An age-dependent association of mannose-binding lectin-2 genetic variants on HIV-1–related disease in children

Kumud K. Singh, PhD,[a] Alexis Lieser, MD,[b] Ping K. Ruan, PhD,[c] Terry Fenton, EdD,[c] and Stephen A. Spector, MD[a] *La Jolla and Orange, Calif, and Boston, Mass*

Background: Mannose-binding lectin (MBL) is part of the lectin pathway of complement activation against various pathogens; however, its role in innate immune responses against HIV-1 infection in children is unknown.

Objective: This study evaluated the effects of mannose-binding lectin-2 *(MBL2)* alleles on HIV-1 disease progression and central nervous system (CNS) impairment in children.

Methods: A cohort of 1037 HIV-1–infected children enrolled in Pediatrics AIDS Clinical Trial Group protocols P152 and P300 before the availability of effective antiretroviral therapy was genotyped for *MBL2* and evaluated for disease progression.

Results: Children with the homozygous variant *MBL2-O/O* genotype were more likely to experience rapid disease progression and CNS impairment than those with the wild-type *AA* genotype. The effects were predominantly observed in children younger than 2 years. In unadjusted Cox proportional hazards models, children younger than 2 years with *MBL2-O/O* experienced more rapid disease progression (*O/O* vs *AA*: relative hazard [RH], 1.54; 95% CI, 1.07-2.22; *P* = .02; *O/O* vs *A/O*: RH, 2.28; 95% CI, 1.09-4.79; *P* = .029). Similarly, children with *MBL2-O/O* were more likely to experience rapid progression to CNS impairment (*O/O* vs *A/A*: RH, 2.78; 95% CI, 1.06-2.69, *P* = .027; *O/O* vs *A/O*: RH, 1.69; 95% CI, 1.07-7.21; *P* = .035). The effects remained significant after adjustment for $CD4^+$ lymphocyte count, plasma HIV-1 RNA, and other genotypes.

Conclusions: *MBL2-O/O* genotypes, which result in lower expression of MBL, are associated with more rapid HIV-1–related disease progression, including CNS impairment, predominantly in children younger than 2 years. These data suggest that *MBL2* variants are associated with altered HIV-1 disease progression, particularly in young children. (J Allergy Clin Immunol 2008;122:173-80.)

Key words: *Mannose binding lectin-2, polymorphisms, HIV-1 disease, central nervous system impairment, children*

Innate immunity comprises antigen-nonspecific defense mechanisms that a host uses immediately or within hours after exposure to a broad spectrum of antigens to eliminate microbes and prevent infection. Unlike adaptive immunity, innate immunity is designed to recognize a few highly conserved structures present in many different microorganisms. Mannose-binding lectin protein (MBL), also called mannose-binding protein, encoded by the mannose binding lectin-2 *(MBL2)* gene, is an important determinant of the innate immune response during an infection.[1-4] MBL is an acute-phase protein that is synthesized by the liver and released into the bloodstream, where it binds to the mannose residues present on some bacteria, yeast, viruses, and parasites. Binding activates the lectin complement pathway and production of C3b protein through MBL-associated serine proteases,[5] which results in opsonization of pathogens, chemotaxis, activation of leukocytes, and direct killing of pathogens.

The *MBL2* gene encodes 32-kd subunits that oligomerize to form 96-kd MBL structural units or "monomers." The "monomers" then further associate to form high-molecular-weight MBL oligomers.[4] Only the high-molecular-weight oligomer structure is capable of activating complement. *MBL2* variants at the following nucleotide positions affect MBL levels: 2 single nucleotide polymorphisms at promoter positions −550-G/C (*H/L* variant) and −221-G/C (*X/Y* variant), one in the 5′ untranslated region +4-C/T (*P/Q* variant) and 3 genetic variants at codons 52, 54, and 57 in exon 1 at nucleotide positions 223-C/T (Arg52Cys, *A/D* allele), 230-G/A (Gly54Asp, *A/B* allele), and 239-G/A (Gly57Glu, *A/C* allele), respectively. *MBL2* exon 1 variants result in single amino acid changes affecting oligomerization of MBL. Homozygous wild-type *(A/A)* sera contain predominantly fully functional MBL, whereas homozygous mutant sera (any combination of *B*, *C*, or *D* alleles) contain mostly low-molecular-weight MBL. Sera containing heterozygous variant alleles (*A/O*, *O* = *B*, *C*, or *D*) contain both high-molecular-weight and low-molecular-weight MBL, with the ratio determined by the promoter type on the normal haplotype (*A* allele).

MBL deficiency was initially recognized as an opsonic defect in children with frequent unexplained infections and has been linked to increased severity and incidence of complications for several inherited immunodeficiency and autoimmune diseases.[6-11] MBL deficiency has also been associated with increased HIV-1 vertical transmission.[12] Recently, an age-dependent association of *MBL2* variants on the susceptibility to acute respiratory tract infection and meningococcal disease was reported.[8,13]

From [a]the Department of Pediatrics, Division of Infectious Diseases, University of California, San Diego, La Jolla; [b]the University of California Irvine Medical Center, Orange; and [c]the Center for Biostatistics in AIDS Research, Harvard School of Public Health, Boston.

Supported by the Pediatric AIDS Clinical Trials Group and the International Maternal Perinatal Adolescent AIDS Clinical Trials (IMPAACT) Network AI068632 and AI-36214 (Virology Core, University of California, San Diego, Center for AIDS Research), and Rest Haven, San Diego, Calif.

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

Presented in part at the XIVth Conference on Retroviruses and Opportunistic Infections, Los Angeles, Calif, February 25-28, 2007.

Received for publication March 17, 2008; revised May 12, 2008; accepted for publication May 16, 2008.

Reprint requests: Stephen A. Spector, MD, Department of Pediatrics, University of California, San Diego, 9500 Gilman Dr, La Jolla, CA 92093-0672. E-mail: saspector@ucsd.edu; or Kumud K. Singh, PhD, Department of Pediatrics, University of California, San Diego, 9500 Gilman Dr, La Jolla, CA 92093-0672. E-mail: kusingh@ucsd.edu.

0091-6749/$34.00

doi:10.1016/j.jaci.2008.05.025

Abbreviations used
CNS: Central nervous system
MBL: Mannose-binding lectin
MBL2: Mannose binding lectin-2
NRTI: Nucleoside reverse transcriptase inhibitor
PACTG: Pediatric AIDS Clinical Trial Group
PFS: Progression-free survival
RH: Relative hazard
WAIS-R: Wechsler Adult Intelligence Scale: Revised
WISC-R: Wechsler Intelligence Scale for Children: Revised

The current study examined the distribution of *MBL2* genotypes/haplotypes and their effect on HIV-1–related disease progression and central nervous system (CNS) impairment in children. We hypothesized that the presence of genetic variants of *MBL2* resulting in production of lower or nonfunctional protein would be associated with susceptibility to HIV infection and disease progression in children. We further hypothesized that younger children with *MBL2* variants would experience more rapid HIV-1 disease than older children. Our findings support an age-dependent association of *MBL2* variants with HIV-1–related disease in children.

METHODS

Patient populations

Two randomized, double-blind, multicenter Pediatric AIDS Clinical Trial Group (PACTG) protocols, P152[14] and P300[15], for seroprevalent children with symptomatic HIV-1 infection were designed to compare the effects of monotherapy with either zidovudine or didanosine and combination therapy with zidovudine plus didanosine and to compare the effects of zidovudine plus lamivudine with didanosine monotherapy and zidovudine plus didanosine combination therapy, respectively. Children from both the studies, PACTG 152 (n = 448) and PACTG 300 (n = 589), were screened for *MBL2* exon 1, untranslated region, and promoter region polymorphisms, retrospectively.

Details of characteristics of children, eligibility criteria, study end points, disease progression, and neuropsychological tests used have been described earlier.[14-16] The neuropsychological and neurodevelopmental tests administered were the Bayley Scales of Infant Development (3-30 months old), the McCarthy Scales of Children's Abilities (31 months to 6 years old), and the Wechsler Intelligence Scale for Children: Revised (WISC-R, for 6-15 years, 11 months old) or the Wechsler Adult Intelligence Scale: Revised (WAIS-R; for >16 years of age). The Mental Developmental Index of Bayley scales, the General Cognitive Index of the McCarthy scales, and the full-scale IQ of the WISC-R or WAIS-R were used to assess cognitive function. The standard score for each test was 100, and the SD was 16 points for the Bayley Mental Developmental Index and the McCarthy General Cognitive Index and 15 points for the WISC-R and WAIS-R. Children were considered to have significant developmental delay if they had a standardized score of 70 or less or (almost equal to) 2 SDs less than the normal test score of 100. Informed consent was obtained from study participants. This study followed the human experimentation guidelines of the US Department of Health and Human Services and the University of California, San Diego review board.

Genotyping

MBL2 genotyping was done by means of real-time PCR with melting curve analysis (LightCycler; Roche, Indianapolis, Ind), as described earlier.[17] The studied genotypes included 2 single nucleotide polymorphisms at promoter positions −550-G/C (*H/L* variant) and −221-G/C (*X/Y* variant), one in the 5′ untranslated region +4-C/T (*P/Q* variant) and 3 genetic variants at codons 52, 54, and 57 in exon 1 at nucleotide positions 223-C/T (Arg52Cys, *A/D* allele), 230-G/A (Gly54Asp, *A/B* allele), and 239-G/A (Gly57Glu, *A/C* allele), respectively.

Statistical analyses

The primary end points for the analyses were either time to progression to first clinical HIV-1–related disease end point or death, which constituted the progression-free survival (PFS). The disease progression included weight-growth failure, ≥2 opportunistic infections, malignancy, and/or a new abnormality of the CNS (eg, neurologic deterioration, a decrease in neurocognitive test scores, and/or brain growth failure). The CNS impairment end point, a subset of PFS, was defined as time from entry on the study to the deterioration in brain growth, psychologic function, and/or neurologic status.[14-16] Table I provides a summary of end points by age group. The cross-tabulations of *MBL2* genotypes/haplotypes by race/ethnicity and age groups were used to evaluate the genotype and allele frequencies.

Analyses of time to PFS and CNS impairment end points were performed by using Kaplan-Meier methods, and proportional hazards models were used to investigate the effects of genotype variants on PFS and CNS end points in univariate and multivariate analyses that included baseline covariates ($CD4^+$ lymphocyte count, HIV-1 RNA, treatments, race, and sex), as well as other host genotypes previously found to alter HIV-related disease. We also controlled for potential effects of treatment and treatment failure by including the change in $CD4^+$ lymphocyte counts and plasma HIV-1 viral load during follow-up as time-varying covariates in the Cox proportional hazard model. Based on the hypothesis that younger children with *MBL2* variants might have a different experience with regard to disease progression from the older children and because the median age of the children in this cohort was 2.3 years, we performed subgroup analysis using age less than 2 years and ≥2 years as the cutoff points to provide a balanced number of children in each age group. The interaction term between age and *MBL2* genotype was also included in the model. Although the results were as hypothesized, with the effects of genotype on outcome most evident within the younger age group, the interaction term between age groups and *MBL2* genotypes that were included in the model was not statistically significant. The log-rank test was used for comparisons of survival curves for different genotypes. All *P* values are 2-sided.

RESULTS

Characteristics of children

Of the 1037 children included in this analysis, 568 (55%) were girls and 469 (45%) were boys. The children evaluated were 60.3% non-Hispanic black, 26.1% Hispanic, and 13.6% non-Hispanic white. Ages ranged from 42 days to 18 years (<2 years, 46%; >2-5 years, 26%; 5-10 years, 19%; and >10 years, 9%). The median age was 2.3 years (10th and 90th percentiles, 0.45 and 9.5 years). Of the 1037 children, 300 (29%) were followed for less than 12 months, 450 (43%) for 12 to 23 months, 197 (19%) for 24 to 35 months, and 90 (9%) for more than 35 months. A total of 226 (22%), of whom 151 (67%) were less than 2 years old, were identified as having HIV-1–related disease progression during the course of the 2 studies. Characteristics of the children by 2 age groups have been summarized in Table I.

Distribution of *MBL2* genotypes and haplotypes

MBL2-O allele frequency was 0.27 in non-Hispanic blacks, 0.25 in Hispanics, and 0.21 in non-Hispanic whites. The prevalence of the homozygous wild type *(A/A)* was similar across all races/ethnicities (see Table E1 in the Online Repository at www.jacionline.org). The heterozygous *A/B* genotype was less common and the heterozygous *A/C* genotype was more frequent in the non-Hispanic blacks when compared with either Hispanics or non-Hispanic whites ($P < .001$). The non-Hispanic black children had the highest *C* allele frequency (non-Hispanic blacks, 0.20; Hispanics, 0.07; and non-Hispanic whites, 0.05). Of note, the homozygous mutant *C/C* genotype was found exclusively in