Mannose-Binding Lectin Genotypes and Susceptibility to Epstein-Barr Virus Infection in Infancy

Jeppe T. Friberg, 1 Ruth F. Jarrett, 2 Anders Koch, 1,* Peter Garred, 3 June M. L. Freeland, 2 Andreas Andersen, 1 and Mads Melbye 1

Department of Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark 1; LRF Virus Centre, Institute of Comparative Medicine, Faculty of Veterinary Medicine, University of Glasgow, Scotland 2; and Department of Clinical Immunology, Rigshospitalet, Copenhagen, Denmark 3

Received 23 December 2009/Returned for modification 15 February 2010/Accepted 28 June 2010

In a cohort study of children <4 years of age in Greenland, mannose-binding lectin (MBL2) genotypes and Epstein-Barr virus (EBV) antibody levels were determined. EBV seropositivity was significantly lower and time to seroconversion increased in MBL-insufficient compared with MBL-sufficient children, indicating that MBL may be involved in primary EBV infection in infancy.

Epstein-Barr virus (EBV) infection is ubiquitous, and the majority of individuals are infected early in life, but in developed countries 25 to 35% remain seronegative until adolescence (2, 12).

The serum protein mannose-binding lectin (MBL) is a part of the innate immune system binding to a variety of infectious agents and promoting opsonophagocytosis and activating the complement system (27). Three variant alleles in exon 1 of the MBL2 gene on chromosome 10 coding for MBL independently reduce the amount of functional MBL subunits in heterozygous individuals 5- to 10-fold (18), while homozygous persons have only trace amounts of dysfunctional MBL in their blood (27). Furthermore, promoter polymorphisms in the MBL2 gene (X and Y) influence the level of functional MBL (18). MBL-deficient individuals are more susceptible to a range of infections, especially in early infancy before the maturation of the adaptive immune system (14), and MBL is known to modulate the response to viral infections, including herpesviruses (3, 4, 23). Thus, MBL increases neutralization of herpes simplex virus type 2 (HSV-2). MBL deficiency is more prevalent in symptomatic HSV-2 patients (4), and MBL-deficient individuals are at greater risk of recurrent HSV-2 infection (23). In contrast, a clear increase in HSV-2 infectivity was observed in mice following pretreatment with MBL, suggesting that MBL opsonization provides an alternative port of virion entry (3). Thus, MBL may facilitate or inhibit HSV-2 infection. The influence of MBL levels on other herpesviruses in humans is unknown, and the aim of this study was to determine whether MBL2 polymorphisms determining MBL levels are associated with EBV infection in unselected children aged 0 to 4 years.

An open cohort study in the west Greenland community of Sisimiut was carried out from 1996 to 1998 (14, 15). Of all children <2 years of age, 294 (87%) participated and were followed regularly. At the end of the study period, or earlier if children left the study, a venous blood sample was drawn into EDTA containers and separated by centrifugation into plasma and blood cells, frozen, and stored at −80°C. Blood was drawn from 252 children, and for 247 children a plasma sample remained stored (mean age at bleeding, 2.3 years; age range, 3 months to 4 years and 2 months). Thus, some children had blood samples drawn later than the end of their monitoring period.

As part of a population-based school survey for EBV infection in 2004, part of the cohort was reexamined. Blood samples were drawn and treated as described above. Prior to centrifugation, whole blood was allocated and stored at −80°C.

MBL2 structural and promoter alleles were detected in the blood samples from 1997 to 1998, as previously described (7, 18). The three variant alleles (B, C, and D) in exon 1 of the MBL2 gene were grouped as allele O, and the normal allele was designated A. By combining these genotypes and the effects of the promoter variants X and Y, we were able to define six MBL genotypes divided into an MBL-sufficient group (YA/YA, YA/XA, YA/O, XA/XA) (n = 234) and an MBL-insufficient group (XA/O and O/O) (n = 13) with virtually undetectable amounts of functional MBL in the blood of the latter group (5, 6, 8).

Levels of plasma IgG and IgM antibodies to the EBV viral capsid antigen (VCA) were determined in the 1997–1998 and 2004 blood samples using enzyme-linked immunosorbent assays (ELISAs) (Novitex, Freiburg, Germany) (9). Each assay included positive and negative controls and replicates of low, intermediate, and high calibrators. A standard curve based on the absorbance values of the calibrators was constructed and used to determine antibody concentrations in units per ml. EBV seropositivity was defined as either a VCA-IgG of >200 U/ml or a VCA-IgM of >500 U/ml.

DNA was extracted from whole blood (500 μl) from the samples collected in 2004 using QIAamp DNA blood minikits (Qiagen, Crawley, United Kingdom). All samples were assayed using a real-time quantitative TaqMan PCR based on the pol gene of EBV (17). Where sufficient material was available, the sample was assayed in duplicate (83.5% of samples).
TABLE 1. Epstein-Barr virus (EBV) seropositivity and median antibody levels in children in Greenland

| Parameter                  | No. of EBV-seropositive samples/total no. of samples (% positive) | Median EBV-VCA IgG levels (U/ml) | P value<sup>a</sup> | P value<sup>b</sup> |
|----------------------------|---------------------------------------------------------------|----------------------------------|---------------------|---------------------|
| Age (yr)<sup>c</sup>       |                                                               |                                  |                     |                     |
| 0–1½                      | 52/76 (68.4)                                                  | 1,147                            | 0.001               | 0.0001              |
| 1½–3                      | 86/94 (91.5)                                                  | 1,377                            |                     |                     |
| 3–4½                      | 71/77 (92.2)                                                  | 1,314                            |                     |                     |
| Sex                       |                                                               |                                  |                     |                     |
| Male                      | 94/114 (82.5)                                                 | 1,358                            | 0.30                | 0.73                |
| Female                    | 115/133 (86.5)                                                | 1,266                            |                     |                     |
| MBL genotype              |                                                               |                                  |                     |                     |
| Insufficient<sup>d</sup>  | 5/13 (38.5)                                                   | 761                              | 0.01                | 0.05                |
| Sufficient<sup>e</sup>    | 204/234 (87.2)                                                | 1,343                            |                     |                     |

<sup>a</sup> P value for difference between the groups using logistic regression with adjustment for sex and age.

<sup>b</sup> P value for difference between the groups using linear regression with adjustment for sex and age.

<sup>c</sup> Age at time of blood sampling, 1997–1998.

<sup>d</sup> XA/O, O/O.

<sup>e</sup> YA/YA, YA/XA, YA/O, XA/XA.

<sup>f</sup> Median IgG levels are shown for EBV-seropositive samples only.

Regression analyses were used to determine the associations between MBL2 genotypes and EBV seropositivity (logistic regression) and log VCA-IgG levels (linear regression), with adjustment for sex and age. The cumulative risk of EBV seroconversion by age according to MBL2 genotype was estimated by a nonparametric maximum likelihood estimator (25). A test of difference in the cumulative distribution according to MBL2 genotype and sex was performed in an additive hazard regression model for current status data including the two variables (16).

The study was approved by the Commission for Scientific Research in Greenland, which acts as an ethics board for Greenland.

At the time of blood sampling in 1997–1998, 84.6% of children (209 of 247) were EBV seropositive (Table 1). The rate of seropositivity increased with age. There was no gender difference in seropositivity or EBV VCA-IgG levels. Seropositivity was significantly lower for the MBL-insufficient group: 5 of 13 children (38.5%), compared with 204 of 234 children (87.2%) for the MBL-sufficient group, and EBV VCA-IgG levels were also lower in the MBL-insufficient than in the MBL-sufficient group. None of the children were EBV VCA-IgM positive. EBV infection, measured by the presence of EBV antibodies, was on average acquired significantly later among MBL-insufficient children (P < 0.0001) (Fig. 1).

In 2004, 47 boys and 68 girls (mean age, 7.8 and 7.7 years, respectively) were reexamined, of whom 6 were MBL insufficient and 109 MBL sufficient. Among MBL-insufficient children, EBV seropositivity (5 of 6 [83%] versus 107 of 109 [98%]), median EBV VCA levels (1,233 versus 1,353 U/ml), and median EBV copy number in whole blood (2.6 versus 6.8 copies per μg DNA) were lower than in MBL-sufficient children (none of these measurements was significant).

These results indicate that polymorphisms in the MBL2 gene determining low levels of functional MBL in serum are associated with a delay in primary EBV infection.

In older children no association has been found between presence of EBV in nasopharyngeal aspirates and intermediate/high levels of MBL (11), but the association between MBL and primary EBV infection has not been investigated. We found that the risk of EBV infection, measured by EBV VCA-IgG/IgM seroconversion, was lower among MBL-insufficient than among MBL-sufficient individuals in early childhood. However, at a later age comparable proportions of EBV seropositivity were observed in the two groups. Thus, MBL insufficiency may retard but not eliminate the susceptibility to EBV infection, especially in infancy.

A sociological explanation for the delayed seroconversion, i.e., a reduced exposure to EBV among MBL-insufficient children, is difficult to imagine, as MBL-insufficient children generally have an increased risk of other (especially upper respiratory tract) infections compared with MBL-sufficient children (14). Although in theory MBL-insufficient children might be kept at home for this reason and thus be less exposed to EBV, absence from childcare centers for both groups was very low and the difference was insignificant (median of 2.5 and 4 days for MBL-sufficient and -insufficient children, respectively, corresponds to a median of 0.4% and 0.8% of their time of observation; P = 0.11).

A possible explanation involves the interaction between EBV and the target B cell. CD21, the B-cell receptor for EBV glycoprotein gp350, is also a complement receptor (CR2) (22), and complement activation with subsequent opsonization of EBV might facilitate B-cell entry. This is partly supported by the observation that infectivity of another herpesvirus, HSV-2, is significantly promoted by high levels of MBL, probably due to opsonization of virions (3). Alternatively, complement activation is required to facilitate entry of EBV-infected B-cells into the germinal center reaction and thus ensure establishment of persistent EBV infection in memory B cells (26).
this scenario, MBL would augment complement activation and germinal center formation.

Both models could explain the low number of EBV copies in blood of MBL-insufficient children. Little is known about the mechanisms responsible for the EBV load in the blood of healthy individuals, but a number of factors, including age and severity of primary infection, efficiency of the immune response in clearing the virus, and the genetic background of the individual, have been proposed to be influential (13).

EBV seroprevalence was above 50% in children below 1 year of age, comparable to observations in other populations (19, 20, 28). The majority of these IgG antibodies constitute maternally acquired anti-EBV IgG that disappears during the first 8 months of age (1, 24). Theoretically, the delayed EBV seroconversion in MBL-insufficient children could reflect an increased clearance of maternally acquired antibodies, but to our knowledge no data imply higher loss of such antibodies in MBL-insufficient children. Moreover, as maternally acquired antibodies rarely persist after 8 months of age, a possible increased clearance of these antibodies cannot explain the difference in EBV seroprevalence between 1 and 2 years of age.

Moreover, an inability among MBL-insufficient children to mount an antibody response could theoretically explain the delayed seroconversion. However, in a study using mice immunized with a tetanus toxoid vaccine, the IgG response in MBL-insufficient mice was found to be heightened compared to that in MBL-sufficient mice (10). The interaction may be more complex, as other results indicate that the modifying effect of MBL on the humoral immune response is influenced by the genetic environment (21). Thus, although it cannot be excluded that a reduced antibody response among MBL-insufficient children may contribute to the observed delay in seroconversion, EBV VCA-IgG levels were found to be comparable between MBL-sufficient and -insufficient children at later ages.

Delayed primary EBV infection, even in infancy, might have important implications. In developed countries 25 to 30% of the population remains EBV-seronegative until adolescence and carries an increased risk of infectious mononucleosis and a subsequent increased risk of Hodgkin’s lymphoma.

Although these findings need confirmation in larger studies, this study was carried out in the second-biggest town of Greenland, with a very high participation rate (87%), and we were able to show a significant difference in EBV infection between MBL-sufficient and MBL-insufficient children even given the limited sample size.

In conclusion, MBL may be involved in primary EBV infection in infancy, since MBL insufficiency seems to retard EBV infection.

We thank the participating children of Sisimiut, their parents, the involved childcare centers and daycare mothers, and the two schools of Sisimiut in 2004 for participating in this project. We also thank the staff at Sisimiut Health Center, Greenland, in particular the chief medical officers Ove Rosing Olsen, the late Peter Dybdahl Andersen, and head nurse Ellis Thierry, for a fruitful collaboration and for providing optimal working conditions.

This study was funded by the Danish Medical Research Council, the Commission for Scientific Research in Greenland (KVUG), the Nordic Cancer Union, the Danish Cancer Foundation, the Gangsted Foundation, the Einer Willumsen Grant, the Dagmar Marshall Foundation, the Aase and Einar Danielsen Foundation, the Foundation for Promotion of Medical Science, the Novo-Nordisk Research Foundation, University Hospital Rigshospitalet, and the Copenhagen Hospital Cooperation Research Foundation.

REFERENCES

1. Biggar, R. J., W. Henle, G. Fleisher, J. Becker, E. T. Lennette, and G. Henle. 1978. Primary Epstein-Barr virus infections in African infants. I. Decline of maternal antibodies and time of infection. Int. J. Cancer 22:239–243.
2. Cohen, J. J. 2000. Epstein-Barr virus infection. N. Engl. J. Med. 343:841–492.
3. Fischer, P. B., S. Ellermann-Eriksen, S. Thiel, J. C. Jensenius, and S. C. Mogensen. 1994. Mannan-binding protein and bovine conglutinin mediate enhancement of herpes simplex virus type 2 infection in mice. Scand. J. Immunol. 39:439–445.
4. Gadjeva, M., S. R. Paludan, S. Thiel, V. Slavov, M. Ruseva, K. Eriksson, G. B. Lowhagen, L. Shi, K. Takahashi, A. Ezekowitz, and J. C. Jensenius. 2004. Mannan-binding lectin modulates the response to HSV-2 infection. Clin. Exp. Immunol. 138:304–311.
5. Garred, P., F. Larsen, H. O. Madsen, and C. Koch. 2003. Mannose-binding lectin deficiency—revisited. Mol. Immunol. 40:73–84.
6. Garred, P., F. Larsen, J. Seyfarth, R. Fujita, and H. O. Madsen. 2006. Mannose-binding lectin and its genetic variants. Genes Immun. 7:85–94.
7. Garred, P., H. O. Madsen, and A. Svejgaard. 1996. Genetics of human mannann-binding protein, p. 139–164. In R. A. B. Ezekowitz, K. Sastry, and K. B. M. Reid (ed.), Collectins and innate immunity. R. G. Landes, Austin, TX.
8. Garred, P., T. Pressler, H. O. Madsen, B. Frederiksen, A. Svejgaard, N. Holby, M. Schwartz, and C. Koch. 1999. Association of mannose-binding lectin gene heterogeneity with severity of lung disease and survival in cystic fibrosis. J. Clin. Invest. 104:431–437.
9. Gartner, R. C., R. D. Hess, D. Bandt, A. Kruse, A. Rethwilm, K. Roemer, and N. Mueller-Lantzsch. 2003. Evaluation of four commercially available Epstein-Barr virus enzyme immunosassays with an immunofluorescence assay as the reference method. Clin. Diagn. Lab. Immunol. 10:78–82.
10. Gutfrohm, H. K., L. M. Stuart, L. Shi, M. C. Carroll, J. Chen, D. L. Kasper, R. A. Ezekowitz, and K. Takahashi. 2009. Dysfunction of mannose-binding lectin greatly increases antibody response in a mouse model of vaccination. Clin. Immunol. 130:264–271.
11. Honore, P., H. O. Madsen, K. Sandvej, A. Koch, and P. Garred. 1999. Lack of association between mannose-binding lectin, acute otitis media and early Epstein-Barr virus infection among children in Greenland. Scand. J. Infect. Dis. 31:363–366.
12. IARC Working Group. 1997. Epstein-Barr Virus and Kaposis sarcoma herpesvirus/human herpesvirus 8. IARC monographs on the evaluation of the carcinogenic risks to humans. World Health Organization, International Agency for Research in Cancer, Lyon, France.
13. Khan, G., E. M. Miyashita, B. Yang, G. J. Babcock, and D. A. Thorley-Lawson. 1996. Is EBV persistence in vivo a model for B cell homeostasis? Immunology 85:173–179.
14. Koch, A., M. Melbye, P. Sorensen, P. Homse, H. O. Madsen, K. Molbak, C. G. Hansen, L. H. Andersen, G. W. Hahn, and P. Garred. 2001. Acute respiratory tract infections and mannose-binding lectin insufficiency during early childhood. JAMA 285:1316–1321.
15. Koch, A., P. Sorensen, P. Homse, K. Molbak, F. K. Pedersen, T. Mortensen, H. Elberling, A. M. Eriksen, O. R. Olsen, and M. Melbye. 2002. Population-based study of acute respiratory infections in children, Greenland. Emerg. Infect. Dis. 8:586–593.
16. Lin, D. Y., D. Oakes, and Z. Ying. 1998. Additive hazards regression with current status data. Biometrika 85:289–298.
17. MacKenzie, J. A. Gallagher, R. A. Clayton, J. Perry, O. B. Eden, A. M. Ford, M. F. Greaves, and R. F. Jarrett. 2001. Screening for herpesvirus genomes in common acute lymphoblastic leukemia. Leukemia 15:415–421.
18. Madsen, H. O., P. Garred, S. Thiel, J. A. Kurtzhals, L. U. Lamm, L. P. Ryder, and A. Svejgaard. 1999. Interplay between promoter and structural gene variants control basal serum level of mannan-binding protein. J. Immunol. 155:3013–3020.
19. Melbye, M., P. Ebbesen, P. H. Levine, and T. Bennike. 1984. Early primary infection and high Epstein-Barr virus antibody titers in Greenland Eskimos at high risk for nasopharyngeal carcinoma. Int. J. Cancer 34:619–623.
20. Morris, M. C., W. J. Edmunds, L. M. Hesketh, A. J. Vyse, E. Miller, P. Morgan-Capner, and D. W. Brown. 2002. Sero-epidemiological patterns of Epstein-Barr and herpes simplex (HSV-1 and HSV-2) viruses in England and Wales. J. Med. Virol. 67:522–527.
21. Ruseva, M., M. Kolev, F. Dagnaes-Hansen, S. B. Hansen, K. Takahashi, A. Ezekowitz, S. Thiel, J. C. Jensenius, and M. Gadjeva. 2009. Mannan-binding lectin deficiency modulates the humoral immune response dependent on the genetic environment. Immunity 127:279–288.
22. Sarrias, M. R., S. Franchini, G. Canziani, E. Argyropoulos, W. T. Moore, A. Sahu, and J. D. Lambris. 2001. Kinetic analysis of the interactions of com-
plement receptor 2 (CR2, CD21) with its ligands C3d, iC3b, and the EBV glycoprotein gp350/220. J. Immunol. 167:1490–1499.
23. Seppanen, M., M. L. Lokki, M. Lappalainen, E. Hiltunen-Back, A. T. Rovio, S. Kares, M. Hurme, and J. Aittoniemi. 2009. Mannose-binding lectin 2 gene polymorphism in recurrent herpes simplex virus 2 infection. Hum. Immunol. 70:218–221.
24. Shapiro, L. R., Y. Hirshaut, D. M. Kanef, and P. Glade. 1972. Epstein-Barr virus in infancy. J. Pediatr. 80:1025–1026.
25. Sun, J. 2005. Interval censoring, p. 2603–2609. In P. Armitage and T. Colton (ed.), Encyclopedia of biostatistics, 2nd ed. John Wiley and Sons, Ltd., New York, NY.
26. Thorley-Lawson, D. A., and A. Gross. 2004. Persistence of the Epstein-Barr virus and the origins of associated lymphomas. N. Engl. J. Med. 350:1328–1337.
27. Turner, M. W. 2003. The role of mannose-binding lectin in health and disease. Mol. Immunol. 40:423–429.
28. Wang, P. S., and A. S. Evans. 1986. Prevalence of antibodies to Epstein-Barr virus and cytomegalovirus in sera from a group of children in the People’s Republic of China. J. Infect. Dis. 153:150–152.