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HLA-DR Specific Monoclonal Antibodies Block Lymphoproliferative Response to Measles Vaccine *in vitro*: a pilot study

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Abstract

Humoral and cell-mediated immune responses are important in protection against measles. Non-response to vaccination has been associated with specific HLA-DR and HLA-DQ alleles; however, little is known about the relative importance of these alleles in the cellular immune response induced by measles virus vaccine. To investigate the role of HLA-DR/DQ class II restriction, a small pilot study was conducted. Lymphoproliferation assays using class II DR and DQ-specific monoclonal antibodies (MoAb) were performed at one week and two weeks post immunization with MMR2 vaccine. The mean stimulation index (SI) was 4.4 and 5.3 at one and two weeks with reductions in SI of 47.6% and 70.2%, respectively, following the addition of DR-specific MoAb ($p < 0.001$). These results clearly show that a significant proportion of the cell-mediated immune response to measles virus vaccine, as measured by SI, is HLA-DR restricted.

Keywords

Cell-mediated immunity (CMI); Vaccine; HLA-restriction; Measles Virus; Immunity; Monoclonal Antibody; Immune memory

Introduction

Measles is one of the most transmissible human infectious diseases. According to the CDC, multiple outbreaks of measles resulted in 215 cases in the U.S in 2011. Although the use of live, attenuated measles vaccines significantly reduces the number of measles cases, disease due to vaccine failure still occurs. Failure to seroconvert occurs in 2 to 10% of healthy adults after immunization [1]. Understanding the immunogenetic mechanism(s) of vaccine response, or failure, is crucial to the development of effective alternate vaccine, such as subunit vaccines or DNA-based vaccines. Studies in our laboratory have demonstrated

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Disclosures

Dr. Poland is the chair of a safety evaluation committee for novel non-measles vaccines undergoing clinical studies by Merck Research Laboratories.

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significant associations between measles antibody nonresponse and specific HLA-DR (DRB1*0301, DRB1*0701) and HLA-DQ (DQA*0201) alleles [2–5]. However, little is known about the relative importance of immune response genes in the development of the CMI response [6,7]. To investigate the role of HLA class II restriction in the cell-mediated immune response to measles virus vaccine (MVV), we conducted a pilot study of five subjects who had lymphoproliferation assays (LPA) using anti (blocking) class II monoclonal antibodies (MoAb) following measles immunization.

Materials and Methods

Study population

Four healthy adult volunteers without a known history of natural measles infection, but who had received one previous dose of measles-mumps-rubella (MMR-II) vaccine, were recruited for blood draw one and two weeks after receiving a second dose of MMR-II. One additional subject, 10 years post second dose of MMR II, was included for comparison. Subjects were recruited through the immunization clinic at the Mayo Clinic after Institutional Review Board approval of the research protocol and after obtaining informed consent from each subject.

Measles virus strain

Subjects were immunized with 0.5 mL of live, attenuated measles virus (MMR-II, Merck, West Point, PA). Monovalent measles virus (Attenuvax®, Merck, West Point, PA), obtained commercially, was used as the stimulating antigen for the lymphoproliferation assays.

Monoclonal antibodies (MoAb)

MoAb were obtained through the Mayo Clinic Monoclonal Antibody Core Facility. L227 (ATCC HB96) specifically blocks HLA-DR, and IVD12 (ATCC HB144) blocks DQα. Monoclonal antibodies were used at a final concentration of 10 µg/mL. Anti- hu br ca IgG1 was used as an irrelevant MoAb.

Lymphoproliferation assay

Experiments leading up to this pilot study were primarily designed to test and optimize the LPA assay. Fresh PBMCs from donors were used to optimize many variables including incubation times, antigen concentrations, and technique according to relevant findings in the literature [1,8,4]. Briefly, peripheral blood mononuclear cells (PBMC) were isolated from healthy donors within one hour of collection using standard Ficoll-Paque™ procedures. Lymphocytes were washed twice in PBS, counted, then resuspended at 1×10^6 cells/mL in RPMI 1640 with HEPES (25 mM), 1% Penicillin-Streptomycin, 5% horse serum, 50 µM 2-ME, and TCM (Celox serum replacement). Samples were plated in triplicate in 96-well, flat-bottom polystyrene microtiter plates. The total volume was 200 µL, and the final concentration of lymphocytes was 200,000 cells/well. Fifty-one plaque-forming units (PFU) of MVV (Attenuvax®, Merck, West Point, PA) were added at the initiation of culture. Phytohemagglutinin (PHA) was used as the positive control at a final concentration of 5 µg/mL. The plates were incubated for four days; 18 hours prior to harvesting, 1 µCi of [³H]-thymidine was added to each well. The cells were harvested onto glass fiber filter paper then underwent scintillation counting. A stimulation index (SI) of each set of samples was calculated as the quotient of the average cpm of the stimulated cells divided by the average cpm of the unstimulated cells. Samples with a SI of 3 or greater are considered to be significant for antigen-specific proliferative activity, and indicative of recall immunity to MVV.

Results

Lymphoproliferative responses against MVV

Lymphoproliferative responses against measles virus were measured at one week and two weeks post immunization as shown in Table 1 and Figure 1. The mean SIs of subjects immunized with MMR to measles virus at one week and two weeks were 4.4 and 5.3, respectively, demonstrating an increase of 17% in the SI to measles virus between week one and week two. The mean SI of the subject immunized 10 years earlier was 10.7. Among the four subjects immunized with MMR, 75% developed significant lymphoproliferative responses at one week and two weeks post immunization (Figure 2). All patients had a positive response to PHA.

HLA-DR and HLA-DQ-restricted lymphoproliferative responses against MVV

At the same time the controls were completed, HLA-restricted lymphoproliferative responses were measured *in vitro* after the addition of monoclonal antibodies, which block HLA-DR (L227), HLA-DQ (IVD12), or both HLA-DR/DQ. Table 1 summarizes the antigen-specific lymphoproliferative responses and percent reduction in SIs.

Anti-HLA-DR monoclonal antibodies and lymphoproliferative responses

As illustrated in Figure 1, the measles-specific lymphoproliferative response to MVV after anti-HLA-DR in the four recently immunized subjects declined by an average of 47.6% (range: 26.3% to 58.2%) one week post immunization and declined by an average of 70.2% (range: 46.5% to 79.3%) in the second week post immunization. A mean reduction in lymphoproliferation after measles virus stimulation of 78% was demonstrated in the subject immunized 10 years earlier. The difference in the mean SI of the wells with L227 compared to the control wells was statistically significant ($p < 0.001$).

Anti-HLA-DQ monoclonal antibodies and lymphoproliferative responses

After anti-HLA-DQ, measured antigen-specific lymphoproliferative responses showed an average decline of 17.7% (range: 9.3% increase to 36.8% decline) at week one and further declined by 32.1% (range: 13.8% to 58.3%) at week two in the immunized subjects. The subject previously immunized 10 years earlier demonstrated a mean reduction in SI of 29.5% (data not shown). The difference in the mean SI of the wells with IVD12 compared to the control wells was not statistically significant ($p = 0.33$).

Anti-HLA-DR/DQ monoclonal antibodies and lymphoproliferative responses

The mean reduction in the antigen-specific lymphoproliferative responses at one week and two weeks post immunization were 47.1% (range: 0% to 78.2% decline) and 68.0% (range: 61.5% to 75.0% decline), respectively. The difference in the mean SI of the wells with L227 and IVD12 compared to the control wells was statistically significant ($p < 0.001$), but not statistically different from the addition of L227 alone. The mean reduction in SI with the addition of anti-HLA-DR/DQ monoclonal antibodies in the subject previously immunized 10 years earlier was 76.9%.

Discussion

In immune adults, memory T cells have been shown to be both CD8+ and CD4+ cells, which are HLA class I and class II-restricted, respectively [9,10]. In late convalescence, the predominant population of cells is measles virus (MV)-specific CD4+ HLA class II-restricted cytotoxic T-lymphocytes (CTLs) [11]. This supports the observations found in this

pilot study that HLA class II-restricted responses after immunization with measles appear to play a significant role in the development of CMI responses.

Antibody nonresponse following vaccination has been linked to specific HLA alleles. [2,5,12–14]. In one study, HLA alleles DRB1*03 and DQA1*0201 was significantly associated with seronegativity even after accounting for the effects of other alleles [5]. It is unknown what role these alleles play in the cell-mediated immune response following vaccination, specifically whether the CMI response following immunization might be inhibited by preventing effective antigen presentation in the context of these alleles. Demotz et al. showed this association as well by demonstrating that the lymphoproliferative response to MV nucleoprotein could be blocked by L243 (anti-DR monoclonal antibody) and that the cells were predominately DRB1*1103-restricted T-cell clones. In addition, anti-DP and anti-DQ monoclonal antibodies did not prevent proliferation [15]. Despite the ability of CNBr-digested nucleoprotein to create MV nucleoprotein-specific T-cells, this was not studied following immunization. The isolated nucleoprotein specific T-cell clones presented in the context of HLA-DR and not -DQ or -DP, but again it is unknown whether HLA-DR monoclonal antibodies would block the response following MV immunization.

Our aim was to investigate the role of HLA class II (HLA-DR and HLA-DQ) restriction in the recall immune response to MVV following immunization after a second dose of MMR-II. We have shown lymphoproliferative responses in controls similar to other investigators [1,16] following both recent and past MMR immunization. Although individual responses to MVV vary, a marked statistically significant reduction in the SI was seen when HLA-DR molecules were blocked prior to a recall immune memory assay, the lymphoproliferation assay ($p < 0.05$), a proxy for cell-mediated immunity. Blocking HLA-DQ MoAb did not provide a statistically significant reduction in the SI, although it appears to have a modest effect on MV-specific lymphoproliferation at two weeks post immunization, which may be due to inadequate statistical power. The combination of L227 and IVD12 did not synergistically inhibit the cellular immune response to MVV greater than L227 alone. Since HLA-DR and -DQ were nonspecifically blocked for the purposes of this study, we did not HLA type individual subjects. Future studies will provide an opportunity for a larger sample size and thereby permit analysis by HLA type.

In conclusion, HLA-DR restricted responses appear to play a significant role in the cellular immune recall response following measles immunization. HLA-DQ restricted responses may also contribute; however, additional work is necessary to confirm these findings. The relationship between HLA and other genes and the magnitude of the adaptive immune response to measles immunization requires further investigation. Further work to characterize the exact immunogenetic mechanisms of the innate and adaptive immune response to measles virus vaccine is underway.

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Abbreviations

MVV	measles virus vaccine
LPA	lymphoproliferation assays
MoAb	monoclonal antibodies

SI	stimulation index
MMR	measles-mumps-rubella
PBMC	peripheral blood mononuclear cells
PFU	plaque forming units
CTL	cytotoxic T lymphocytes

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Figure 1. Recently immunized subjects

Comparison of the mean lymphoproliferative responses to MVV one week and two weeks following a second dose of measles vaccine in column A to the mean lymphoproliferative responses following the addition of anti-DR monoclonal antibody (column B), anti-DQ monoclonal antibody (column C), and both anti-DR and anti-DQ monoclonal antibodies (n=4). MVV stimulated PBMC: A: No MoAb. B: +Anti-DR MoAb. C: +Anti-DQ MoAb. D: +Anti-DR/DQ MoAb.

†p < 0.001 (Column B compared to A)

♦p = .34 (Column C compared to A)

★p < 0.001 (Column D compared to A)

Figure 2. Stimulation indices s/p MMR

Stimulation indices among individuals following MMR at one and two weeks.

Table 1Summary of the stimulation indices^a

	SI	SI with 95% confidence interval over % Reduction in SI		
	MVV ^b	L227 ^c	IVD12 ^d	L227/IVD12
1 week s/p MMR	4.4 (2.2–6.5)	2.1 (1.3–2.9) 47.6 (33.4–61.8)	4.3 (.7–7.8) 17.3 (–10.5–45.2)	1.9 (1.1–2.6) 47.1 (0.2–94.1)
2 weeks s/p MMR	5.2 (2.6–7.8)	1.5 (.7–2.4) 69.9 (54.5–85.2)	3.5 (1.8–5.1) 31.4 (13.3–49.4)	1.8 (.6–2.9) 68.0 (62.3–73.6)
10 years s/p MMR	10.7	78	29.5	76.9

^aResults reported above are based on the mean of the absolute differences of the SIs in immunized subjects.^bMVV: Measles vaccine virus^cL227: HLA-DR blocking antibody^dIVD12: HLA-DQ blocking antibody