. The −56T allele in the IFNGR1 promoter results in higher IFNGR1 transcriptional activity and represents a genetic risk factor for atopic cataracts. (*Invest Ophthalmol Vis Sci.* 2007;48:583–589) DOI:10.1167/iovs.06-0991

Atopic dermatitis (AD) is a chronic inflammatory skin disease. In the acute stage, there is local infiltration by T-helper type 2 (Th2) cells; the subsequent infiltration by T-helper type 1 (Th1) cells produces chronic AD lesions.[1] Genetic epidemiologic studies on monozygotic twins[2] and genetic association studies[3,4] suggested a genetic susceptibility for AD. Because the severe complications of AD manifest in the eye as keratoconjunctivitis,[5] retinal detachment,[6] cataract,[7] and keratoconus, it is important to identify the genetic risk factors for ocular AD. Our group previously reported several atopy-related genes including high-affinity IgE receptor beta (FCERB),[8] interleukin 13 (IL-13),[9] and interferon gamma receptor (IFNGR),[10] and elucidated their functional roles. In the present study, we genotyped these candidate genes and compared the results in patients with AD with and without ocular AD and normal control subjects. As we found a strong association between the −56C/T single nucleotide polymorphism (SNP) in the promoter region of IFNGR1 and ocular AD, we further investigated the role of this SNP. IFNGR comprises the two transmembrane subunits IFNGR1 and IFNGR2. IFNGR1 is encoded by a 30-kb gene (chromosome 6) consisting of 7 exons,[11] and its expression is essential for ligand binding and signaling through Jak1 and STAT1; IFNGR2 transduces IFN-γ (IFNG) signals through Jak2.[11,12] Reduced IFNGR1 expression results in diminished JAK1/JAK2/STAT1 signaling,[13] and the expression of IFNGR1 is downregulated by *Mycobacterium* infections[14] and the TLR2 ligand[13]—factors known to counteract atopic diseases.[15,16] There is growing evidence of a role for IFNG in the effector phase of chronic AD[1] and allergic conjunctivitis.[17] Furthermore, overexpression of the IFNG gene in mouse epidermis produces eczema-like phenotypes,[18] and its overexpression in the lens induces cataracts in transgenic mice.[19] We found that among patients with ocular AD, in those with atopic cataracts, there was a strong genetic association with the IFNGR1 −56C/T SNP. Because the SNPs that placed individuals at high risk for ocular AD manifested higher IFNGR1 promoter activity in lens epithelial cells (LECs), we investigated the IFNGR1 mRNA levels in LECs obtained at cataract surgery.

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Supported by grants from the Japanese Millennium Project and the Eye Bank Association of Japan. AM was a recipient of a Bausch & Lomb overseas research fellowship.

Submitted for publication August 22, 2006; revised October 13, 2006; accepted December 19, 2006.

Disclosure: **A. Matsuda**, None; **N. Ebihara**, None; **N. Kumagai**, None; **K. Fukuda**, None; **K. Ebe**, None; **K. Hirano**, None; **C. Sotozono**, None; **M. Tei**, None; **K. Hasegawa**, None; **M. Shimizu**, None; **M. Tamari**, None; **K. Namba**, None; **S. Ohno**, None; **N. Mizuki**, None; **Z. Ikezawa**, None; **T. Shirakawa**, None; **J. Hamuro**, None; **S. Kinoshita**, None

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TABLE 1. Clinical Characters of the Ocular AD Patients

	Ocular AD	Control
Total subjects	78	282
AD + cataract*	48	—
AD + retinal detachment (RD)†	15	—
AKC‡	35	—
Mean age	27.18 (6–48 y)	38.21 (19–68 y)
Male:Female ratio	1.2:1.0	1.5:1.0

* Cataract only, 30 cases; caratact + RD, 10 cases; cataract + AKC, 6 cases; catract + RD + AKC, 2 cases.

† RD only, 3 cases; cataract + RD, 10 cases; cataract + RD + AKC, 2 cases.

‡ AKC only, 27 cases; cataract + AKC, 6 cases; cataract + RD + AKC, 2 cases.

MATERIALS AND METHODS

Antibodies and Cell Lines

We purchased anti-human major histocompatibility complex (MHC) class II antibody from Dako Japan (Kyoto, Japan) and Alexa-488 goat anti-mouse IgG antibody from Invitrogen Japan (Tokyo, Japan). Human immortalized LECs (SRA01/04), obtained from RIKEN cell bank (Tsukuba, Japan),[20] were maintained with 10% fetal bovine serum (FBS) in minimum essential medium (MEM, Invitrogen).

Subjects

In all patients with AD, the disease was diagnosed according to the criteria of Hanifin and Rajka.[21] Peripheral blood was obtained from 78 patients with (Table 1) and 186 without ocular AD.[4] The patients were recruited at Juntendo University Hospital, Yamaguchi University Hospital, Takao Hospital, Kyoto Prefectural University Hospital, Japan Red Cross Society Nagoya 2nd Hospital, Hokkaido University Hospital, and Yokohama City University Hospital. Atopic keratoconjunctivitis (AKC) was diagnosed according to the criteria of the Japanese Ophthalmological Society, and atopic cataracts were detected by slit lamp examination. The control subjects were 282 randomly selected, population-based individuals 19 to 68 years of age (mean, 38.21) with no atopy-related diseases. All study subjects were ethnic Japanese. According to the rules of the process committee at SNP Research Center of RIKEN, written informed consent was obtained from all participants; parental consent was obtained for individuals younger than 16 years. The study was conducted in accordance with the tenets of the Declaration of Helsinki.

Screening for Genetic Polymorphisms

Genetic polymorphisms screening was carried out essentially as previously described.[3,4] The IFNGR1 genomic region targeted for SNP discovery included a 2.5-kb continuous region 5′ to exon 1 (promoter region) and 11 exons, each with a minimum of 200 bases of flanking intronic sequences. We designed primer sets on the IFNGR1 genomic sequence (GenBank: AL050337; http://www.ncbi.nlm.nih.gov/Genbank; provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD). Each polymerase chain reaction (PCR) was performed with 5 ng of genomic DNA from 24 individuals (12 patients with AD and 12 control subjects). Sequence reactions were performed (Big Dye Terminator ver. 3.1 using a 3700 DNA analyzer; Applied Biosystems [ABI], Foster City, CA).

Genotyping

Initial screening genotyping of SNPs in the *FCER1B*, *IL13*, and *IFNGR1* gene regions has been described.[9,10,22] We further genotyped the *IFNGR1* gene by allele frequency (minor allele frequency [MAF] >10%) and based on intragenic linkage disequilibrium (LD) information. We genotyped the SNPs either with the invader assay,[23] by PCR-RFLP, direct sequencing, or with a genotyping assay (Taqman; ABI). An invader assay was performed with multiplex PCR products as the template. Genotyping was performed on a sequence-detection system (Prism 7700; ABI), according to the manufacturer's protocol.

Statistical Analysis

Statistical analysis was carried out essentially as previously described.[4] Allele frequencies in patients with AD and the control subjects were compared by the contingency χ^2 test. $P < 0.01$ (also in the case of multiple comparisons after Bonferroni adjustment) was considered to be statistically significant. Odds ratio (OR) and 95% confidence interval (CI) were also calculated.

Reporter Gene Assay. Reporter gene assay was carried out essentially as previously described.[4] A pair of 753-base IFNGR1 promoter sequences was subcloned continuous to exon 1 into pGL3 basic vector (Promega Corp., Madison, WI). Two clones were made; the first was −611G, −56C, and the second was −611G, −56T. After all subcloned plasmids were verified by direct sequencing, pGL3-IFNGR1 promoter plasmids and pRL-TK (as an internal control for transfection efficiency) were transfected into immortalized human LECs (Lipofectamine 2000; Invitrogen). The medium was changed 24 hours later, and the LECs were stimulated with lipopolysaccharide (LPS, 1 μg/mL, L4391; Sigma-Aldrich) or recombinant human IFN-γ (1000 U/mL, R&D Systems). Luciferase activity was measured with a dual luciferase reporter assay kit (Promega) at 36 hours after transfection.

TABLE 2. Genotyping of Candidate Genes for Ocular AD

6886G/A (E237G)			329G/A (R110Q)			−56C/T		
FCERB1	**Normal**	**Ocular AD**	***IL13***	**Normal**	**Ocular AD**	***IFNGR1***	**Normal**	**Ocular AD**
GG	7	2	GG	130	28	CC	69	14
GA	72	24	GA	120	37	CT	153	32
AA	200	51	AA	28	12	TT	60	32
NS*			NS			$P = 0.0004$†		

* NS, no significant associations.

† *P* values for comparisons of genotype −56TT versus −56CT + −56CC between cases and controls.

TABLE 3. List of *IFNGR1* SNPs Identified in a Japanese Population

SNP			Position	Amino Acid	MAF (%)
1	5′-Promoter	A/T	−766	—	2
2	5′-Promoter	C/T	−731	—	2
3	5′-Promoter	A/G	−611	—	12
4	5′-Promoter	C/T	−255	—	2
5	5′-Promoter	C/T	−56	—	46
6	Exon 1	G/A	40	Val/Met	2
7	Exon 1	G/A	48	Arg/Arg	2
8	Intron 1	C/T	95	—	46
9	Intron 1	A/G	130	—	48
10	Exon 2	C/T	12300	Ile/Ile	4
10	Intron 6	G/A	18693	—	2
12	Intron 6	C/T	20488	—	2
13	Intron 6	A/G	20685	—	35
14	Exon 7	T/G	20877	Ser/Ser	2
15	Exon 7	T/C	21227	Pro/Leu	2
16	Exon 7	A/G	21499	3′-UTR	2
17	Exon 7	A/G	21503	3′-UTR	2
18	Exon 7	G/A	21514	3′-UTR	2
19	Exon 7	A/C	21663	3′-UTR	4

MAF, minor allele frequency.

IFNGR1 Overexpression in LECs

Full length human *IFNGR1* cDNA was generated by PCR and then subcloned into pcDNA-V5-His vectors (Invitrogen), and the sequence was verified by direct sequences. LECs in six-well culture plates were transfected with 500 ng of the plasmid/well, using 1 μL of transfection reagent (Lipofectamine 2000; Invitrogen) according to the manufacturer's protocol. Twenty-four hours later, the culture medium was changed; the cells were stimulated with 1 μg/mL LPS and 1000 U/mL human IFN-γ. Twelve hours after stimulation, they were washed extensively with phosphate-buffered saline (PBS). Total RNA was extracted with an RNA isolation kit (NucleoSpin II; Macherey-Nagel, Duren, Germany), and cDNAs were prepared using random primers and the reverse transcriptase (Revertra Ace; both from Toyobo, Osaka, Japan) according to the manufacturer's protocol.

Reverse Transcription and Real-Time PCR Analysis

Reverse-transcription (RT) and real-time PCR analysis was carried out essentially as previously described.[24] Anterior capsules, obtained at cataract surgery with written informed consent, were immediately stored in stabilizer (RNAlater reagent; Ambion, Austin, TX) to protect the RNA. The procedure was approved by the ethics committees of Kyoto Prefectural University of Medicine. Total RNA was isolated with the (Micro RNA extraction kit; Qiagen Japan, Tokyo) from the anterior capsules or LECs, and then cDNA was prepared as described earlier. We used real-time PCR probes and primers specific for human IFNGR1, inducible nitric oxide synthase (iNOS), and GAPDH (Assay-on-Demand gene expression products; ABI). Real-time PCR analysis was performed on a sequence-detection system (Prism 7300; ABI). The relative expression of IFNGR1 in LECs was quantified by the standard curve method using GAPDH expression in the same cDNA as the control.

Immunohistochemistry

Lens capsules obtained at cataract surgery were frozen in OCT compound, cryostat sections were cut, mounted on slides, and fixed in 4% paraformaldehyde in PBS. Nonspecific staining was blocked (30 minutes) with blocking buffer (10% normal goat serum, and 1% bovine serum albumin [BSA] in PBS). Anti-MHC class II monoclonal antibody (1:200 dilution) was then applied and incubated overnight at 4°C. After they were washed with PBS, the slides were incubated for 30 minutes with Alexa 488-conjugated anti-mouse IgG. The slides were inspected under a confocal microscope (Leica, Tokyo, Japan).

RESULTS

Genotyping of the Candidate Genes

First, we screened for SNPs in the *FCER1B*, *IL13*, and *IFNGR1* gene regions. Although we observed no associations between the SNPs in the *FCER1B* and *IL13* regions (Table 2), there was a statistically significant association between the −56C/T SNP in the *IFNGR1* region and ocular AD ($P = 0.0004$).

SNP Discovery and Case–Control Association Study in the *IFNGR1* Region

Our genotyping of the candidate genes prompted the additional screening for other SNPs in the *IFNGR1* region. As shown in Table 3, we detected 19 SNPs. Their position is numbered relative to their position in the published *IFNGR1* gene sequence (GenBank: AL050337). Position 1 is the adenine

TABLE 4. Pair-Wise LD Calculated for Common SNPs and Tag SNP Typings (MAF > 10%)

SNPs	r^2
3–5	0.1074
3–8	0.1074
3–9	0.0988
3–13	0.0499
5–8	1
5–9	0.9197
5–13	0.464
8–9	0.9197
8–13	0.464
9–13	0.5045

−611G/A (SNP 3)			206856A/G (SNP 13)		
IFNGR1	Normal	Ocular AD	*IFNGR1*	Normal	Ocular AD
GG	257	59	AA	139	49
GA	23	13	AG	123	24
AA	2	0	GG	20	5
$P = 0.02$*			NS		

* Comparisons of genotype −611GG versus −611GA + −611AA between cases and controls.

TABLE 5. Genotype Frequencies and Case Control Analysis of *IFNGR1* −56 C/T SNPs in Ocular AD

				Genotype TT versus CT + TT between Cases and Controls		
	−56CC	−56CT	−56TT	OR (95% CI)	χ^2	*P*
Healthy controls (n = 282)	69 (25%)	153 (54%)	60 (21%)	—	—	—
Atopic dermatitis (n = 192)	48 (25%)	102 (53%)	42 (22%)	1.04 (0.60–1.62)	0.024	0.88
Ocular AD (n = 78)	14 (21%)	32 (37%)	32 (42%)	2.57 (1.51–4.39)	12.53	0.0004
Atopic cataract (n = 48)	5 (10%)	19 (40%)	24 (50%)	3.70 (1.96–6.97)	17.83	0.000024
AKC (n = 35)	8 (21%)	14 (36%)	13 (43%)	2.19 (1.04–4.59)	4.4	0.035

of the first methionine. Among the 19 SNPs, there were five common SNPs with MAF greater than 10%. We selected SNP 3 (−611G/A) and SNP 5 (−56C/T) in the promoter region and SNP 13 (20685 A/G) in intron 6, as tag SNPs because of intragenic pair-wise LD expressed as r^2 (Table 4). SNP 206856A/C (No. 13) did not show an association with ocular AD, SNP −611G/A (No. 3) exhibited marginal association not stronger than SNP -56C/T (No. 5). Therefore, we focused on SNP −56C/T. There was a significant association between the −56C/T SNP and ocular AD (raw P = 0.0004, OR = 2.57, 95% CI = 1.51–4.39), the association became stronger for the atopic cataracts (raw P = 0.000024, OR = 3.70, 95% CI = 1.96 to 6.97; Table 5). All the genotype frequencies of the SNPs were concordant with Hardy-Weinberg equilibrium.

Haplotype Analysis of *IFNGR1* (−611/−56) SNPs

We also tested the distribution of two-locus haplotypes in AD and control samples (Table 6). Among the two-locus haplotypes of SNPs in the promoter region (−611G/A and −56T/C), the −611G/−56C haplotype showed decreased risk for ocular AD (G-C versus others; P = 0.00,003, OR = 2.26). The IFNGR1 haplotype −611G/−56C showed decreased risk for atopic cataracts (G-C versus others; P = 0.000003, OR = 3.16), to a degree that was greater than that of single SNP genotype association (−56TT versus others, P = 0.00002).

Reporter Gene Analysis

Using pGL3-basic vector, we prepared a construct for −611G/−56C, the major haplotype, and for −611G/−56T, the common haplotype among patients with AD. The primers used for subcloning were 5′-aggtgagatcattagacatt-3′ (forward) and 5′-gctgctaccgacggtcgctggct-3′ (reverse). All assays were performed in triplicate. In Figure 1, a representative result of three independent experiments is shown as the mean ± SD. In the absence of stimulation, luciferase activity was not significantly different between −56C/T SNPs containing constructs in the LECs. The genotype −56T containing construct induced stronger IFNGR1 promoter activity than the −56C construct when stimulated for 12 hours with IFNG or IFNG+LPS (P = 0.01 and 0.02, respectively, by Student's t-test).

IFNGR1 Overexpression Experiment in LECs

LECs transfected with an IFNGR1 expression plasmid showed approximately a fivefold increase in IFNGR1 expression (Fig. 2, left). iNOS mRNA expression was only observed in cells stimulated with IFNG+LPS. iNOS gene expression was upregulated approximately threefold in IFNGR1-overexpressing LECs compared with mock-transfected LECs (Fig. 2, right).

Real-Time PCR Analysis

cDNAs were synthesized from total RNA isolated from the anterior lens capsules of patients undergoing surgery for atopic (n = 5) and senile cataracts (n = 5). The expression of IFNGR1 mRNA was significantly higher in the atopic than the senile cataracts (Fig. 3, P = 0.00005 by Mann-Whitney's U-test).

Anti-MHC Class II Immunostaining of Lens Epithelium

Anti-MHC class II immunohistochemistry was performed on senile and atopic cataract lens capsules. LECs in atopic but not in senile cataracts were positive for MHC class II immunostaining (Fig. 4).

DISCUSSION

Although Nishimura et al.[25] reported genetic linkage in allergic conjunctivitis, ours is the first genetic association study of ocular AD, which tends to be more severe and longer lasting than allergic conjunctivitis without AD. Initial genotyping screening showed that atopy- or AD-related genes did not necessarily show an association with ocular AD. Among our candidate SNPs, we found a strong genetic association between the IFNGR1 −56C/T SNP and ocular AD. We previously reported an association between IFNGR1 SNPs and the serum IgE concentration in patients with atopic asthma.[10] Herein, we

TABLE 6. Structure and Frequencies of Two-Locus Haplotype in *IFNGR1*

	Frequency				
Haplotype	Control	Ocular AD	Atopic Cataract	*P**	*P*†
−611G/−56C	0.52	0.32	0.25	0.000030	0.0000030
−611G/−56T	0.44	0.56	0.64	0.014	0.00032
−611A/−56T	0.048	0.058	0.054	0.850	1.590
−611A/−56C	0	0.064	0.05	NA	NA

NA, not applicable.
* Ocular AD versus control.
† Atopic cataract versus control.

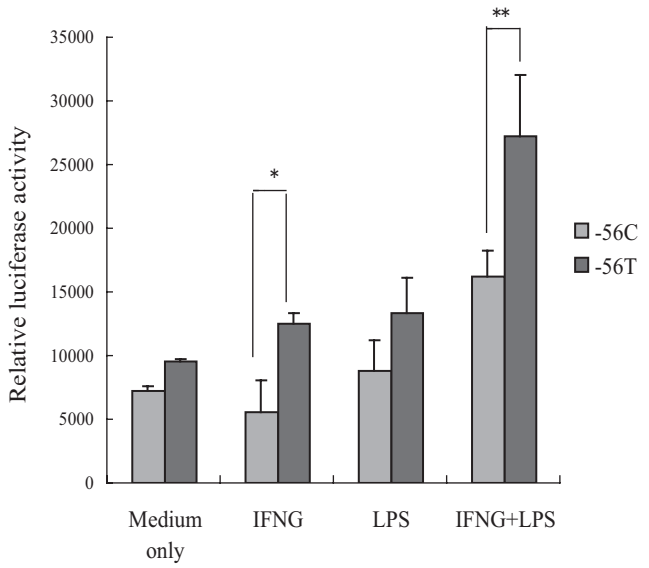

FIGURE 1. Reporter gene assay of IFNGR1 promoter region. IFNGR1 promoter-pGL3 vector consisting of −611G/−56C or -611G/−56T SNPs were transfected into human lens epithelial cells. Twenty-four hours after transfection, the cells were stimulated with human recombinant IFNG and/or LPS. Relative luciferase activity was measured 12 hours after stimulation. $^{*}P = 0.01$, $^{**}P = 0.02$; Student's *t*-test.

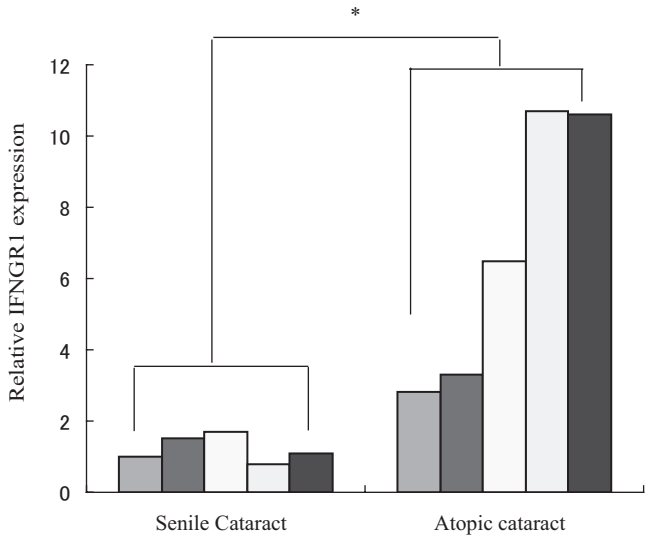

FIGURE 3. Real-time PCR analysis of IFNGR1 mRNA expression in human lens anterior capsules. Total RNA was extracted from the anterior lens capsule of atopic/senile cataracts. cDNA was synthesized from the total RNA. Real-time PCR analysis was performed with expression assay probes. The amount of relative expression was normalized to that of GAPDH ($^{*}P = 0.00003$; Mann-Whitney test.)

document that the association between the SNPs and ocular AD was stronger than in the previous study. As surprisingly, there was no association between SNP and AD without ocular complications (Table 4), we postulated that in addition to its effect on serum IgE, the IFNGR1 gene plays some role as an organ-specific susceptibility gene for ocular AD. In African populations, there is a genetic associations between the IFNGR1 −56C/T SNP and *Helicobacter pylori* infection,[26] and cerebral malaria[27]; the −56T genotype was associated with higher serum *H. pylori* antibody concentrations, and −56C/T heterozygosity was protective against cerebral malaria infection. These results suggest that the −56C/T SNP plays some functional role(s) not only in the Japanese, but also in the African population.

We examined the association between atopic cataracts and the IFNGR1 SNP, because cataract formation was observed in IFNG transgenic mice,[19] and IFNG treatment of LECs induced apoptosis,[28] one of the pathologic features of atopic cataracts.[29] Using a reporter gene assay, we first examined the effect of the −56C/T SNP in human immortalized LECs.[20] We made 753-bp IFNGR1 promoter region constructs to analyze the −611G/−56C and −611G/−56T haplotypes, because these two major haplotypes make up more than 90% of all haplotypes (Table 5). After LEC stimulation with IFNG and LPS, we found significantly higher transcriptional activity in the presence of the −56T allele, the risk allele for atopic cataracts, than the −56C allele (Fig. 1). This result is consistent with the findings of Juliger et al.,[30] whose reporter gene assay showed a higher level of IFNGR1 transcriptional activity with the −56T allele, and is well matched to our haplotype association study which showed that −611G/−56C is a protective and −611G/−56T is a risk haplotype for ocular AD induction (Table 5). In our experiments, we used LPS/IFNG stimulation because LPS/

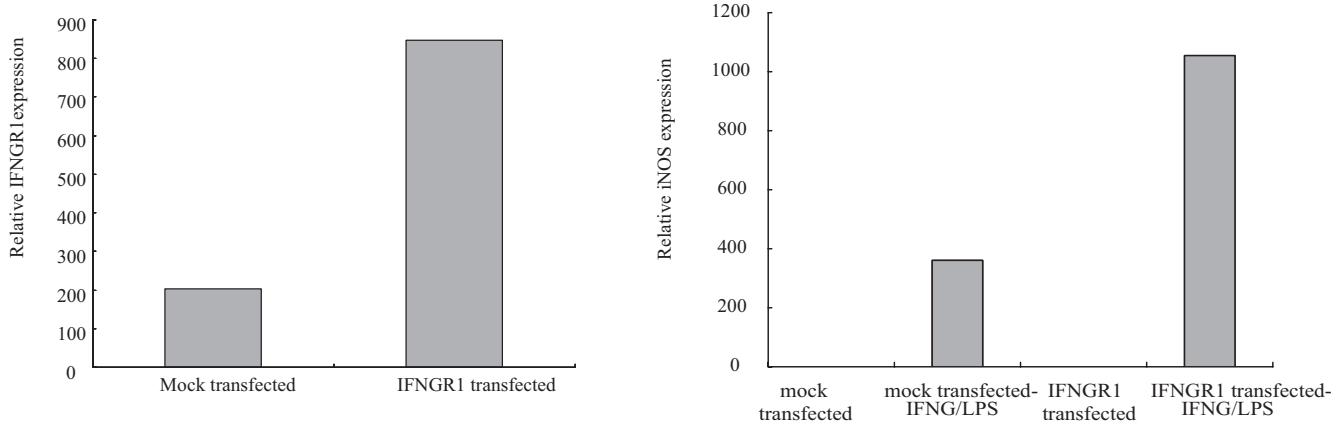

FIGURE 2. The effect of IFNGR1 overexpression for iNOS expression in LECs. *Left*: real-time PCR analysis of IFNGR1 expression of IFNGR1-overexpressing LECs. An approximately five-fold overexpression of IFNGR1 mRNA was detected. *Right*: real-time PCR analysis of iNOS mRNA expression. IFNGR1-transfected LECs showed higher iNOS expression than that of mock-transfected LECs when stimulated with IFN-γ+LPS. No iNOS expression was observed without IFN-γ+LPS stimulation.

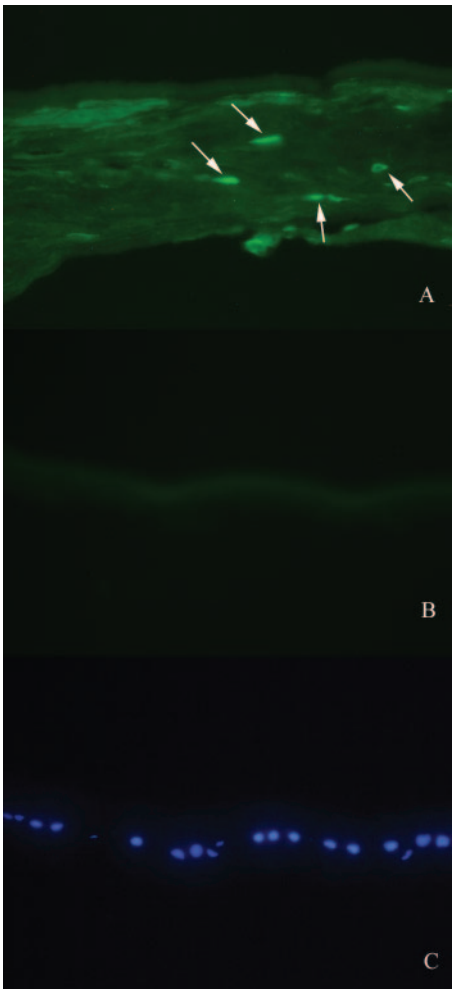

FIGURE 4. Immunohistochemical staining of human anterior lens capsules with MHC class II antibody. Cryosections of anterior lens capsules were immunostained with anti-MHC class II antibody. (**A**) In an anterior lens capsule of an atopic cataract, positive immunostaining was observed in some of the lens epithelial cells (*arrows*). (**B**, **C**) Anterior lens capsule of a senile cataract; no MHC class II staining was observed. The existence of lens epithelial cells was verified with nuclear DAPI staining. Original magnification, ×400.

IFNG treatment of LECs induced iNOS expression,[31] a known cataract-inducing factor.[32] As a downstream signal of IFNG, iNOS has been intensively studied in macrophages,[33] and in airway[34] and lens epithelium.[31] To clarify the role of IFNGR1 in the induction of iNOS in LECs, we transfected LECs with IFNGR1 and stimulated them with IFNG+LPS. Cells that overexpressed IFNGR1 generated higher amounts of iNOS mRNA (Fig. 2), a finding consistent with that reported by Li et al.[31] As the NOS inhibitor could prevent the development of cataracts in selenite-treated rats,[32] iNOS expression may play a role in the genesis of cataracts.

Furthermore, LECs from atopic cataracts manifested higher IFNGR1 mRNA expression than did LECs from senile cataracts (Fig. 3), and LECs from atopic cataracts were positive for MHC class II staining (Fig. 4). Our results are consistent with those of Egwuagu et al.,[35] who showed that ectopic MHC class II expression due to IFNG overexpression resulted in ocular disease including cataract formation. Based on these considerations, we postulate that IFNG-IFNGR signals are active in the development of atopic cataracts and that higher IFNGR1 expression may be a predisposing factor for atopic cataracts. We are in the process of measuring IFNG concentration in aqueous humor samples from patients with atopic and senile cataracts.

Although topical steroids are frequently used to treat ocular atopic conditions, they are causative reagents for cataracts.[36] Therefore, treatment of ocular AD with inhibitors of T-cell activation (e.g., cyclosporine and tacrolimus) or with NOS inhibitors may be more successful in preventing IFNG-mediated atopic cataract formation. Our findings identified a genetic risk factor for ocular complications in patients with AD. We are planning additional genotyping and functional studies on other candidate genes and are investigating antiapoptotic molecules Bcl-2[29] and major basic protein.[37] The roles of glutathione should also be investigated because of a possible relationship with subcapsular cataracts.[38]

Acknowledgments

The authors thank Kazuhiko Mori, Tomomi Nishida, and Naoko Inomata for collecting DNA and lens epithelium; Miki Kokubo, Hiroshi Sekiguchi, and Natsuko Tenno for excellent technical support; Tadao Enomoto and Akihiko Miyatake for collecting normal control DNAs; and Julian M. Hokpkin for invaluable continuous support.

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