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Human leukocyte antigen and interleukin 2, 10 and 12p40 cytokine responses to measles: Is there evidence of the HLA effect?

Inna G. Ovsyannikova [a], Jenna E. Ryan [a], Robert M. Jacobson [a,b], Robert A. Vierkant [c], V. Shane Pankratz [c], Gregory A. Poland [a,d,*]

[a] *Mayo Clinic Vaccine Research Group, Mayo Clinic College of Medicine, Rochester, MN 55905, USA*
[b] *Department of Pediatric and Adolescent Medicine, Mayo Clinic College of Medicine, Rochester, MN 55905, USA*
[c] *Department of Health Science Research, Mayo Clinic College of Medicine, Rochester, MN 55905, USA*
[d] *Program in Translational Immunovirology and Biodefense, Mayo Clinic College of Medicine, Rochester, MN 55905, USA*

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Abstract

HLA class I and class II associations were examined in relation to measles virus-specific cytokine responses in 339 healthy children who had received two doses of live attenuated measles vaccine. Multivariate linear regression modeling analysis revealed suggestions of associations between the expression of DPA1*0201 ($p = 0.03$) and DPA1*0202 ($p = 0.09$) alleles and interleukin-2 (IL-2) cytokine production (global p-value 0.06). Importantly, cytokine production and DQB1 allele associations (global p-value 0.04) revealed that the alleles with the strongest association with IL-10 secretion were DQB1*0302 ($p = 0.02$), DQB1*0303 ($p = 0.07$) and DQB1*0502 ($p = 0.06$). Measles-specific IL-10 secretion associations approached significance with DRB1 and DQA1 loci (both global p-values 0.08). Specifically, suggestive associations were found between DRB1*0701 ($p = 0.07$), DRB1*1103 ($p = 0.06$), DRB1*1302 ($p = 0.08$), DRB1*1303 ($p = 0.06$), DQA1*0101 ($p = 0.08$), and DQA1*0201 ($p = 0.04$) alleles and measles-induced IL-10 secretion. Further, suggestive association was observed between specific DQA1*0505 ($p = 0.002$) alleles and measles-specific IL-12p40 secretion (global p-value 0.09) indicating that cytokine responses to measles antigens are predominantly influenced by HLA class II genes. We found no associations between any of the alleles of HLA A, B, and Cw loci and cytokine secretion. These novel findings suggest that HLA class II genes may influence the level of cytokine production in the adaptive immune responses to measles vaccine.

Keywords: Measles vaccine; HLA; Interleukin-2; Interleukin-10; Interleukin-12p40; ELISA

1. Introduction

Measles is the most transmissible virus known in humans and requires 95–98% vaccine uptake to prevent persisting endemicity [1,2]. A single measles vaccine fails to induce antibody in 2–10% of people [3–6], and only 80.8% of 1490 children developed seropositivity following a single dose of measles containing vaccine given at 12 months or later in life [7]. Further, only 81.5% of those who were seronegative responded after a second dose [7]. Measles outbreaks still occur in highly vaccinated populations because of cases introduced from outside the country and primary and possibly secondary vaccine failure [2,8,9]. The importance of measles vaccine failure has become more apparent, and an understanding of this failure may provide a basis for improved vaccines. Thus, examining variability in measles vaccine-induced immunity is critical for its continued use as a tool for measles eradication.

Both CD4 and CD8 T cells are activated by measles virus infection, and cytokine production by these two T cell subsets is essential for the development and regulation of

* Corresponding author. Fax: +1 507 266 4716.
E-mail addresses: ovsyannikova.inna@mayo.edu (I.G. Ovsyannikova), ryan.jenna@mayo.edu (J.E. Ryan), jacobson.robert@mayo.edu (R.M. Jacobson), vierkant.robert@mayo.edu (R.A. Vierkant), pankratz.vernon@mayo.edu (V.S. Pankratz), poland.gregory@mayo.edu (G.A. Poland).

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humoral and cellular immunity [10–12]. Two main T helper subsets have been recognized: Th1 cells producing cytokines favoring cell-mediated immunity (IFN-γ, IL-2, TNF-α, and IL-12), and Th2 cells producing cytokines necessary for humoral immune responses (IL-4, IL-5, IL-6, IL-10, and IL-13) [13,14]. Measles virus infection induces a Th2 type response and it is likely that cytokine imbalances play a role in immunosuppression [15–18]. In contrast, vaccination with measles virus preferentially induces Th1 type immune responses [19–21]. Therefore, alterations in cytokine levels during vaccination can have dramatic effects on vaccine efficacy.

Our previous work has suggested that humoral and cellular immune responses to measles vaccine are genetically restricted by HLA genes [22–24]. Further, we demonstrated that the HLA genes may also play a role in measles-specific cytokine responses and demonstrated significant associations with measles-induced IFN-γ and IL-4 secretion and HLA alleles [25,26]. We hypothesized that variations in IL-2, IL-10, and IL-12p40 cytokine responses to measles vaccine are associated in part with genetic polymorphisms of the HLA genes. The aim of this hypothesis-generating study was to investigate IL-2, IL-10, and IL-12p40 cytokine immune responses to measles in healthy children and to determine whether associations exist between these cytokines and HLA class I and class II alleles following two doses of measles vaccine.

2. Materials and methods

2.1. Study subjects

As previously described, between December 2001 and August 2002 we enrolled 346 healthy children (age 12–18 years) identified through the Minnesota Independent School District 535 registration rolls [27]. For this sub-study, HLA and cytokine data were available on 339 subjects. Measles vaccination was part of a routine program and all enrolled participants had documentation in their medical records of having received two doses of live measles–mumps–rubella (MMR) vaccine (Merck Research, West Point, PA) containing the attenuated Edmonston B strain of measles virus. Mayo Clinic's Institutional Review Board granted approval for the study, and blood samples were drawn after informed consent, permission, and assent were obtained as appropriate from the subjects and their parents.

2.2. In vitro IL-2, IL-10, and IL-12p40 cytokine responses to measles

Peripheral blood mononuclear cells (PBMC) were immediately separated from heparinized venous blood by Ficoll-Hypaque (Sigma, St. Louis, MO) density gradient centrifugation as described elsewhere [25,26]. Cells were resuspended in RPMI 1640 culture media (Celox Laboratories Inc., St. Paul, MN) containing 10% dimethyl sulfoxide (Sigma) and 20% fetal calf serum (FCS; Hyclone, Logan, UT), frozen at −80 °C, and stored in liquid nitrogen.

After cryopreservation, PBMC were thawed, resuspended in RPMI 1640 culture media supplemented with 10% normal human serum (NHS; Irvine Scientific, Santa Ana, CA), 100 U/ml penicillin (Sigma), and 100 μg/ml streptomycin (Sigma) as previously described [25,26]. For determination of secreted IL-2 and IL-10, PBMC (4×10^5 cells/well) were cultured in duplicate with or without the Edmonston B vaccine strain of measles virus (multiplicity of infection [MOI] of 0.1) diluted in RPMI 1640 culture media supplemented with 1% NHS. Additionally, 2×10^5 PBMC were incubated in triplicate in the same media at a MOI of 0.5 for determination of IL-12p40. Cell-free supernatants were removed after 6 days and measles virus-specific IL-2, IL-10, and IL-12p40 responses were quantitatively determined by ELISA following the manufacturer's protocol (BD Pharmingen, San Diego, CA). The sensitivity of each ELISA assay was 4 pg/ml. The intra-assay coefficient of variation (CV) for IL-2, IL-10, and IL-12p40 assays in our laboratory were 42%, 48%, and 44%, respectively. Median background levels of IL-2, IL-10, and IL-12p40 cytokine production in cultures not stimulated with measles virus were subtracted from the median measles-induced responses to produce corrected secretion values. Negative corrected values indicate that the unstimulated secretion levels were, on average, higher than the stimulated secretion levels.

2.3. Molecular HLA typing

Genomic DNA was extracted from blood samples using the Puregene extraction kit (Gentra Systems Inc., Minneapolis, MN). HLA class I A locus typing was performed using the SeCore HLA-A locus sequencing kit (Dynal Biotech, Brown Deer, WI), followed by sequence-specific primer (SSP) UniTray typing and AmbiSolv, which consisted of specific primer mixes selected to resolve common ambiguities, when needed. HLA class I B locus typing was performed using the reference strand conformation analysis (RSCA) Multi-Dye B locus kit, followed by the ABI B locus sequencing kit, and the SSP UniTray and AmbiSolv (Dynal Biotech). HLA class I Cw locus typing was performed using the Cw high resolution SSP Unitray (Dynal Biotech). Any ambiguities were resolved using the Forensic Analytical Cw locus sequencing kit and AmbiSolv when needed.

HLA class II DRB1 locus typing was performed using the DRB1 high resolution RSCA and SSP Unitray typing kits (Dynal Biotech). HLA-DPA1 locus typing was performed primarily using the Dynal AllSet DPA1 Unitray. HLA-DQA1, HLA-DQB1 and HLA-DPB1 loci typing were performed primarily using the appropriate SSP high resolution Unitray typing kits with the entire locus on a single tray (Dynal Biotech). Any ambiguities resulting from molecular techniques, including SSP and RSCA, were resolved by AmbiSolv primer mixes. RSCA samples were