Innate and adaptive host responses and their genetic control in tuberculosis : studies in Indonesia, a highly TB endemic setting

Sahiratmadja, E.K.

TB drugs are stored by name of patients.
Strong and continued commitment from both the medical officer and the patients is needed as the TB treatment is lengthy (6 months)

Submitted

5

Analysis of Genes in Innate Immune Pathways Finds Novel Associations with Pulmonary Tuberculosis Risk in Indonesia

Sonia Davila,[1] Martin Hibberd,[1] **Edhyana Sahiratmadja**,[3,5]
Carine Bonnard,[2] Bachti Alisjahbana,[6] Reinout van Crevel,[7]
Esther van de Vosse,[4] Tom H.M. Ottenhoff [3] and Mark Seielstad [2]

[1] Infectious Diseases, Genome Institute of Singapore, Singapore
[2] Population Genetics, Genome Institute of Singapore, Singapore
[3] Dept. Immunohematology and Bloodtransfusion, Leiden University Medical Center, Leiden, The Netherlands
[4] Dept. Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands
[5] Eijkman Institute for Molecular Biology, Jakarta, Indonesia
[6] Div. Tropical and Infectious Diseases, Dept. Internal Medicine Faculty of Medicine, University of Padjadjaran, Bandung, Indonesia
[7] Dept. Internal Medicine, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands

SUMMARY

Background. Infection by *Mycobacterium tuberculosis* is a major global health burden. Despite high rates of exposure, only 5-10% of those infected will develop active tuberculosis (TB) disease, suggesting a significant role for genetic variation in the human immune response to this infection. To investigate this, we have performed a comprehensive association study of genes involved in innate immune recognition, with subjects from a high incidence population in Jakarta, Indonesia.

Methods and findings. A population based association study was carried out in 762 Indonesian subjects (375 pulmonary TB cases vs. 387 healthy controls) using a candidate-gene approach. Successful genotyping of 906 single nucleotide polymorphisms (SNPs) in 45 genes was performed. We found evidence for association with 21 of the polymorphisms in nine genes - most prominently Toll-like receptor-8 (*TLR8*) and complement (*C3*, *C1QB)* genes. After splitting subjects by gender, analysis of *TLR8*, located on Chromosome Xp22, identified a putatively functional variant which confers a higher risk of active disease to males, with an odds ratio of 1.8 (95% CI: 1.2-2.7). In the complement pathway, two neighboring polymorphisms within *C3* showed association with disease risk, conferring a protective effect to carriers of the minor alleles. We also found a protective effect among carriers of the minor alleles at rs735239 (-871G), and rs2287886 (-139G) located in the promoter region of DC-SIGN (*CD209*), a C-type lectin receptor. Both SNPs are contained within a haplotype block that showed the same protective effect.

Conclusions. These results provide evidence, for the first time, of a role for the *TLR8* and *C3* genes in susceptibility to TB. Furthermore, sex-specific genetic effects appear to be involved, at least in part, in the vulnerability to infection. In addition, replication of an association between a promoter variant of *CD209* and susceptibility to TB first reported in a South African population is confirmed here in an Asian population, strongly suggesting that polymorphisms in this gene are important determinants of outcome.

INTRODUCTION

Although one-third of the world's population is infected with *M. tuberculosis* [1] fewer than 10% of these infected individuals will develop clinical disease.[2] The immunological mechanisms that distinguish the majority of individuals, who successfully contain these organisms, from the minority, who develop progressive mycobacterial disease are largely unknown.

It is becoming increasingly clear that innate immunity plays a crucial role in directing many aspects of the host response, including the ensuing adaptive response; thereby making it a primary defense mechanism. The initial phase of this process is pathogen identification involving a wide range of pattern recognition molecules. We and others have postulated that pathogen recognition could be a key component in determining the outcome of infection.[3,4] To address this, we have carried out a SNP-based case-control candidate gene approach to investigate the possible role of these innate immune pathways in susceptibility to pulmonary tuberculosis. The candidate genes chosen encode for proteins that are concentrated along related pathways, as evidence is building in a number of diseases, including TB[5] and meningococcal disease,[6] that variations in more than one gene in a related pathway may have similar functional consequences, and thus result in a similar phenotype upon infection.

In particular, we have concentrated on three groups of innate immune molecules, the Toll-like receptor (TLR) pathway,[7-11] the mannose binding lectin (MBL)[12-15] and some of its downstream partners in the complement cascade,[5,16] and other pattern recognition receptors - including cytoplasmic receptors,[17,18] C-type lectin receptors [19] and scavenger receptors .[20]

For the TLR pathway, all functional receptors, except *TLR5*, were studied. The latter was excluded due to a low level of polymorphism and to sequence duplications which could make SNP genotyping difficult. TLRs signal via cytoplasmic TLR adaptors; hence, we included *MyD88*, *TOLLIP*, *TIRAP* and *TICAM1*&*2* and downstream members *IRAK4*&*1* and *Ly96* (MD2).[21] Furthermore, CD14,[22] a surface molecule that partners with TLR4 to increase the PAMP repertoire, was also incorporated in the study. Bacterial surface components have been reported as ligands for a wide range of other host receptors, including those in our study.[20,23] In particular, CARD15 has been shown to recognize *M. tuberculosis* [18] while promoter polymorphisms in *CD209*,

a receptor in the C-type-lectin family encoding for DC-SIGN, have been reported to be associated with susceptibility to TB.[24,25]

We have undertaken the most comprehensive genetic investigation of innate immune recognition molecules involved in the response to *M. tuberculosis* infection to date and have implicated several genes in the disease process.

METHODS

Subject Recruitment

439 new pulmonary tuberculosis patients above 15 years of age were recruited from an outpatient tuberculosis clinic in central Jakarta (Indonesia). Diagnosis was based on clinical presentation and chest X-ray examination, confirmed by sputum microscopy positive for mycobacteria.[26] 490 randomly selected control subjects with the same sex and age (±10%), were recruited from neighbouring households. First-degree relatives of patients were excluded. Control subjects with signs and symptoms suggesting active tuberculosis or a history of prior anti-TB treatment were also excluded. Self and parental ethnicities were recorded upon recruitment. A Javanese origin encompassed three groups - the Jawa, Betawi, and Sunda - and altogether comprised more than 80% of the total sample. The non-Javanese category included individuals born on other Indonesian islands. Subjects were considered of mixed ethnicity when one parent was of Javanese ethnic origin and the other non-Javanese.

Subjects were tested for diabetes mellitus ($n = 35$) and HIV coinfection ($n = 0$), both of which are considered to be major risk factors for tuberculosis development. (Details described elsewhere [27]). Briefly, subjects with levels of fasting blood glucose over 126 mg/dL were considered to have diabetes. HIV testing was performed using dipstick test (Abbott, Determine). In order to define a homogeneous phenotype, patients suspected of extra-pulmonary tuberculosis ($n = 27$) were not considered in the analyses. Controls with suspected tuberculosis after chest X-ray examination ($n = 24$) or a history of tuberculosis ($n = 7$) were also excluded.

Genomic DNA was extracted from whole blood following a protocol described elsewhere.[28] After genotyping, 74 samples were excluded because of sample duplication and/or familial relationships not originally reported, but identified by RelPair.[29]

Consent forms approved by local Institutional Review Boards of the Medical Faculty of University of Indonesia and the Eijkman Institute for Molecular Biology in Jakarta were signed upon recruitment by all participants.

SNP Genotyping

Selection of SNPs was carried out using an in-house database, GISSNP, which integrates data from public databases (Ensembl, Celera, dbSNP build 123/126) as well as proprietary data. Polymorphisms with the following characteristics were preferentially chosen: putative functional variants resulting in changes in the protein sequence, minor allele frequencies over 5%, average spacing of one SNP every 1 to 2 kilobases. To screen for possible regulatory elements, flanking regions five kilobases upstream and downstream of the gene were also covered.

Design of a custom Oligo Pool Assay (Illumina®, San Diego, CA, United States) was implemented following the manufacturer's specifications. Analysis of 856 SNPs was performed with a BeadStation 500G Genotyping System (Illumina®). Genotypes were analyzed with Beadstudio software also from Illumina. Fifty SNPs were genotyped with a Sequenom primer extension-based protocol described elsewhere.[30,31] The genotype concordance among the two systems used for genotyping in this study has been reported to be over 99.5%.[31] Assessment of genotypes was done by laboratory personnel without any prior knowledge of the diagnosis of the subjects.

Statistical Analysis

Hardy-Weinberg equilibrium was calculated in the control group using *HelixTree® v4.4.1* (GoldenHelix Inc., Bozeman, MT, United States) and *Exemplar* (Sapio Sciences, LLC, York, PA, United States). Similarly, allelic association analysis was carried out in both software packages. Allelic *p*-values were calculated by means of a 2x2 chi-square table, whereas genotypic *p*-values were derived from a 3X2 chi-square table. A two-sided Fisher Exact test, when counts in any cell fell below five, as well as genotype association analysis and odds ratios were calculated with *Exemplar*. Allelic analysis of SNPs located on Chromosome X was performed with *Haploview v3.31*.[32] A likelihood ratio test was applied to calculate genotypic associations of SNPs on Chromosome X.

The statistical significance of nominal *p*-values was assessed by permutation analysis (n = 10,000). *Exemplar* applied a SNP level correction in

allelic and genotypic *p*-values, whereas *Haploview v3.31* was applied for permutation analysis of variants located on Chromosome X.

Haplotype blocks and linkage disequilibrium plots were constructed with *Haploview v3.31* using the default algorithm proposed by Gabriel *et al.*[33] Applying a pairwise method, tagSNPs from associated genes were obtained after setting a standard cut-off value of $r^2 > 0.8$.[34]

Sample duplications and family relationships - full siblings and parent-offspring - were identified through RelPair.[29] In such instances, one member of each relative pair was dropped from the analysis.

Analysis of population stratification was performed after genotyping 299 SNPs in 698 samples (330 cases, 368 controls) of our study group. One of these was out of HWE and, thus, excluded from the analysis. These SNPs were chosen to be more than 10 kilobases away from any known gene, to have average minor allele frequencies around 30% and to be in linkage equilibrium with one another. The level of population stratification was calculated using the approach of Devlin and Roeder.[35] Briefly, an inflation factor was calculated as the median of the chi-square values for all 298 SNPs, divided by 0.675 and then squared.

RESULTS

In order to investigate the underlying genetic factors affecting host susceptibility to pulmonary TB, a collection of single nucleotide polymorphisms (SNPs) in 45 candidate genes involved in relevant aspects of the innate immune response was analyzed (Table 1). We successfully genotyped 906 SNPs, located within these genes and in putative regulatory flanking regions. Nearly 20% ($n = 184$) of the genotyped polymorphisms were monomorphic in our study population (Table 1). Variants with call-rates below 90% ($n = 27$) were not considered. Thirty-eight SNPs showed deviations from Hardy-Weinberg equilibrium (HWE) in the control group, and were removed from further analysis (Table 1). Association analyses were applied to the remaining 657 polymorphic SNPs with reliable genotypes.

Study population

The demographic and clinical data (Table 2) of the 375 patients and 387 controls included in the study showed a comparable median age for both groups, controls being slightly older. Males comprised 60% of both the affected and the control groups. A smaller number of patients presented evidence of BCG scarring compared to the control group [38% vs. 43%]. The self-reported ethnicity of each subject and his/her parents was carefully considered in an effort to avoid spurious associations arising from population stratification.

In order to detect traces of population stratification a large subset of subjects included in this study were genotyped for an independent set of 299 SNPs. The correction factor calculated according to the method of Devlin and Roeder and described in the methods section resulted in a value below 1 (0.82), which indicated that there was no significant population stratification in our study group.[36]

Allelic association analysis

After genotyping the 762 subjects, allelic association analysis identified 20 SNPs from nine genes with *p*-values below 0.05 (Table 3). Polymorphisms from the complement pathway (*C3*, *C1QB*, *CR1*), scavenger receptors (*SCARB1*, *SCARB2*, *SCARF1*) and Toll-Like receptor (*TLR8*) genes were heavily represented. Five of the top 20 polymorphisms were coding variants, four of which alter the predicted protein sequence. Seven of the disease-associated SNPs were located within introns. The rest of the associated variants were found in putative regulatory regions, within five kilobases upstream or downstream of a gene (Table 3).

Allelic analysis of polymorphisms within *TLR8* detected an association with a missense variant, rs3764880 (Met1Val), which appears to ablate the putative start codon in one of the transcripts encoded by this gene. An additional three SNPs located in the upstream region of the gene also showed significant association with disease (Table 3).

The similarity of their allele frequencies suggests the possibility of strong linkage disequilibrium (LD) with the missense variant, Met1Val, discussed above. Given that *TLR8* is located on the X chromosome, we performed separate tests in each sex (Table 4). We found a strong association with the minor allele of the functional polymorphism, rs3764880A, with susceptibility to pulmonary TB in males [OR (95% CI) = 1.8 (1.2-2.7), *p*-value = 0.007]

Table 1.
Candidate Genes and SNPs Analyzed

	Genes	Genotyped SNPs	SNPs out of HWE	Monomorphic SNPs (%)
Toll-Like receptors (TLR), adaptors & pathway	*TLR1*	16	1	9 (56.2)
	TLR2	12	-	2 (16.7)
	TLR3	9	2	4 (44.4)
	TLR4	28	-	12 (42.8)
	TLR6	8	-	-
	TLR7	12	-	3 (25)
	TLR8	16	-	6 (37.5)
	TLR9	15	-	5 (33.3)
	TLR10	19	1	3 (15.8)
	MYD88	9	-	4 (44.4)
	TICAM1	11	-	4 (36.4)
	TICAM2	7	-	-
	TIRAP	9	-	2 (22.2)
	TOLLIP	12	-	1 (8.3)
	TREM1	20	-	4 (20)
	TREM2	10	-	3 (30)
	CD180	19	1	-
	CD14	11	-	4 (36.4)
	Ly86	56	-	4 (7.1)
	Ly96	22	1	4 (18.2)
	LBP	30	1	4 (13.3)
	IRAK1	15	-	5 (33.3)
	IRAK4	14	-	7 (50)
Complement	*C1QA*	6	1	-
	C1QB	10	2	3 (30)
	C1QC	7	-	-
	CD93	15	-	4 (26.7)
	C2	21	-	3 (14.3)
	C3	42	1	11 (26.1)
	CR1	55	3	7 (12.7)
	ITGAM	35	1	8 (22.8)
	ITGAX	26	3	5 (19.2)
	MBL2	19	-	3 (15.8)

Other Pattern Recognition Receptors	*CARD4*	40	2	10 (25)
	CARD15	20	-	7 (35)
	CD209	20	2	6 (30)
	CLEC4M	12	-	3 (25)
	FCGR2A	20	6	3 (15)
	MARCO	24	2	2 (8.3)
	SCARA3	23	1	-
	SCARB1	52	1	4 (7.7)
	SCARB2	36	2	4 (11.1)
	SCARF1	13	1	4 (30.7)
	SFTPB	15	1	4 (26.6)
	SFTPC	15	2	3 (20)
TOTAL	45	906	38	184 (20.3)

Table 2.
Demographic and Clinical Data of the Study Population

	TB Patients (n=375)	Controls (n=387)
Age years (median)	14-75 (28)	15-70 (32)
Gender (male:female)	228 (60.8%) : 147(39.2%)	232 (60%) : 155 (40%)
BCG Scar Present	143 (38%)	168 (43%)
Recurrent TB	14 (3.7%)	-
Self reported ethnicity		
Javanese	326 (86.9%)	314 (81.1%)
Non Javanese	29 (7.7%)	21 (5.4%)
Mixed	19 (5.1%)	29 (7.5%)
Unknown	1 (0.3%)	23 (5.9%)

whereas this trend was not observed in females. Of possible significance, the minor allele (A) in this Indonesian population was reversed compared to reported frequencies in Caucasian and African groups, but was consistent with Asian populations.[37] Around 70% of the Indonesian subjects carried the G allele (Val), whereas this same allele has been reported in 30% of Caucasian and African populations. Very similar significant association values were found in the three promoter variants, attributable to strong LD in this region.

Table 3.
Allelic Distribution and Description of SNPs with p-values <0.05 in TB Patients and Controls

dbSNP rs#	Alleles	No. of Cases[a]	MAF Cases	No. of Controls[a]	MAF Controls	Gene	Location[b]	p-value	Permutation p-value[c]	O.R. (95% CI)
rs11569562	C>T	221	0.3	291	0.38	*C3*	Intron	0.0016	0.0015	0.7 (0.57-0.87)
rs12040131	C>G	13	0.02	31	0.04	*C1QB*	Downstream	0.0095	0.015	2.32 (1.2-4.48)
rs3764879	G>C	153	0.3	124	0.23	*TLR8*	Upstream	0.01	0.038	1.4 (1.06-1.84)
rs3788935	G>A	152	0.3	125	0.23	*TLR8*	Upstream	0.014	0.05	1.4 (1.07-1.86)
rs714521	T>C	321	0.44	290	0.38	*LY86*	Intron	0.015	0.017	0.77 (0.63-0.95)
rs3761624	G>A	152	0.3	126	0.24	*TLR8*	Upstream	0.016	0.059	1.4 (1.06-1.8)
rs3764880	G>A	152	0.3	126	0.24	*TLR8*	M1V, 5'UTR	0.016	0.59	1.4 (1.06-1.8)
rs344555	G>A	155	0.21	203	0.27	*C3*	Intron	0.017	0.019	0.74 (0.58-0.94)
rs17001654	C>G	14	0.02	30	0.04	*SCARB2*	Intron	0.022	0.031	2.08 (1.1-3.9)
rs735239	A>G	69	0.09	99	0.13	*CD209*	Upstream	0.028	0.14	1.43 (1.03-1.99)
rs7951	C>T	28	0.04	48	0.06	*C3*	A1437A	0.032	0.041	1.67 (1.04-2.7)
rs2274567	A>G	271	0.38	320	0.44	*CR1*	H1208R, H1658R	0.032	0.043	1.25 (1.02-1.55)
rs7525160	G>C	274	0.38	329	0.43	*CR1*	Upstream	0.033	0.036	1.25 (1.02-1.54)
rs2293067	A>G	295	0.41	351	0.46	*SCARF1*	Intron	0.035	0.038	1.25 (1.02-1.53)
rs12831105	G>A	117	0.16	94	0.12	*SCARB1*	Intron	0.036	0.044	0.73 (0.55-0.98)
rs9896488	G>T	51	0.07	76	0.1	*SCARF1*	Upstream	0.042	0.053	1.47 (1.01-2.1)
rs2272011	T>C	81	0.11	112	0.15	*SCARF1*	A425V	0.042	0.044	1.37 (1.01-1.86)
rs3744644	G>C	34	0.04	653	0.07	*SCARF1*	E639D	0.043	0.027	1.57 (1.01-2.45)
rs11260027	G>A	179	0.24	221	0.29	*CD209*	Downstream	0.044	0.043	1.27 (1.0-1.6)
rs2294460	C>T	90	0.12	122	0.16	*LY86*	Intron	0.046	0.054	1.35 (1.0-1.8)

[a] Number of chromosomes carrying the minor allele.
[b] Locations for all the transcripts encoded by a gene are shown.
[c] Number of Permutations = 10,000.

We detected association with pulmonary TB for six polymorphisms located in three genes of the complement pathway. A coding variant from the complement component 3 gene (*C3*), rs7951 (Ala1437Ala), showed a moderately elevated frequency in the control group compared to the affected subjects (*p*-value = 0.032). Moreover, two variants located in introns of the *C3* gene were found to have a lower frequency of the minor alleles in the affected group indicating a protective effect against disease among carriers of these alleles. For instance, carrying the minor allele of rs11569562, situated in intron 38, conveyed a lower risk of TB [allelic OR (95% CI) = 0.7 (0.57-0.87); *p*-value = 0.0016]. The other polymorphism, located roughly 600 bp distant in intron 37, rs344555, displayed the same effect.

A rare variant, overall frequency in our study population below 5%, situated downstream of C1QB, appeared to confer susceptibility to pulmonary TB [OR (95% CI) = 2.32 (1.2-4.48), *p*-value = 0.0095]. Two of the genotyped SNPs within *CR1* conferred a slight risk of disease among carriers of the major allele (Table 3).

Six polymorphisms from three genes belonging to the scavenger receptor family showed significant allelic association (Table3). Two missense polymorphisms within *SCARF1*, A425V and E639D, appeared to increase susceptibility to infection. The E639D polymorphism displays a more significant odds ratio than the A425V polymorphism [allelic OR (95% CI) = 1.57 (1.01-2.45)]. It is worth mentioning that an associated SNP, rs9896488, located about 3.9 kb upstream of *SCARF1*, is also a coding variant, Ala32Ala, within a gene involved in late endocytic transport, Rab interacting lysosomal protein (*RILP*).

A polymorphism in the promoter region of *CD209*, rs735239, showed a significant difference in allele distribution between both groups. The allelic odds ratio (Table 3) indicated that infection is more likely among carriers of the major allele (A).

In order to address the significance of our findings, a permutation analysis of the allelic *p*-values was carried out (Table 3). Fourteen of the initial 20 polymorphisms passed the permutation test (n = 10,000), with their *p*-values remaining statistically significant at an adjusted *p*-value < 0.05. The same analysis was applied separately by gender to polymorphisms in *TLR8*. In this case, four of the original polymorphisms on Chromosome X maintained statistical significance at an adjusted *p*-value < 0.05 (Table 4) in males.

Table 4.
Allele Distribution of *TLR8* Polymorphisms among TB Patients and Controls in Males and Females

dbSNP ID	Males					Females		
	No. of Cases (%)[a]	No. of Controls (%)[a]	p-value	Permutational p-value[b]	No. of Controls (%)[a]	O.R. (95% CI)	No. of Cases (%)[a]	p-value
rs376487977	77 (34.6)	49 (21.7)	0.0024	0.012	1.9 (1.2-2.9)	76 (27.1)	74 (24.3)	NS
rs378893576	76 (34.3)	50 (22.1)	0.0039	0.017	1.8 (1.2-2.8)	76 (27.1)	74 (24.3)	NS
rs3761624	76 (34.3)	51 (22.4)	0.007	0.02	1.8 (1.2-2.7)	76 (27.1)	74 (24.3)	NS
rs3764880	76 (34.3)	51 (22.4)	0.007	0.02	1.8 (1.2-2.7)	76 (27.1)	74 (24.3)	NS

[a] Number of chromosomes carrying the minor allele.
[b] Number of Permutations=10,000.

Genotype Association Analysis

Association analyses of genotypes (Table 5) implicated all of the variants identified by the tests of allelic association. Furthermore, one additional significantly associated SNP - rs2287886 (*CD209*) was detected. In agreement with the allelic results, homozygosity for the minor allele of the *C3* polymorphisms, rs11569562 (TT), and rs34455 (AA) had a strong protective effect against pulmonary TB [OR (95% CI) = 0.42 (0.25-0.69), *p*-value = 0.0013; OR (95% CI) = 0.37 (0.19-0.73), *p*-value = 0.01, respectively]. Due to a very low minor allele frequency of the rare allele of variant rs12040131 from *C1QB* no homozygotes were observed. Nevertheless, more homozygotes for the major allele were identified in the affected group compared to controls, indicating susceptibility to disease amongst carriers.

Analysis of genotypes for polymorphisms located on Chromosome X was done using a likelihood ratio test. Four variants on *TLR8* were found to be more frequent in cases than controls, indicating susceptibility to disease for carriers of the minor allele. Due to the fact that males only carry one copy of each allele, the genotype association outcome was expected to be the same as for the previous allele association result. Thus, we focused on analyzing genotypes of female subjects (Table 6). The observed number of homozygotes for the associated missense polymorphism, rs3764880 (AA), may have been too low to detect an effect (14 affected vs. 9 controls). Nevertheless there was an apparent trend towards the same outcome observed in the overall sample, with affected females showing 5% more homozygotes for the minor allele, compared to the control group.

Two promoter variants within *CD209* showed significant differences in genotype distributions between cases and controls. In the case of rs735239 [OR (95% CI) = 1.5 (1.1- 2.2), *p*-value = 0.04] the major allele showed association with susceptibility to disease. The second associated SNP, rs2287886, conferred a protective effect to those subjects homozygous for the minor allele [OR (95% CI) = 0.45 (0.26-0.79), *p*-value = 0.017]. After performing permutation analyses ($n = 10.000$), all associated variants retained statistically significant genotype association values (Table 5).

Haplotype analysis

Assessing the haplotype structure of genes with associated polymorphisms identified some redundant SNPs among the 21 significantly associated variants. Construction of haplotypes helped to establish regions of high

Table 5.
Genotype Distributions of SNPs with p-values < 0.05

dbSNP rs#	Gene	p-value	Permutational p-value[a]	O.R. (95% CI)[b]	1[c] Case: Control	2[c] Case: Control	3[c] Case: Control	Associated genotype
rs11569562	*C3*	0.0013	0.0006	0.42 (0.25-0.69)	165:144	173:181	24:55	TT (3)
rs344555	*C3*	0.01	0.009	0.37 (0.19-0.73)	219:209	131:139	12:32	AA (3)
rs2287886	*CD209*	0.017	0.018	0.45 (0.26-0.79)	182:183	166:157	19:41	GG (3)
rs12040131	*C1QB*	0.035	0.024	2.3 (1.18-4.48)	349:350	13:29	0:0	CC (1)
rs9896488	*SCARF1*	0.038	0.032	1.4 (0.95-2.07)	311:309	51:71	0:0	GG(1)
rs17001654	*SCARB2*	0.038	0.044	2.05 (1.07-3.9)	348:351	14:29	0:0	CC (1)
rs735239	*CD209*	0.04	0.044	1.5 (1.1-2.2)	302:286	59:89	5:5	AA (1)
rs714521	*Ly86*	0.047	0.049	0.7 (0.51-0.95)	109:145	185:180	68:55	TT (1)

[a] Number of Permutations = 10,000.
[b] Two genotypes were collapsed and compared to the associated genotype.
[c] (1) Homozygous for the major allele, (2) Heterozygous,
(3) Homozygous for the minor allele.

linkage disequilibrium and to define polymorphisms located within the same haplotype block. Tagging SNPs were identified with *Haploview v3.31* using the default settings [33] after setting a standard threshold of $r^2 \geq 0.8$. Variants within *TLR8* passed the cut-off value, with one SNP tagging the other three associated polymorphisms.

Only genes with significantly associated haplotypes are reported here. Investigation of the gene structure of *TLR8* showed two distinct haplotype blocks in our population. As expected from our initial studies, all four associated polymorphisms appeared in the same haplotype block (Block1). Performing separate association analysis of haplotypes in males and females confirmed the single SNP associations. The frequent haplotype (H1) harboring rs3764880G, showed a pronounced protective effect against disease among male carriers [OR (95% CI) = 0.55 (0.36-0.83)].

We investigated whether any linkage disequilibrium pattern could be revealed between the associated variants of *CD209*. Analysis of haplotype structure and frequencies detected three haplotype blocks. Within block 3, towards the 5' region of the gene, one haplotype (H2) presented a marked difference in distributions between cases and controls (Table 7). This haplotype, with an 11% frequency in our study population, contained two -

Table 6.
Genotype Distribution of *TLR8* Polymorphisms among TB Patients and Controls in All and female

dbSNP ID	All[a]	Females			
	p-value	1[b] Case:Control	2[b] Case:Control	3[b] Case:Control	p-value
rs3764879	0.016	78:87	48:56	14:9	NS
rs3788935	0.019	78:87	48:56	14:9	NS
rs3761624	0.026	78:87	48:56	14:9	NS
rs3764880	0.026	78:87	48:56	14:9	NS

[a] Indicates the summation of males and females
[b] (1) Homozygous for the major allele, (2) Heterozygous, (3) Homozygous for the minor allele.

promoter variants previously discussed: rs735239 (-871G) and rs2287886 (-139G). In accordance with published reports,[24] this haplotype (H2) was found to be more common in the control group [OR (95% CI) = 0.75 (0.59-0.96)].

The complement gene, *C3*, was divided into five haplotype blocks. Block 1 contained polymorphisms from intron 37, rs344555, and intron 38, rs11569562, associated with disease susceptibility in previous analysis. The most common haplotype (H1) within this block (Table 7), carrying the major alleles for both polymorphisms, was found with higher frequency in affected subjects [OR (95% CI) = 1.35 (1.1-1.7), *p*-value = 0.052]. Furthermore, carriers of the second most frequent haplotype harboring the minor alleles of both SNPs were shown to be more frequent in the control group, thus protecting against disease.

One of the scavenger receptor genes (*SCARF1*), displayed association at the haplotype level as well. The gene was divided into two blocks. Within the first block (Table 7) comprising 8 kb, the second most common haplotype (H2), with an overall frequency of 36.5% in our study population, showed association with the case group [OR (95% CI) = 1.25 (1.01-1.54), *p*-value = 0.039]. Coding polymorphisms reported in Table 3, A425V and E639D, were included in this haplotype. A smaller additional block (Block 2) contained two associated haplotypes that conferred opposite effects: H1, showed susceptibility to infection, and H3 that displayed protection against disease. The associated SNP, rs2293067, was contained in both haplotypes.

Table 7.
Haplotype Analysis and Distribution of Polymorphisms in Patients and Controls in *Cd209*, *C3* and *SCARF1*[a]

Gene	Haplotype	Frequency (%)[b]	Cases (%)	Controls (%)	p-value	O.R. (95% CI)[b]
CD209[c]						
Block 3 (1kb)	H1-AAAGT	70.6	72.1	68.8	0.15	1.17 (0.94-1.46)
	H2-GAGAA	11.4	9.8	13.1	0.04	0.71 (0.52-0.98)
	H3-GGAGT	7.9	7.2	8.5	0.33	0.83 (0.57-1.21)
	H4-GAAAA	6.3	7	5.7	0.24	1.29 (0.85-1.96)
	H5-GGAGA	3.5	3.5	3.5	0.92	1.01 (0.58-1.75)
C3[d]						
Block 1 (2kb)	H1-AGG	64.7	68.3	61.4	0005	1.35 (1.09-1.68)
	H2-CAA	24	21.4	26.4	0.023	0.75 (0.59-0.96)
	H3-AAG	9.6	8.4	10.9	0.1	0.74 (0.52-1.06)
SCARF1[e]	H1-GCGAA	50.3	49.4	51.1	0.5	0.93 (0.76-1.14)
Block 1 (8 kb)	H2-CCGAA	36.5	39.1	34	0.039	1.25 (1.01-1.54)
	H3-CGGAG	5.3	4.3	6.3	0.081	0.66 (0.41-1.05)
	H4-CCAGG	5.2	4.7	5.7	0.37	0.82 (0.51-1.3)
Block 2 (0.7kb)	H1-AG	56.3	58.9	53.8	0.045	1.23 (1.00-1.51)
	H2-GG	21.9	21.3	22.5	0.5	0.93 (0.72-1.19)
	H3-GA	21.6	19.4	23.6	0.049	0.77 (0.60-0.99)

[a] Only blocks with associated haplotypes are shown.
[b] Frequency of one haplotype against the rest of haplotypes together.
[c] Alleles in haplotypes of *CD209* are ordered from rs2287886 to rs7359874.
[d] Alleles in haplotypes of *C3* are ordered from rs37957 to rs344555.
[e] Alleles in haplotypes of *SCARF1* are ordered from rs4790732 to rs9896488.

DISCUSSION

Here we describe an association study aiming to identify polymorphisms which confer increased susceptibility to pulmonary TB in an Indonesian population. The novelty of our approach stems from the comprehensive genetic coverage of a large number of genes whose protein products are involved in pathogen recognition and related pathways. Our results show novel

genetic associations of key genes implicated in the innate immune response, such as *TLR8*, scavenger receptors, and members of the complement family. Moreover, we have confirmed a recent report describing an association between variants in DC-SIGN (*CD209*) and TB.[24]

Toll-like receptor 8

The cloning and characterization of human *TLR7/8/9* revealed significant similarity of their protein sequences,[38,39] defining, together with *TLR3*, a new sub-family within the Toll-like receptor genes. In contrast to the other TLRs, their protein products are localized intracellularly rather than at the cell surface.[40] Even though only TLR9 has been experimentally proven to recognize mycobacterial DNA,[41] single-stranded RNA derived from pathogens has been proposed as a likely ligand of TLR7 and TLR8.[42,43] The translocation of TLR9 from the endoplasmic reticulum to the lysosome following CpG binding has recently been described.[44] TLR8 and TLR9 are very closely related to each other, raising the possibility that both receptors share a similar mode of activation. *M. tuberculosis* is an intracellular pathogen that resides in characteristic phagosomes, which are not acidic, generally do not mature into phagolysosomes[45] but do interact with early endosomes, where the bacteria could encounter TLR8. TLR8 is among the least studied members of the toll-like receptor family, as it lacks a functional homolog in mouse,[43] but our results with TB suggest it should be the focus of concerted study in human systems.

For the first time, the association of multiple polymorphisms co-inherited within a distinct haplotype of *TLR8* with susceptibility to pulmonary TB is shown. *TLR7* and *TLR8* are in close proximity on Chromosome X, which suggests that any allele conferring susceptibility to disease may well have a higher impact among males who carry only one copy of the gene. Indeed, the genetic association was more significant in affected males. Hence, inferences about gender-specific effects could possibly be drawn from our findings, where male carriers of the minor allele of rs3764880 showed an increased susceptibility to pulmonary TB. One might expect to find the same association among females homozygous for the same allele, and a tendency towards an altered distribution of affected females homozygous for the minor allele (10%) was indeed observed when compared to female controls (6%). Failure to detect a significant association in this case may be attributed to reduced

power resulting from the small number of observed homozygotes in our study group (Table 6). Studies involving larger cohorts will be necessary to clarify this genetic effect.

Two *TLR8* transcript variants have been characterized thus far.[38,39] One of the associated polymorphisms in this study, rs3764880 (Met1Val), abolishes a putative start codon within the alternative transcript variant 2, (Figure 1). Asian populations appear to have an unusually elevated derived allele frequency for this missense variant compared to other ethnic groups.[37] Such a significant rise in allele frequency, presumably occurring in Asia, could indicate an important selective advantage or disadvantage for this allele in some environments. In agreement with the genetic association of *TLR8* with susceptibility to TB, preliminary data from microarray expression studies indicated increased expression levels of variant 2 transcript in patients (Hibberd ML, unpublished data). The effect, if any, of replacing the first methionine of transcript 2 by valine has not been established. Nevertheless, taking into account that TLR8 is a membrane protein; the initial amino acids are predicted to act as signal peptide. Therefore, removal of the sequence could well affect the intracellular trafficking or proper folding of the protein .[46]

CD209

The role of DC-SIGN, the protein product of *CD209*, in pathogen recognition has been extensively studied and a number of independent reports have shown genetic association between several of its polymorphisms and host susceptibility to various infectious agents, such as HIV,[25] dengue[47] and *M. tuberculosis*.[24] The latter reported two promoter variants, -871G and -336A, that confer protection against TB among homozygous subjects in a South African cohort.

In this study we validated the association of rs735239 (-871G) to TB in an Indonesian population. However, due perhaps to vast differences in minor allele frequencies of rs480483 (-336A) among populations, 11% in Indonesians *vs.* 38% in South African colored, this variant did not show a significant association with TB in our study group. Differences in allele frequencies in the polymorphisms of the gene among distinct populations have been suggested to result from selective pressure by infectious agents.[24] On the other hand a polymorphism located towards the starting codon of the

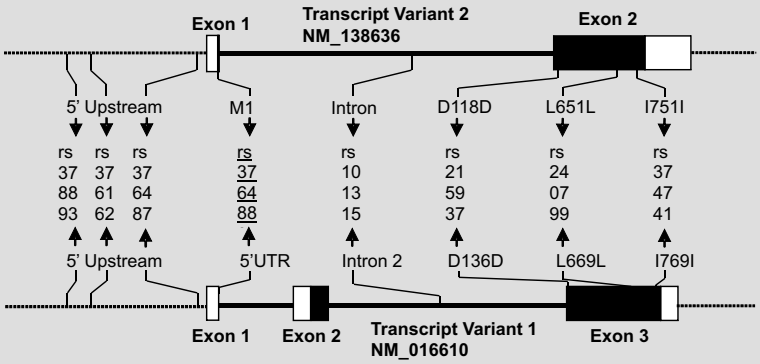

Figure 1.
Transcript variants of TLR8 and location of genotyped SNPs within both transcripts.
Exons are shown as rectangles. Filled areas represent translated sequences, open areas indicate untranslated regions. The associated polymorhpism resulting in a coding change exclusive of transcript variant 2 (rs3764880) is highlighted.

gene, rs2287886 (-139G), was found to have a protective effect against TB disease amongst homozygous carriers. It is worth pointing out that variant -336A, which does not display any significant association in our cohort, is however included in the associated haplotype (Block 3-H2) detected in the promoter region of DC-SIGN (Table 7) together with -871G and -139G.

Cell-free experiments have reported that the -336A allele alters transcript levels leading to increased expression of DC-SIGN, which plays an important role in uptake of *M. tuberculosis*.[47] A hypothesis that needs to be experimentally tested could be that these changes on protein expression would lead to alterations of bacteria uptake or impairment of intracellular signaling after binding to DC-SIGN. Furthermore, the effect of these associated variants from the 5' end of the gene on *CD209* transcription should be further investigated.

Complement component genes

In this study we have identified genetic variants within three genes of the complement pathway which show significant association with pulmonary TB in an Indonesian population. These findings emphasize the role of this pathway in the uptake and management of *M. tuberculosis* after infection. A protective effect against disease was shown for two polymorphisms within introns 37 and 38 of *C3*. Moreover, a haplotype that carries both alleles confirmed the identified association. Evidence of activation of the complement pathway has been reported upon recognition of *M. tuberculosis* by C3 in human bronchoalveolar fluid .[5]

Phagocytosis of bacteria by the macrophage complement receptors, CR1 among others,[48] and interactions between complement components, such as binding of CR1 and C1q are well established.[49] One polymorphism of *CR1* showing association with disease, rs2274567, is located on a Sushi domain, a motif that is known to be involved in protein-protein and protein-ligand interactions.[50] Changes of the amino acid sequence in this motif have been proven to abolish the binding of specific ligands to their receptor.[50]

Scavenger receptor genes

Variants from two scavenger receptors genes class B, *SCARB1* and *SCARB2*, involved in lipid metabolism, were detected in our genetic analysis. Polymorphisms of *SCARB1* have been associated with levels of lipoproteins in plasma in diabetic patients,[51] but its role in mycobacterial infection has yet to be elucidated.

Unexpectedly, a gene not included in our initial selection, *RILP* (Rab7-interacting lysosomal protein), but located less than 5 kb upstream of *SCARF1*, showed association for one coding SNP. RILP is implicated in controlling late endocytic transport. It has been described that early expression of RILP during *Salmonella* infection impaired intracellular replication of the pathogen.[52] Moreover, disrupting interaction of RILP with Rab7 seems to provide an optimal space for replication of bacteria.[53,54]

Further genetic and functional studies on each of these associated polymorphisms will help to characterize and gain a deeper understanding of their roles in this infectious disease.

In summary, we have reported a comprehensive genetic study of a collection of genes coding for proteins involved in pathogen recognition, which resulted in detection of novel genetic associations with pulmonary tuberculosis risk in an Indonesian population.

Acknowledgements
We thank Anugrah Maya and Erita Istriana for helping in clinical work at PPTI-Jakarta supervised by Dr. Halim Danusantoso; Iskandar Adnan from Eijkman Institute for Molecular Biology Jakarta for helping in the DNA isolation supervised by Prof. Sangkot Marzuki and Dr. Herawati Sudoyo. We thank Prof R.H.H. Nelwan from University of Indonesia for long-standing collaboration in Jakarta. We are grateful to our colleagues at the Genome Institute of Singapore: Meah Wee Yang and Jason Ong for genotyping; Rick Ong for helping on the management of the genotyping data and, Frans Verhoeff for maintaining the GISSNP database. This work has been supported by funding from the Agency for Science & Technology and Research of Singapore (A*STAR) and by the Royal Netherlands Academy of Arts and Sciences (KNAW, project 99MED01), the Netherlands Organization for Scientific Research (NWO), and the Scientific Research for the Tropic (WOTRO, PRIOR-project). The funding agencies had no role in any aspect of the direction of the study or preparation of the manuscript.

References

1. The World Health Organization (2006) Tuberculosis.Fact sheet number 104. World Health Organization.
2. Bloom BR, Small PM (1998) The evolving relation between humans and Mycobacterium tuberculosis. N Engl J Med 338: 677-678.
3. Aderem A, Underhill DM (1999) Mechanisms of phagocytosis in macrophages. Annu Rev Immunol 17: 593-623.
4. van Crevel R, Ottenhoff TH, van der Meer JW (2002) Innate immunity to Mycobacterium tuberculosis. Clin Microbiol Rev 15: 294-309.
5. Ferguson JS, Weis JJ, Martin JL, Schlesinger LS (2004) Complement Protein C3 Binding to Mycobacterium tuberculosis Is Initiated by the Classical Pathway in Human Bronchoalveolar Lavage Fluid. Infect Immun 72: 2564-2573.
6. Hibberd ML, Sumiya M, Summerfield JA, Booy R, Levin M (1999) Association of variants of the gene for mannose-binding lectin with susceptibility to meningococcal disease. Meningococcal Research Group. Lancet 353: 1049-1053.
7. Bulut Y, Michelsen KS, Hayrapetian L, Naiki Y, Spallek R, et al. (2005) Mycobacterium Tuberculosis Heat Shock Proteins Use Diverse Toll-like Receptor Pathways to Activate Pro-inflammatory Signals 10.1074/jbc.M411379200. J Biol Chem 280: 20961-20967.
8. Ben-Ali M, Barbouche MR, Bousnina S, Chabbou A, Dellagi K (2004) Toll-like receptor 2 Arg677Trp polymorphism is associated with susceptibility to tuberculosis in Tunisian patients. Clin Diagn Lab Immunol 11: 625-626.
9. Medzhitov R (2001) Toll-like receptors and innate immunity. Nat Rev Immunol 1: 135-145.
10. Takeda K, Akira S (2005) Toll-like receptors in innate immunity. Int Immunol 17: 1-14.
11. Akira S, Takeda K (2004) Toll-like receptor signalling. Nat Rev Immunol 4: 499-511.
12. Selvaraj P, Jawahar MS, Rajeswari DN, Alagarasu K, Vidyarani M, et al. (2006) Role of mannose binding lectin gene variants on its protein levels and macrophage phagocytosis with live Mycobacterium tuberculosis in pulmonary tuberculosis. FEMS Immunol Med Microbiol 46: 433-437.
13. Soborg C, Madsen HO, Andersen AB, Lillebaek T, Kok-Jensen A, et al. (2003) Mannose-binding lectin polymorphisms in clinical tuberculosis. J Infect Dis 188: 777-782.
14. Hoal-Van Helden EG, Epstein J, Victor TC, Hon D, Lewis LA, et al. (1999) Mannose-binding protein B allele confers protection against tuberculous meningitis. Pediatr Res 45: 459-464.
15. Bernig T, Taylor JG, Foster CB, Staats B, Yeager M, et al. (2004) Sequence analysis of the mannose-binding lectin (MBL2) gene reveals a high degree of heterozygosity with evidence of selection. Genes Immun 5: 461-476.
16. Fitness J, Floyd S, Warndorff DK, Sichali L, Malema S, et al. (2004) Large-scale candidate gene study of tuberculosis susceptibility in the Karonga district of northern Malawi. Am J Trop Med Hyg 71: 341-349.
17. Ogura Y, Bonen DK, Inohara N, Nicolae DL, Chen FF, et al. (2001) A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature 411: 603-606.
18. Ferwerda G, Girardin SE, Kullberg B-J, Le Bourhis L, de Jong DJ, et al. (2005) NOD2 and Toll-Like Receptors Are Nonredundant Recognition Systems of Mycobacterium tuberculosis. PLoS Pathogens 1: e34.
19. Neyrolles O, Gicquel B, Quintana-Murci L (2006) Towards a crucial role for DC-SIGN in tuberculosis and beyond. Trends Microbiol 14: 383-387.

20. Arredouani MS, Palecanda A, Koziel H, Huang YC, Imrich A, et al. (2005) MARCO is the major binding receptor for unopsonized particles and bacteria on human alveolar macrophages. J Immunol 175: 6058-6064.
21. Divanovic S, Trompette A, Atabani SF, Madan R, Golenbock DT, et al. (2005) Inhibition of TLR-4/MD-2 signaling by RP105/MD-1. J Endotoxin Res 11: 363-368.
22. Pacheco E, Fonseca C, Montes C, Zabaleta J, Garcia LF, et al. (2004) CD14 gene promoter polymorphism in different clinical forms of tuberculosis. FEMS Immunol Med Microbiol 40: 207-213.
23. Ferguson JS, Voelker DR, McCormack FX, Schlesinger LS (1999) Surfactant protein D binds to Mycobacterium tuberculosis bacilli and lipoarabinomannan via carbohydrate-lectin interactions resulting in reduced phagocytosis of the bacteria by macrophages. J Immunol 163: 312-321.
24. Barreiro LB, Neyrolles O, Babb CL, Tailleux L, Quach H, et al. (2006) Promoter variation in the DC-SIGN-encoding gene CD209 is associated with tuberculosis. PLoS Med 3: e20.
25. Martin MP, Lederman MM, Hutcheson HB, Goedert JJ, Nelson GW, et al. (2004) Association of DC-SIGN promoter polymorphism with increased risk for parenteral, but not mucosal, acquisition of human immunodeficiency virus type 1 infection. J Virol 78: 14053-14056.
26. The World Health Organization (2005) Global Tuberculosis Control. Surveillance, planning, financing.: World Health Organization.
27. Alisjahbana B, van Crevel R, Sahiratmadja E, den Heijer M, Maya A, et al. (2006) Diabetes mellitus is strongly associated with tuberculosis in Indonesia. Int J Tuberc Lung Dis 10: 696-700.
28. Sambrook JR, DW. (2001) Molecular cloning: a laboratory manual. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.
29. Epstein MP, Duren WL, Boehnke M (2000) Improved inference of relationship for pairs of individuals. Am J Hum Genet 67: 1219-1231.
30. Skipper L, Li Y, Bonnard C, Pavanni R, Yih Y, et al. (2005) Comprehensive evaluation of common genetic variation within LRRK2 reveals evidence for association with sporadic Parkinson's disease. Hum Mol Genet 14: 3549-3556.
31. Einarsdottir K, Humphreys K, Bonnard C, Palmgren J, Iles MM, et al. (2006) Linkage disequilibrium mapping of CHEK2: common variation and breast cancer risk. PLoS Med 3: e168.
32. Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21: 263-265.
33. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, et al. (2002) The structure of haplotype blocks in the human genome. Science 296: 2225-2229.
34. de Bakker PI, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, et al. (2005) Efficiency and power in genetic association studies. Nat Genet 37: 1217-1223.
35. Devlin B, Roeder K (1999) Genomic control for association studies. Biometrics 55: 997-1004.
36. Kohler K, Bickeboller H (2006) Case-control association tests correcting for population stratification. Ann Hum Genet 70: 98-115.
37. The International HapMap Project (2003) The International HapMap Project. Nature 426: 789-796.
38. Du X, Poltorak A, Wei Y, Beutler B (2000) Three novel mammalian toll-like receptors: gene structure, expression, and evolution. Eur Cytokine Netw 11: 362-371.
39. Chuang TH, Ulevitch RJ (2000) Cloning and characterization of a sub-family of human toll-like receptors: hTLR7, hTLR8 and hTLR9. Eur Cytokine Netw 11: 372-378.

40. Heil F, Ahmad-Nejad P, Hemmi H, Hochrein H, Ampenberger F, et al. (2003) The Toll-like receptor 7 (TLR7)-specific stimulus loxoribine uncovers a strong relationship within the TLR7, 8 and 9 subfamily. Eur J Immunol 33: 2987-2997.
41. Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, et al. (2000) A Toll-like receptor recognizes bacterial DNA. Nature 408: 740-745.
42. Diebold SS, Kaisho T, Hemmi H, Akira S, Reis e Sousa C (2004) Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science 303: 1529-1531.
43. Heil F, Hemmi H, Hochrein H, Ampenberger F, Kirschning C, et al. (2004) Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. Science 303: 1526-1529.
44. Latz E, Schoenemeyer A, Visintin A, Fitzgerald KA, Monks BG, et al. (2004) TLR9 signals after translocating from the ER to CpG DNA in the lysosome. Nat Immunol 5: 190-198.
45. Crowle AJ, Dahl R, Ross E, May MH (1991) Evidence that vesicles containing living, virulent Mycobacterium tuberculosis or Mycobacterium avium in cultured human macrophages are not acidic. Infect Immun 59: 1823-1831.
46. Beuret N, Rutishauser J, Bider MD, Spiess M (1999) Mechanism of endoplasmic reticulum retention of mutant vasopressin precursor caused by a signal peptide truncation associated with diabetes insipidus. J Biol Chem 274: 18965-18972.
47. Sakuntabhai A, Turbpaiboon C, Casademont I, Chuansumrit A, Lowhnoo T, et al. (2005) A variant in the CD209 promoter is associated with severity of dengue disease. Nat Genet 37: 507-513.
48. Schlesinger LS (1993) Macrophage phagocytosis of virulent but not attenuated strains of Mycobacterium tuberculosis is mediated by mannose receptors in addition to complement receptors. J Immunol 150: 2920-2930.
49. Klickstein LB, Barbashov SF, Liu T, Jack RM, Nicholson-Weller A (1997) Complement receptor type 1 (CR1, CD35) is a receptor for C1q. Immunity 7: 345-355.
50. Wei X, Orchardson M, Gracie JA, Leung BP, Gao B, et al. (2001) The Sushi domain of soluble IL-15 receptor alpha is essential for binding IL-15 and inhibiting inflammatory and allogenic responses in vitro and in vivo. J Immunol 167: 277-282.
51. Osgood D, Corella D, Demissie S, Cupples LA, Wilson PW, et al. (2003) Genetic variation at the scavenger receptor class B type I gene locus determines plasma lipoprotein concentrations and particle size and interacts with type 2 diabetes: the framingham study. J Clin Endocrinol Metab 88: 2869-2879.
52. Marsman M, Jordens I, Kuijl C, Janssen L, Neefjes J (2004) Dynein-mediated vesicle transport controls intracellular Salmonella replication. Mol Biol Cell 15: 2954-2964.
53. Harrison RE, Brumell JH, Khandani A, Bucci C, Scott CC, et al. (2004) Salmonella impairs RILP recruitment to Rab7 during maturation of invasion vacuoles. Mol Biol Cell 15: 3146-3154.
54. Via LE, Deretic D, Ulmer RJ, Hibler NS, Huber LA, et al. (1997) Arrest of mycobacterial phagosome maturation is caused by a block in vesicle fusion between stages controlled by rab5 and rab7. J Biol Chem 272: 13326-13331.