Measles virus infection diminishes preexisting antibodies that offer protection from other pathogens

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Measles virus is directly responsible for more than 100,000 deaths yearly. Epidemiological studies have associated measles with increased morbidity and mortality for years after infection, but the reasons why are poorly understood. Measles virus infects immune cells, causing acute immune suppression. To identify and quantify long-term effects of measles on the immune system, we used VirScan, an assay that tracks antibodies to thousands of pathogen epitopes in blood. We studied 77 unvaccinated children before and 2 months after natural measles virus infection. Measles caused elimination of 11 to 73% of the antibody repertoire across individuals. Recovery of antibodies was detected after natural reexposure to pathogens. Notably, these immune system effects were not observed in infants vaccinated against MMR (measles, mumps, and rubella), but were confirmed in measles-infected macaques. The reduction in humoral immune memory after measles infection generates potential vulnerability to future infections, underscoring the need for widespread vaccination.

During a recent measles outbreak in the Netherlands, families in communities with low vaccination rates consented to provide blood samples. Plasma was collected before and after laboratory-confirmed MV infection from 77 unimmunized children with a mean age of 9 (SD ± 2) years, plus five unimmunized children who remained uninfected during the study (24). Of the 77 children, 34 were reported to have mild measles and 43 to have severe measles [detailed in (24)]. The mean time between sample collections was 10 weeks, and mean time of collection after MV infection was 7 weeks (table S1).

To measure the diversity and magnitude of the epitope-specific antibody repertoires in these children and controls, we used VirScan (25), a phage-display immunoprecipitation and sequencing (PhiP-Seq) technology (26) developed for virome-wide detection of antibodies against viral epitopes. VirScan primarily detects antibodies to short contiguous epitopes as opposed to conformational epitopes. The cells producing antibodies to all epitopes are phenotypically similar, aside from their antibody product. Thus, changes in the antibody repertoire detected by VirScan represent changes across the spectrum of antibodies, and these include neutralizing and non-neutralizing antibodies. For this study, we generated an expanded VirScan library that encodes the full proteomes of most known human pathogenic viruses (~400 species and strains) plus many bacterial proteins. For each sample, we obtained a comprehensive measure of the individual’s antipathogen antibody repertoire diversity (i.e., the total epitope hits across all pathogen peptides). We also derived an antibody epitope binding signal (EBS), which is...
a relative measure of antibody titer for each epitope.

We used VirScan to profile the immune memory antibody repertoires before (time 1) and after (time 2) MV infection. Paired samples were also obtained from four control cohorts (n = 119 paired specimens; table S1). These samples were derived from: (i) approximately age-matched controls sampled ~1 year apart (control A; n = 28 paired specimens); (ii) age-matched controls with samples collected ~3 months as the measles cohorts (control B; n = 31); (iii) adult controls with samples collected ~1 year apart (control C; n = 22); (iv) young children before and after their first measles-mumps-rubella (MMR) vaccination (MMR vaccinated; n = 33); and (v) unvaccinated children from the same community as the MV cases but who remained seronegative for MV (MV negative; n = 5). Control cohorts A, B, and C were individuals with no known exposure to MV.

Measles modulates the diversity of the antibody repertoire and causes loss of preexisting antibodies

We assessed changes in antibody repertoire diversity (measured as the total number of unique pathogen epitopes recognized, or epitope hits) before and after measles relative to those observed in controls, standardizing the total number of epitope hits per individual by cohort for comparison (Fig. 1A). We detected substantial reductions in the number of pathogen epitopes recognized after measles but limited changes in the absence of measles. MV infections were associated with a mean reduction of ~20% in the overall diversity or number of MV-specific epitopes in children after measles infection or MMR vaccination (Fig. 1C). No changes in the total IgG, IgA, or IgM levels were detected, as determined by quantitative ELISA (enzyme-linked immunosorbent assay) (fig. S2). These results suggest...
that, rather than a simple loss of total IgG, there is a restructuring of the antibody repertoire after measles.

To measure the full effect of measles on the pre-measles repertoire and to circumvent interference from new exposures during follow-up, we next restricted analysis to epitopes detected at the first time point and quantified retention or loss of epitope recognition. After severe or mild measles, children lost a median of 40% (range: 11 to 62%) or 33% (range: 12 to 73%), respectively, of their total preexisting pathogen-specific antibody repertoires (Fig. 2A and fig. S3). In contrast, controls retained ~90% of their repertoires over similar or longer durations.

Controlling for interval duration in a binomial random effects model (see materials and methods), we estimated the per-pathogen probability of antibody retention for each child (Fig. 2, B and C). Loss of antibodies after MV infection varied widely for specific pathogens and between children. A small fraction of MV-infected individuals retained antibodies similar to the bottom quartile of the controls. However, the most-affected 20% of children lost >50% of the pathogen-specific antibodies for most pathogens. In some of these children, up to 70% loss was detected for specific pathogens.

Antibody repertoire retention (~90%) was similar in control cohorts A and B despite the longer sampling interval in B compared with A (1 year versus 3 months). The retained antibody repertoire could represent a core stable LLPC repertoire (~90%), and the 10% that was lost could represent a transient repertoire derived from IgG-secreting B cells and plasmablasts. This result is consistent with previous studies that showed no effect of immunosuppressive therapy, such as B cell-depleting anti-CD19 or anti-CD20 treatment, on retention of the majority of the antibody repertoire (17, 19, 21, 22). Therefore, measles is associated with greater loss of antibody-mediated epitope recognition than can be explained by ablation of B cells, suggesting a direct effect on the LLPC compartment.

**Measles decreases the strength of epitope recognition**

Simply counting epitope numbers recognized before and after measles underestimates immune memory impairment because epitope recognition can be detected even when a large fraction of cellular clones producing the relevant antibody are eliminated. To quantify the relative abundance of particular antibodies, we generated an EBS metric, which is a z-score that measures the relative enrichment of an epitope in a VirScan immunoprecipitation (see materials and methods). Thus, EBS represents a VirScan analog of relative antibody titers and can be measured over time to detect changes in epitope recognition. Significant increases in EBS for a given epitope usually indicate a new exposure during the follow-up interval. In controls, significant reductions are normally detected when a recent exposure has occurred before the first time point, resulting in a peak around time 1 followed by an expected rapid decay of the antibody signal that is detected at time 2.

To evaluate changes in EBS for each pathogen species in each child, EBS signals were clustered by pathogen species and child (EBS<sub>pc</sub>) and a paired t test (paired per epitope) was used to test for significant differences at time 2 versus time 1. False discovery rate (fdr)-adjusted P values, sample size, and effect size (calculated as the fold change of the geometric mean at time 2 versus time 1) are shown for each EBS<sub>pc</sub> cluster (Fig. 3A and fig. S4). Significant changes in EBS<sub>pc</sub> were detected in children in each cohort. Among controls, these changes were distributed equally, below and above a fold change of 1, indicating recent pathogen exposures before time 1 or during the follow-up interval, respectively. Unlike the controls, after measles, the significant changes in pathogen-specific EBS<sub>pc</sub> were both more frequent and nearly uniformly negative with median fold changes (0.58 and 0.62), indicating ~40% reductions in EBSs (Fig. 3A). The effect was stronger after severe versus mild MV infections (P < 0.001). We confirmed these results by comparing the fold changes in EBS<sub>pc</sub> to quantitative ELISA assays for adenovirus, enterovirus, and respiratory syncytial virus (RSV) in a selection of children who had decreased or increased EBS, and found good correlation (correlation coefficient r = 0.73; P < 0.001) (fig. S5, A and B).

To further clarify cohort-level changes, we combined the epitopes from all individuals and evaluated changes on a per-pathogen-species basis across each cohort (fig. S6). Among MV-infected individuals, the median fold change in EBS measured across all species was a strongly negative effect (median fold change: 0.69 or −31%; P < 0.0001). In contrast, among controls, the antibody EBS
was significantly changed only for relatively few pathogen species, and these were roughly balanced between positive and negative effects.

**Measles disrupts recognition of pathogen epitopes across the cohort**

At the cellular level, MV would not be expected to preferentially infect particular antibody-secreting cells on the basis of their pathogen targets or the type of antibodies produced (i.e., neutralizing or not). We therefore tested for cohort-wide differences in EBSs on a per-epitope basis. Across approximately 1100 epitopes each from the control A and B cohorts, none significantly changed (Fig. 3, B and C, and fig. S7), indicating that antibody epitope recognition across a group of individuals is remarkably stable, even when changes are detected at the pathogen species level, as in fig. S6. In contrast, EBS was substantially reduced in 12% of the 855 epitopes evaluated after mild MV infections and in 39% of the 1079 epitopes evaluated after severe MV infections (P < 0.0001) (Fig. 3, B and C). Only one epitope in mild measles and three in severe measles showed a significant increase, <0.2% overall.

VirScan detects neutralizing antibodies when those antibodies target short contiguous epitopes encoded in the phage display. A well-characterized neutralizing short contiguous epitope is the 24-amino-acid target of the anti-RSV monoclonal antibody therapies palivizumab and motavizumab. Among 22 MV-infected children with antibodies against this neutralizing epitope and without evidence of new RSV exposure, we detected a fold change in EBS of 0.59 (SD ± 0.18; P < 0.001) (fig. S8), in line with the observations above and indicating that the effects of MV are the same for the neutralizing and non-neutralizing antibody repertoires.

**The MMR vaccine does not impair the immune repertoire**

A marked increase in the overall antibody repertoire diversity was noted in MMR-vaccinated controls (Fig. 1), indicating that a similar loss of epitopes recognized for each species per paired sample) are indicated by the symbol shape. For each pathogen, the geometric mean (gMean) EBS was calculated at each time and the fold change at time 2 versus time 1 was calculated and indicated by the position of each point along the y axis. Density distributions adjacent to the points reflect the distribution of points that were significantly changed on the basis of a fold change in epitope binding signal (EBS) was measured for each epitope. (A) Each point represents one pathogen species per sample pair. Changes in EBS for all epitopes recognized per pathogen per sample pair were compared using a Wilcoxon matched-pairs signed rank test. Fdr-adjusted P values (adjusted for a fdr of 5%) are indicated by color of each symbol. The total number of epitopes included in each paired test (i.e., the number of epitopes recognized for each species per paired sample) are indicated by the symbol shape. For each pathogen, the geometric mean (gMean) EBS was calculated at each time and the fold change at time 2 versus time 1 was calculated and indicated by the position of each point along the y axis. Density distributions adjacent to the points reflect the distribution of points that were significantly changed on the basis of a fold change in epitope binding signal. Only pathogen with antibodies targeting >5 epitopes were included. (B) Change in gMean EBS per epitope, across all paired samples per cohort. Format as in (A), except here each point represents a single epitope, and the gMean EBS is calculated across all paired samples with the epitope detected. The color of each point represents the fdr-adjusted Wilcoxon matched-pairs signed rank test P value, and shapes indicate the number of paired samples per cohort with antibodies against the particular epitope). Only epitopes recognized in >6 paired samples were included. (C) Scatter plot showing each epitope (point) shown in (B), but instead of plotting the fold change, the gMean EBS at time 1 (x axis) versus time 2 (y axis) is plotted. The P values (colors) and samples sizes (shapes) are indicated. MV-specific epitopes were included and highlighted in green. Epitopes significantly changed in a positive direction are labeled with pathogen species name, NS, not significant; PIV-4, parainfluenza virus-4.

Thus, this higher-resolution analysis failed to detect immune impairment at the epitope level. Combined with increased epitope diversity after MMR vaccination (Fig. 1A), this analysis supports decades of observations that MMR vaccines do not increase susceptibility to subsequent infections (29, 30).
Nevertheless, because of the naturally rapidly increasing antibody diversity in this young cohort at the time of MMR receipt, we cannot definitively rule out the potential for minor reductions of antibody-producing cells with measles vaccination.

Reconstruction of the antibody repertoire with high-transmission pathogens is spatially clustered

Although antibody diversity and abundance were negatively affected for almost all pathogens, a subset of children had increased EBS and/or epitope hits for particular pathogens after measles (Figs. 1, D and E, and 3A; and figs. S4, S8, and S11), suggestive of potential recovery is associated with pathogen transmission. Moreover, six of these children (75%) shared a household with another child with increased adenovirus C EBS. We found similar clustering effects for other respiratory pathogens: influenza A virus [of eight children with increased influenza A EBS, five (63%) were from a single postal code representing only 26% of the measles cases (OR 5.8; \( P < 0.001 \)]; RSV [of nine with increased EBS, five (56%) were from the same household; Fig. 4B]; and S. pneumoniae and Neisseria meningitides. In these children, the probability of retaining preexisting antibodies for these pathogens was relatively high (fig. S12).

If reconstruction of the antibody repertoire requires new exposures, children experiencing repertoire reconstruction for transmissible pathogens should cluster spatially. Using postal codes and household identifiers, we observed clustering of pathogen-specific repertoire reconstruction at both the postal code or school level and the household level (Fig. 4). Among 19 children with increased adenovirus C antibody EBS, eight (42%) came from a single postal code (number 7) that included only 12 (16%) of the 77 measles cases (Fig. 4A). Thus, children in this postal code had significantly increased odds [odds ratio (OR) 9.4; Fisher’s exact test, \( P < 0.001 \)] of recovering their adenovirus C repertoire versus other postal codes, suggesting recovery is associated with pathogen transmission. Moreover, six of these children (75%) shared a household with another child with increased adenovirus C EBS. We found similar clustering effects for other respiratory pathogens: influenza A virus [of eight children with increased influenza A EBS, five (63%) were from a single postal code representing only 26% of the measles cases (OR 5.8; \( P < 0.05 \)]; RSV [of nine with increased EBS, five (56%) were from the same postal code (OR 4.5; \( P < 0.05 \)]; and S. pneumoniae and Neisseria meningitides [of thirteen, seven (54%) came from a single postal code (OR 4.5; \( P < 0.05 \)] and two shared a household]. Combined, these indicate local pathogen transmission and suggest that reconstruction of the antibody repertoire occurs on a per-pathogen basis and is associated with new exposures.

In contrast to the highly transmissible respiratory pathogens above, where mean EBS was increased after measles infection, we found that for chronic viruses [i.e., HHV-4 and human herpesvirus 5 (cytomegalovirus; HHV-5)], there was no evidence of spatial clustering. Further, only 6 out of 12 individuals (50%) with increased HHV-4 antibody EBS also developed antibodies against new HHV-4 epitopes. HHV-5 behaved similarly, with only 33% of individuals with increased HHV-5 antibody EBS also developing new antibodies to previously untargeted HHV-5 epitopes. The lack of development of antibodies targeting new epitopes, despite increases in EBS of existing antibodies for these two pathogens, is different from what we observed for the more-transmissible viruses noted above, in which 87% of pathogen-specific increases in EBS at time 2 were associated with development of new antibodies against previously unrecognized pathogen-specific epitopes. An increase in EBS without addition of new epitope recognition points toward reinfection with or reactivation of the same virus.

Two additional notable pathogens for which positive changes in EBS were often detected after measles were the bacteria S. pneumoniae and N. meningitides.
and *S. aureus*. Acute viral respiratory infections, including measles, increase susceptibility to respiratory bacterial infections (6, 31–33). After measles, new acquisitions of bacteria or increased replication of already colonizing bacteria could elicit antibody responses and reinstate antibody diversity and protection against these pathogens. In line with this, we observed net increases in antibody diversity, including development of antibodies against new epitopes, and increases in EBS for *S. aureus* [the only pathogen for which a net positive change in antibody diversity or EBS was detected after measles; 45 of 77 children (58%) had increased EBS] (Fig. 1 and fig. S11). Increases were also observed for *S. pneumoniae*, although the overall cohort-level effect remained negative.

**Reconstruction of the antibody repertoire through reexposure after measles carries risks**

Despite the potential benefits of reinstating the antibody repertoire, exposure to pathogens after measles—especially when in the presence of diminished preexisting immune memory—can carry risks. Ten of 43 children (23%) with severe measles were diagnosed with acute otitis media (AOM; most commonly pneumococcal), which was associated with a threefold increase in odds of carrying greater pneumococcal antibody diversity after measles compared with children without an AOM diagnosis. In addition, two children with mild measles and AOM and one with a diagnosis of bacterial pneumonia had increased pneumococcal antibody diversity at follow-up. Combined, these effects suggest that the antibody repertoire begins rebuilding soon after measles, through pathogen exposures, and that pathogen exposure after measles, while serving to reinstate immune memory, may pose excess risk.

**Experimental MV infection confirms a decrease in previously acquired immune memory**

To confirm the findings above using a controlled experimental setting with longer follow-up, we used VirScan to profile the antibody repertoire in plasma collected before and 5 months after experimental MV infection of four rhesus macaques (34). The overall diversity of the antibody repertoire was decreased an average of 26% (range: 21 to 35%), and reductions were distributed across pathogens (Fig. 5A). Notable exceptions were observed for simian foamy virus and Epstein-Barr virus, both of which can reactivate during immune dysregulation and thus may be early contributors to repertoire reconstruction. As expected, we detected increases in recognition of MV epitopes (Fig. 5B). Out of 21 MV epitopes recognized after measles, only one (phosphoprotein/V protein 29) was recognized before infection and was reduced by 40%. This likely represented a cross-reactive epitope that was diminished along with the rest of the repertoire.

Each monkey lost, on average, 40 to 60% of its preexisting antibody repertoire (Fig. 5C), and this loss persisted for at least 5 months after MV infection. In each monkey, the fractions lost were distributed across the pathogens, which is expected for a virus that indiscriminately infects antibody-secreting cells.

**Fig. 5. Measles virus infection in macaques deletes preexisting immune memory.** Four rhesus macaques (14Y, 31Y, 46Y, and 50Y) were infected with the Bihoven strain of wild-type MV [detailed in (34)], and plasma samples were collected before and 5.1 months after infection. (A) Heatmap showing the percentage change in total epitopes recognized per pathogen in each monkey from time 1 to time 2. Pathogens with >10 unique epitopes recognized at either time point per macaque are shown. The color and text indicate percent change in total epitopes recognized per pathogen species before versus after infection. (B) Heatmap showing the signal strength of anti-measles antibodies (EBS) for each epitope for which at least one monkey developed an antibody. Colors and text of each cell represent the respective EBS values. Letters indicate the MV protein (F, fusion protein; H, hemagglutinin; N, nucleoprotein; and P, phosphoprotein), and the numbers indicate the position along the protein of the first amino acid of the 56-amino-acid peptide. (C) The fractions of epitopes recognized before MV infection that remained 5 months after are shown (0 months after infection is baseline and thus set to 1 for all pathogens). Each gray line indicates a different pathogen, and the dark black line indicates the average across the pathogens. The boxplot summarizes the interquartile range and median fraction retained across the pathogens.
Cumulative evidence supports the establishment of an immune amnesia state after measles

Using VirScan, we quantified the effects of measles on antigen-presenting antigen repertoire in plasma obtained before and after natural and experimental MV infections. We found that measles is associated with large reductions in both the diversity of the antigen repertoire and magnitude of the binding signal, likely reflecting reduced antibody titers resulting from diminished numbers of cells producing the respective antibody. The reduction of diversity may be greater than we report because even if particular antibody-producing cells have been eliminated, an antibody half-life of ~3 weeks (35) means that residual antibodies were still detectable at the time of sampling. Our findings show that after recovery from MV infections, individuals enter a state in which immune functionality is restored, but memory cell elimination induced by measles may alter previously acquired memory.

MV can infect 20% to 70% of memory cells, including B cells, T cells, and plasma cells in the lymphoid tissue and peripheral blood during the first 3 to 10 days after infection (7, 10, 24). Clinical trials of systemic administration of engineered MV for oncolytic therapy of multiple myeloma also demonstrated efficient entry of MV into the bone marrow and plasma cell infection (36). In wild-type MV infections, lymphocytes undergo rapid proliferation during immune activation (37), and lymphocyte counts (and other immunological markers) return to normal levels within weeks of infection. Thus, the immunological toll of measles is often considered limited to that period. After experimental MV infection in monkeys, we found that we could no longer detect up to 60% of the antibody repertoire, possibly reflecting reduced antibody titers resulting from the lasting damage to the immune system. The WHO recently reported that between 2000 and 2017, MV vaccines have prevented more than 21 million deaths directly attributable to measles (41). These findings suggest that the number of deaths averted might be much greater, and they attest to the immense public health value of the measles vaccine.

REFERENCES AND NOTES

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an adviser for MPM Capital, none of which affect this work. M.J.M. has served as a member of Sanofi advisory board for RSV therapeutics. D.E.G. is a member of the GlaxoSmithKline Vaccine Research and Development Advisory Board. S.J.E., T.K., and H.B.L. are inventors on a patent application filed by The Brigham and Women’s Hospital (US20160320406A) that covers the use of the VirScan library to identify pathogen antibodies in blood. All other authors declare no competing interests. Data and materials availability: The VirScan library is available under a material transfer agreement. Data are available in the main text, the supplementary materials, or on Dryad (42).

SUPPLEMENTARY MATERIALS

Figs. S1 to S12
Table S1
References (43–45)

View/request a protocol for this paper from Bio-protocol.

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The toll of measles on the immune system
Many of the deaths attributable to measles virus are caused by secondary infections because the virus infects and functionally impairs immune cells. Whether measles infection causes long-term damage to immune memory has been unclear. This question has become increasingly important given the resurgence in measles epidemics worldwide. Using a blood test called VirScan, Mina et al. comprehensively analyzed the antibody repertoire in children before and after natural infection with measles virus as well as in children before and after measles vaccination. They found that measles infection can greatly diminish previously acquired immune memory, potentially leaving individuals at risk for infection by other pathogens. These adverse effects on the immune system were not seen in vaccinated children. Science, this issue p. 599