ORIGINAL ARTICLE

Duration of Humoral Immunity to Common Viral and Vaccine Antigens

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ABSTRACT

BACKGROUND

Maintenance of long-term antibody responses is critical for protective immunity against many pathogens. However, the duration of humoral immunity and the role played by memory B cells remain poorly defined.

METHODS

We performed a longitudinal analysis of antibody titers specific for viral antigens (vaccinia, measles, mumps, rubella, varicella–zoster virus, and Epstein–Barr virus) and nonreplicating antigens (tetanus and diphtheria) in 45 subjects for a period of up to 26 years. In addition, we measured antigen-specific memory B cells by means of limiting-dilution analysis, and we compared memory B-cell frequencies to their corresponding serum antibody levels.

RESULTS

Antiviral antibody responses were remarkably stable, with half-lives ranging from an estimated 50 years for varicella–zoster virus to more than 200 years for other viruses such as measles and mumps. Antibody responses against tetanus and diphtheria antigens waned more quickly, with estimated half-lives of 11 years and 19 years, respectively. B-cell memory was long-lived, but there was no significant correlation between peripheral memory B-cell numbers and antibody levels for five of the eight antigens tested.

CONCLUSIONS

These studies provide quantitative analysis of serologic memory for multiple antigens in subjects followed longitudinally over the course of more than one decade. In cases in which multiple exposures or repeated vaccinations were common, memory B-cell numbers did not correlate with antibody titers. This finding suggests that peripheral memory B cells and antibody-secreting plasma cells may represent independently regulated cell populations and may play different roles in the maintenance of protective immunity.

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Recovery from acute viral or microbial infection often results in long-term or even lifelong immunity.[1-3] Although the importance of sustaining protective humoral immunity is widely recognized, the mechanisms involved in this maintenance remain unclear.[3-10] To address the issue of antibody maintenance after infection or vaccination, we conducted a longitudinal analysis of antibody titers against multiple antigens with the use of serum samples banked from a combination of scheduled (annual) and event-based collections. On the basis of these studies, we determined whether antigen-specific antibody responses were measurably boosted through environmental exposure, infection, or vaccination. Moreover, we determined the duration of serologic memory over long periods of time during which specific boosting of antibody titers was not observed.

METHODS

SUBJECTS

We recruited human subjects from the Oregon National Primate Research Center through campuswide e-mail, inviting them to provide serum samples for banking at enrollment and to provide additional serum samples from scheduled blood draws. Fifty-one people responded, providing written informed consent and completing an extensive medical-history questionnaire before providing samples for study. No inclusion criteria were used other than a requirement that the subject have at least three serum samples banked for 3 years or more before the study began. On the basis of this criterion, 6 subjects were excluded, and 45 subjects provided a total of 630 serum samples for analysis.

Samples were drawn annually as part of a centerwide, comprehensive program to permit serologic testing of people working in close proximity with nonhuman primates. In the event of an exposure to an animal (e.g., a scratch or bite), an additional serum sample was drawn. Blood samples were also drawn at weekly intervals after smallpox vaccination in a subgroup of eight subjects in order to monitor the acute phase of the immune response. The study was approved by the institutional review board of Oregon Health & Science University.

ELISA AND MEMORY B-CELL MEASUREMENT

Measurement of antibodies by means of enzyme-linked immunosorbent assay (ELISA) was performed as previously described.[11,12] Antigens included vaccinia (WR strain prepared in our laboratory), measles (Edmonston strain, Biodesign), mumps (Enders strain, Biodesign), rubella (HPV77 strain, Viral Antigens), varicella–zoster virus (Rod strain, Biodesign), Epstein–Barr virus gp125 (Viral Antigens), tetanus toxin (C-fragment), and diphtheria toxin (EMD Biosciences). International serum standards were obtained from the National Institute for Biological Standards and Controls in Hertfordshire, England. An optimized limiting-dilution assay was used to measure memory B cells.[13]

STATISTICAL ANALYSIS

Before the final analysis, antibody data were censored by excluding unvaccinated subjects, seronegative subjects, and subjects with test results that were equivocal (<200 ELISA units). A total of one to nine subjects were excluded, depending on the antigen. We removed data points obtained during or shortly after an acute immune response before determining antibody half-life estimates. For example, if more than a doubling of the titer was observed between contiguous time points (hereafter referred to as an antibody spike), then that time point and those from the next 3 years were removed from the analysis so that the rapid initial decrease in antibodies typically observed shortly after antigenic exposure[14-17] would not influence the estimated long-term decay rate. A subject had to have at least three data points for a period of 3 years or more in order to be included in the final analysis. Tetanus and diphtheria vaccines are frequently administered in combination,[18] so if an antibody spike was observed between contiguous data points for one antigen, a reexposure was assumed for the reciprocal antigen and removed from the analysis. Since tetanus–diphtheria toxoid revaccination is recommended every 5 to 10 years, contiguous data points separated by more than 5 years were not used for the analysis of these antigens.

To address possible changes before and after acute immune responses, the analysis was performed first for only the longest period of observation and then for all the data, with each period treated as an independent longitudinal series.

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The results of these two analyses did not differ. For each antigen described in this study, we present the results of the analysis in which all data were used.

Rates of antibody decay were estimated with the use of a longitudinal mixed-effects model (PROC MIXED; SAS version 9.1, SAS Institute). The model had a fixed effect for age and gaussian-distributed random intercepts and slopes (see the Supplementary Appendix, available with the full text of this article at www.nejm.org). Random components were assumed to be uncorrelated. Measures were logarithmically transformed (on a natural log scale) before analysis, resulting in a single-exponent decay model of the untransformed data. Visual inspection indicated that the linear model fitted the data for the subjects well. We assessed the influence of data for any one subject on the analysis with the use of cross-validation. The results were not influenced by any one person's series.

Half-life estimates were obtained by transforming the decay rate and the boundaries of the 95% confidence interval obtained from the fixed-effects slope component of the mixed-effects model. If a specific antibody level does not significantly decay or shows even a small insignificant increase over time (e.g., <1%), then the calculated half-life becomes infinity. To confirm the results from our mixed-effects model, we also fitted least-squares linear regression models to each series. The results from the two approaches were nearly identical (see the Supplementary Appendix). For each antigen, we determined whether the population slope was equal to zero in the mixed-effects model. The P values presented are two-sided, and a P value of less than 0.05 was considered to indicate statistical significance. The comparisons of memory B-cell frequencies and associated serum antibody titers were modeled through least-squares linear regression after $\log_{10}$ transformation.

Table 1. Characteristics of the Subjects.*

Variable	Subjects (N=45)
Sex — no. (%)	
Male	21 (47)
Female	24 (53)
Race or ethnic group — no. (%)†	
White (not of Hispanic origin)	38 (84)
Asian or Pacific Islander	4 (9)
Hispanic	1 (2)
Mixed race	2 (4)
Duration of sample coverage‡	
<5 yr — no. (%)	0
5–10 yr — no. (%)	12 (27)
11–15 yr — no. (%)	10 (22)
16–20 yr — no. (%)	13 (29)
>20 yr — no. (%)	10 (22)
Mean — yr	15.2±6.4
Range — yr	5–26
Age — yr	
At first observation	
Mean	37±8
Range	23–59
At last observation	
Mean	52±10
Range	29–67
Serum samples per subject — no.	
Mean	14.0±8.3
Range	3–35

* Plus–minus values are means ±SD. Because of rounding, not all percentages total 100.
† Race or ethnic group was reported by each subject. Two subjects reported that they were of mixed race (white and Asian or Pacific Islander, and white and American Indian).
‡ Years of sample coverage were rounded to the nearest whole integer.

RESULTS

SERUM SAMPLES AND CHARACTERISTICS OF THE SUBJECTS

A total of 630 serum samples from 45 subjects were obtained for this study or were obtained from the Oregon National Primate Research Center serum bank (Table 1). The majority of these serum samples were obtained from scheduled collections, with 50 samples (7.9%) obtained after an unscheduled event (e.g., exposure to an animal). Each subject provided, on average, 14.0 serum samples (median, 11) during an average period of 15.2 years (median, 15.6). The majority of subjects had received smallpox vaccination during childhood and had recovered from viral infections, including measles, mumps, rubella, Epstein–Barr virus, and varicella–zoster infections. The

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subjects in this cohort had common coexisting conditions but no specific immune deficiencies (Table 1 of the Supplementary Appendix). The duration of serum antibody production was determined with the use of a mixed-effects model of longitudinal analysis. Overall, antigen-specific antibody responses were long-lived, and we found no significant differences in antibody-maintenance patterns according to sex (Table 2).

VACCINIA

One potential mechanism for maintaining long-term immunity to a nonpersisting pathogen is through intermittent reexposure. To examine this possibility, we evaluated antibody responses after smallpox vaccination (Fig. 1A). A total of 39 of 43 subjects born before 1972 (91%) were seropositive for vaccinia (Fig. 1A in the Supplementary Appendix), which is consistent with the findings of a previous study.[11] Before 2003, when eight subjects received booster vaccination, there were only two instances of an antibody spike that was indicative of vaccination or exposure (0.3 event per 100 person-years). This finding is consistent with the discontinuation of routine vaccination in 1972 and the absence of endemic orthopoxviruses in North America that are known to infect humans. Excluding serum samples obtained after smallpox vaccination in 2003, a putative protective level of antiviral antibody[11,21] was present in 28 of the 45 subjects (62%), and the level of vaccinia-specific antibodies decreased slowly, with an estimated half-life of 92 years (95% confidence interval [CI], 46 to infinity; P=0.049).

MEASLES

Measles is rare in the United States, but it remains a serious threat through importation.[22] An analysis of measles-specific antibodies revealed that five subjects had spikes in serum antibody levels (Fig. 1B in the Supplementary Appendix). Two subjects received documented measles–mumps–rubella (MMR) vaccinations; one seroconverted, whereas the other seropositive subject had antibody titers that did not change after vaccination. Four subjects unknowingly contracted a cross-reactive but uncharacterized paramyxovirus infection from exposure to diseased nonhuman primates during a 1999 outbreak. On the basis of the antibody titers from the last available blood

Table 2. Duration of Antigen-Specific Serum Antibody Production.*

Antigen	Protective Titer	Subjects Protected†	Antibody Half-Life‡			P Value
			Total Population	Men	Women	
	IU/ml	*no. (%)*	*year (95 percent confidence interval)§*			
Tetanus¶	0.01	42 (93)	11 (10–14)	12 (10–16)	10 (8–14)	0.23
Diphtheria¶	0.01	40 (89)	19 (14–33)	26 (17–51)	14 (8–42)	0.11
VZV	NA	NA	50 (30–153)	63 (28–∞)	41 (23–212)	0.51
Vaccinia	3.8	28 (62)	92 (46–∞)	99 (48–∞)	85 (31–∞)	0.91
Rubella	10.0	39 (87)	114 (48–∞)	85 (43–∞)	190 (35–∞)	0.60
EBV	NA	NA	11,552 (63–∞)	No decay (84–∞)	3648 (35–∞)	0.99
Mumps	NA	NA	542 (90–∞)	124 (53–∞)	No decay (89–∞)	0.16
Measles	0.2	41 (91)	3014 (104–∞)	369 (67–∞)	No decay (74–∞)	0.56

* International serum standards were obtained from the National Institute for Biological Standards and Controls (Hertfordshire, England). For some antigens, a protective level of antibodies was not known or international serum standards were not available. NA denotes not available, VZV varicella–zoster virus, and EBV Epstein–Barr virus. P values are for the comparison of the antibody half-life between men and women.

† These values were based on the last available serum samples drawn before measles–mumps–rubella vaccination or serologic boosting during the 1999 paramyxovirus outbreak.

‡ For some antigens, antibody responses showed no decay, and an antibody half-life could not be determined. The F test associated with the mixed-effects model was used to compare the serum antibody half-lives for men and women, and no significant differences were observed. Thus, although differences in the magnitude of antibody responses may be influenced by sex,[20] once an effective antibody response has developed, it will be maintained equally well in both men and women.

§ Human IgG has a pharmacologic half-life of 21 days[19] (range, 11 to 42), and continuous replenishment is required if levels are to be maintained for more than 21 days. The antigen-specific antibody half-life (in years) was derived from the annual percent change, determined with the use of a longitudinal mixed-effects model.

¶ The numbers of subjects protected against tetanus and diphtheria represent conservative estimates, since the putative protective levels for both antigens are below the limit of detection by enzyme-linked immunosorbent assay.

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sample drawn before a recent MMR vaccination or serologic boosting during the 1999 outbreak of primate paramyxovirus, 43 of the 45 subjects (96%) were seropositive, with a putative protective level of at least 0.2 IU of antimeasles antibodies per milliliter[23] in 41 subjects (91%) (Fig. 1B in the Supplementary Appendix). The decrease in measles-specific antibodies (Fig. 1B) was not significant (P=0.94) and is likely to be maintained for life (estimated half-life, 3014 years; 95% CI, 104 to infinity).

MUMPS

Although typically less severe than measles, mumps is another childhood disease with the potential for serious complications.[18] In this cohort, 41 of 45 subjects (91%) were seropositive for mumps (Fig. 1C in the Supplementary Appendix). Two subjects received MMR vaccinations during the period of observation, and two other subjects had spikes in antibody levels, one in 1998 and the other in 2004. These spikes may have been the consequence of exposure to naturally occurring mumps rather than MMR vaccination. This incidence rate of 0.3 event per 100 person-years is consistent with a steady decrease in prevalence[18] since routine vaccination began in 1977. Antibody responses to mumps (Fig. 1C), like those to measles, were long-lived (estimated half-life, 542 years; 95% CI, 90 to infinity) and showed no significant decrease (P=0.69).

RUBELLA

Rubella was a leading cause of birth defects in the United States before immunization programs were implemented in the 1970s.[18] A total of 40 of the 45 subjects in our cohort (89%) were seropositive for rubella (Fig. 1D in the Supplementary Appendix). We identified two subjects with documented MMR vaccinations and one subject with a spike in preexisting rubella-specific titers in 2003. Because of an 8-year gap between contiguous serum samples, it is unclear whether the change in this latter subject was due to a natural case of rubella or variability in serum titers. In any case, a spike in rubella-specific antibodies is uncommon, with a low overall incidence rate of 0.15 event per 100 person-years. A putative protective level of at least 10 IU of antirubella antibodies per milliliter[24] was reached in 39 of the 45 subjects (87%). Rubella-specific immunity (Fig. 1D) was maintained, with an estimated half-life of 114 years (95% CI, 48 to infinity) and no significant rate of decrease (P=0.15).

After reviewing the medical histories of the subjects, we identified six subjects in whom only vaccine-induced immunity against measles had developed, four subjects in whom vaccine-induced immunity against mumps had developed, and seven subjects in whom vaccine-induced immunity against rubella had developed. Exclusion of these subjects from the longitudinal analysis shown in Figure 1 had no substantial effect on the calculated duration of serum antibody responses (Table 2 of the Supplementary Appendix). Moreover, although reexposure to the same or a serologically cross-reactive virus may boost antibody titers, these data with regard to vaccinia, measles, mumps, and rubella indicate that repetitive environmental exposures and infections are not absolutely required for maintaining long-term antiviral antibody responses.

EPSTEIN–BARR VIRUS AND VARICELLA–ZOSTER VIRUS

In contrast to acute viral infections, chronic and latent viral infections may either persist or be reactivated from latency, thereby "boosting" immune responses in the infected person. Antibody titers were determined for two latent herpesviruses, Epstein–Barr virus and varicella–zoster virus. A total of 37 of the 45 subjects (82%) were seropositive for Epstein–Barr virus, with seroconversion in 1 subject occurring during the observation period (Fig. 1E in the Supplementary Appendix). Four seropositive subjects had antibody titers that spiked during observation, indicating that reactivation or reexposure events had occurred, but at a relatively low rate (0.6 event per 100 person-years). Humoral immunity against Epstein–Barr virus (Fig. 1E) showed no significant decrease (P=0.99) and is likely to be maintained for life (estimated half-life, 11,552 years; 95% CI, 63 to infinity).

Unlike antibody responses to Epstein–Barr virus, antibody responses to varicella–zoster virus showed frequent fluctuations (Fig. 1F in the Supplementary Appendix). All 45 subjects were seropositive for varicella–zoster virus, and 10 of the 45 subjects (22%) had antibody spikes (1.6 events per 100 person-years). Two subjects described an episode of shingles at or near the time of the observed spike in antibody responses to varicella–zoster virus, one subject may have been exposed to recently vaccinated children, six subjects do

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not recall having shingles or any known exposure to patients with varicella–zoster virus, and information was not available for one subject. Immunity (Fig. 1F) decreased slowly, with an estimated half-life of 50 years (95% CI, 30 to 153; P=0.005). Thus, although the infection is latent with evidence of the most frequent reexposure and reactivation events, varicella–zoster virus induced the most short-lived antibody response of the viruses we examined.

Figure 1 (facing page). Antibody Responses after Viral Infection or Vaccination with Nonreplicating Protein Antigens.

Results of longitudinal analyses of serum titers of antibodies against eight antigens in 45 subjects are shown: vaccinia (Panel A), measles (Panel B), mumps (Panel C), rubella (Panel D), Epstein–Barr virus (EBV) (Panel E), varicella–zoster virus (VZV) (Panel F), tetanus (Panel G), and diphtheria (Panel H). To determine the antibody half-life during the maintenance phase of the immune response, data were censored by removing subjects with seronegative and equivocal samples. For seropositive subjects, time points at or up to 3 years after an antibody spike were removed before analysis. The estimated antibody half-lives, 95% confidence intervals (CI), interquartile ranges, and associated P values were obtained with the use of a mixed-effects model of longitudinal analysis. The shaded regions represent the cutoff between seropositive and seronegative serum titers as determined by enzyme-linked immunosorbent assay (ELISA), and the dotted lines indicate the putative protective levels of antibodies, if known. EU denotes ELISA units and IU international units. IU standards were not available for mumps, EBV, and VZV.

TETANUS AND DIPHTHERIA

Tetanus and diphtheria vaccination has been recommended since the 1940s, primarily with the combined tetanus–diphtheria toxoid vaccine for adults. This recommendation has resulted in a sharp decrease in the incidence of both diseases[18] and prolonged maintenance of antibody titers (Fig. 1G and 1H). A titer of more than 0.01 IU of antitetanus antibodies per milliliter is considered to be protective.[25] ELISA titers of more than 0.16 IU per milliliter correlate well with neutralizing activity; a titer of 0.16 IU per milliliter is the lowest level reliably detected by means of ELISA[26] and is similar to our detection limit of 0.15 IU per milliliter (200 ELISA units). Protective antitetanus responses (Fig. 1G in the Supplementary Appendix) were clearly identified in 42 of the 45 subjects (93%), but this response rate may not reflect the true number of protected persons, since 0.01 IU is below the limit of detection by ELISA. Frequent tetanus boosters resulted in 31 instances of an antibody spike in 27 subjects (4.9 events per 100 person-years) (Fig. 1G in the Supplementary Appendix). Tetanus-specific antibodies decreased rapidly, with an estimated half-life of 11 years (95% CI, 10 to 14; P<0.001), which is similar to the decrease shown in a model reported more than 40 years ago.[27]

To determine whether the rapid antibody decay observed with tetanus held true for other protein antigens, we measured immunity against diphtheria (Fig. 1H). Antidiphtheria antibody titers of more than 0.01 IU per milliliter are considered to be protective,[28] and in 40 of the 45 subjects (89%) diphtheria-specific antibodies remained above our detection limit (200 ELISA units, or 0.04 IU per milliliter) (Fig. 1H in the Supplementary Appendix). It is possible that the remaining five subjects also had sustained protective antidiphtheria immunity but that it could not be definitively measured in these studies. Diphtheria-specific antibody spikes were observed, typically in parallel to responses against tetanus, as expected because of the combined tetanus and diphtheria formulation recommended for adult vaccination. An analysis of diphtheria-specific antibodies indicated an estimated half-life of 19 years (95% CI, 14 to 33; P<0.001). These results suggest that antibody maintenance is greatly influenced by the nature of the antigen, with these proteins eliciting quantitatively shorter antibody responses than those observed after viral infection.

ANTIBODY MAINTENANCE IN INDIVIDUAL SUBJECTS

One model of antibody maintenance predicts that long-term responses are maintained by non–antigen-specific stimulation, also referred to as "bystander activation" of memory B cells during antigenically unrelated infections.[3,8,29] To determine the potential effects of heterologous infection and vaccination, we measured humoral responses to eight antigens in four subjects followed longitudinally for more than 25 years and at weekly intervals after smallpox vaccination (Fig. 2). Common immunologic events, including tetanus and diphtheria booster immunization (in Subjects 1

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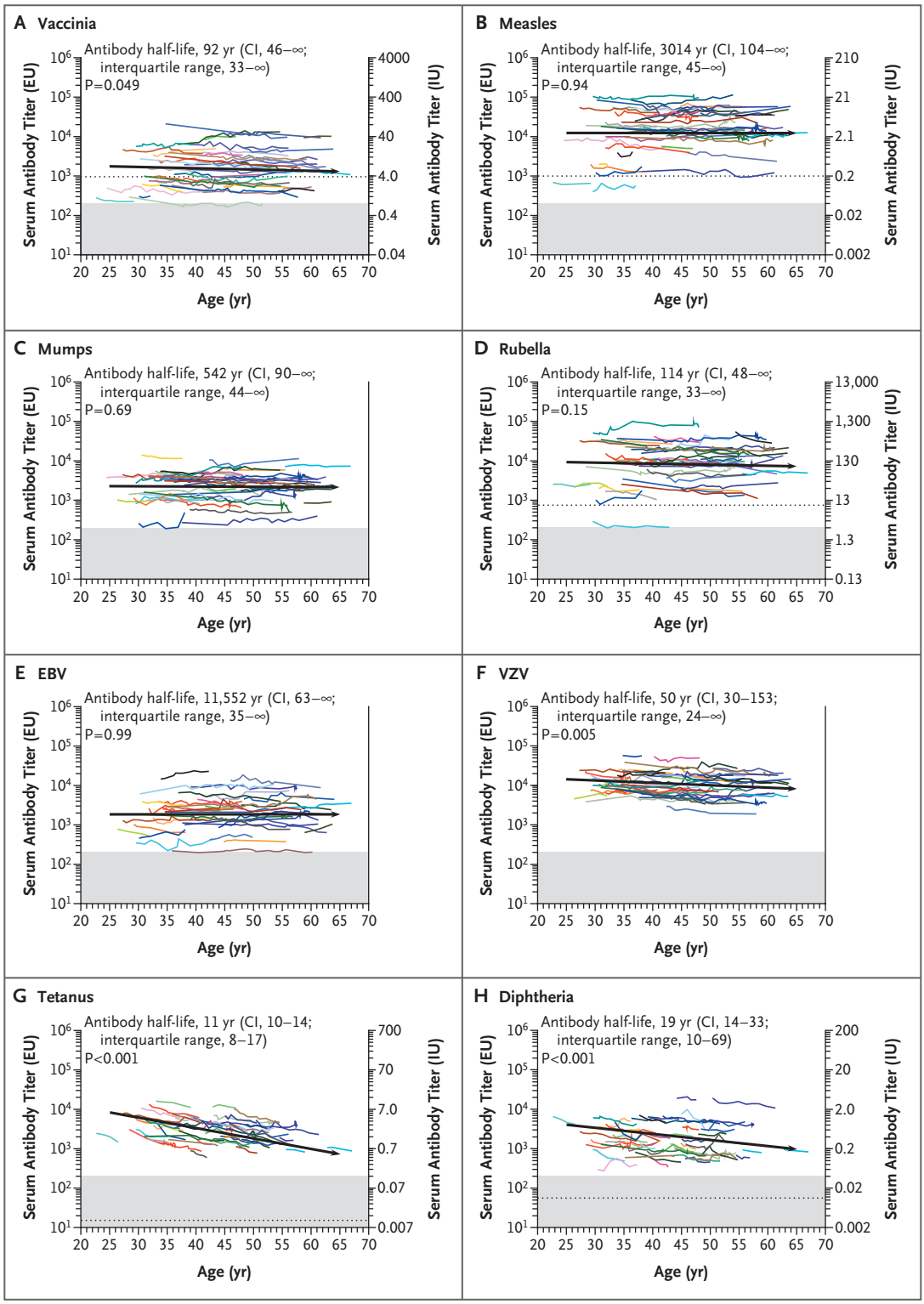

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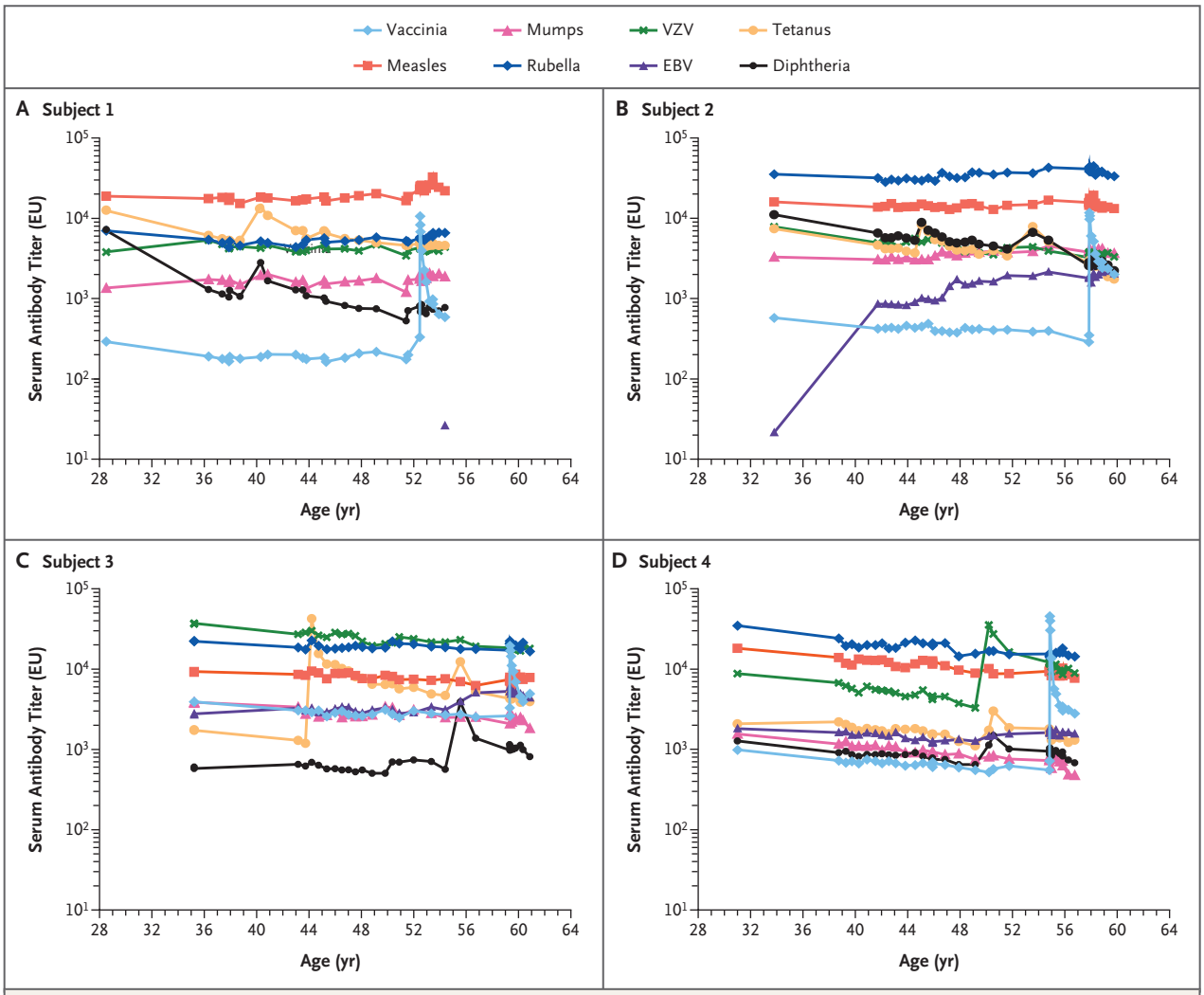

Figure 2. Longitudinal Analysis of Serum Antibody Titers in Representative Subjects.

Serum antibody responses against eight antigens were followed in four subjects with serum samples for a period of more than 25 years. Each subject received a booster smallpox vaccination in 2003, and serum antibody levels were monitored at close intervals after immunization (days 7, 14, 21, 30, and 60 after vaccination) to determine whether a defined viral infection would result in increased antibody production against other nonspecific antigens. Subject 1 (Panel A) was seronegative for Epstein–Barr virus (EBV), as indicated by a single data point of less than 200 ELISA units (EU). Subjects 2, 3, and 4 (Panels B, C, and D) were seropositive for EBV. VZV denotes varicella–zoster virus.

through 4), Epstein–Barr virus seroconversion (in Subject 2), and varicella–zoster virus reexposure or reactivation (in Subject 4), occurred during the period of observation but showed little effect on other antigen-specific antibody responses. After a defined infection with vaccinia (i.e., booster smallpox vaccination), there was little or no alteration in antibody responses to seven other antigens (<6% average change) (Fig. 2), despite vaccinia-specific antibody responses that increased by approximately 4000% at the peak of the response. These results are consistent with those in six subjects who received booster smallpox vaccination and two subjects who received primary smallpox vaccination and later received either live yellow-fever vaccination or MMR vaccination (data not shown). Together, these findings suggest that nonspecific bystander activation is an unlikely mechanism in the maintenance of long-term antibody responses.

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Individual variations in antibody maintenance (Fig. 1) indicated that although the antigen itself plays an important role, it is not the sole factor determining the longevity of antibody responses. The findings in Subject 1 (Fig. 2A) were largely representative of those in the overall cohort, with no measurable decrease in measles-specific antibodies and short-lived tetanus-specific and diphtheria-specific responses (estimated half-life, 14 and 12 years, respectively). Subject 2 (Fig. 2B) had a similar pattern, with no decrease in measles-specific antibodies, whereas tetanus and diphtheria responses had half-lives estimated at 13 and 11 years, respectively. Subject 3 (Fig. 2C) had a measles-specific antibody response that underwent a slow but measurable decrease (estimated half-life, 68 years), with tetanus antibodies showing the most rapid rate of decrease (estimated half-life, 8 years). Diphtheria-specific antibody titers did not decrease over the course of two decades, in sharp contrast to tetanus-specific antibody titers. The findings in Subject 4 (Fig. 2D) are particularly intriguing because, with the exception of antibodies against Epstein–Barr virus (which showed no evidence of a decrease), all antibody responses decreased at relatively similar rates (estimated half-life, 14 to 31 years). Thus, the antigen, as well as one or more currently unknown host-specific factors, has a role in determining the duration of antibody-response patterns.

B-CELL MEMORY AND ASSOCIATION WITH ANTIBODY LEVELS

Plasma cells either maintain antibody levels independently[30-32] or may require replenishment by the proliferation and differentiation of memory B cells. A requirement for all memory B-cell–dependent theories of antibody maintenance is that a correlation must exist between memory B-cell levels and antibody levels.[3,8,29] With the use of a limiting-dilution analysis,[13] we found that memory B cells in the circulation were remarkably long-lived (Fig. 3A). Previous studies have shown that within 1 month after vaccination, memory B-cell numbers in the circulation are representative of the memory B-cell frequencies observed in other lymphoid compartments such as the spleen.[33]

We next compared memory B-cell frequencies with their corresponding serum antibody titers for eight antigens. A significant correlation between memory B-cell levels and antibody levels was observed after acute infection with measles, mumps, and rubella but not vaccinia (Table 3). There was also no significant correlation between memory B cells and antibodies against varicella–zoster virus or Epstein–Barr virus (viruses that maintain latent reservoirs) or for the tetanus–diphtheria vaccine antigens (Table 3). The strength of the correlation varied widely among antigens, suggesting that memory B-cell frequencies were a poor predictor of serum antibody levels (Fig. 3B). For example, only 3% of the variability in antibody levels against tetanus could be explained by memory B-cell frequencies (R^2=0.03). This finding suggests that memory B-cell–dependent replenishment of short-lived plasma cells is not likely to be a global mechanism for antibody maintenance.

DISCUSSION

We investigated the longitudinal maintenance of antibody responses to eight antigens over a period of up to 26 years. Antibody responses after live viral infections had half-lives of 50 years or more, with many showing no measurable decrease. In contrast, responses to nonreplicating protein antigens (tetanus and diphtheria) decreased at a relatively rapid pace (estimated half-lives, 11 to 19 years), suggesting that within a particular person, antigen-specific mechanisms play a substantial role in determining the duration of humoral immunity.

On average, subjects were 52 years of age at the conclusion of the study, and most had contracted natural measles, mumps, or rubella infections during childhood. It is unknown whether vaccine-induced immunity is as long-lived as that induced by natural infection. Although the titers of vaccine-induced antibody responses appear to be lower than those reached after natural infection (data not shown), a longitudinal analysis of immunity against measles, mumps, and rubella suggests that serologic memory may be similar to natural infection (Supplementary Appendix). However, this result is based on a very small sample (two to five subjects), and further studies on antibody maintenance will be required before statistically meaningful comparisons can be made. This information is particularly important in contemporary populations in which asymptomatic reboosting of immunity by circulating natural or wild-type viruses appears to be relatively rare.

Two principal mechanisms for maintaining

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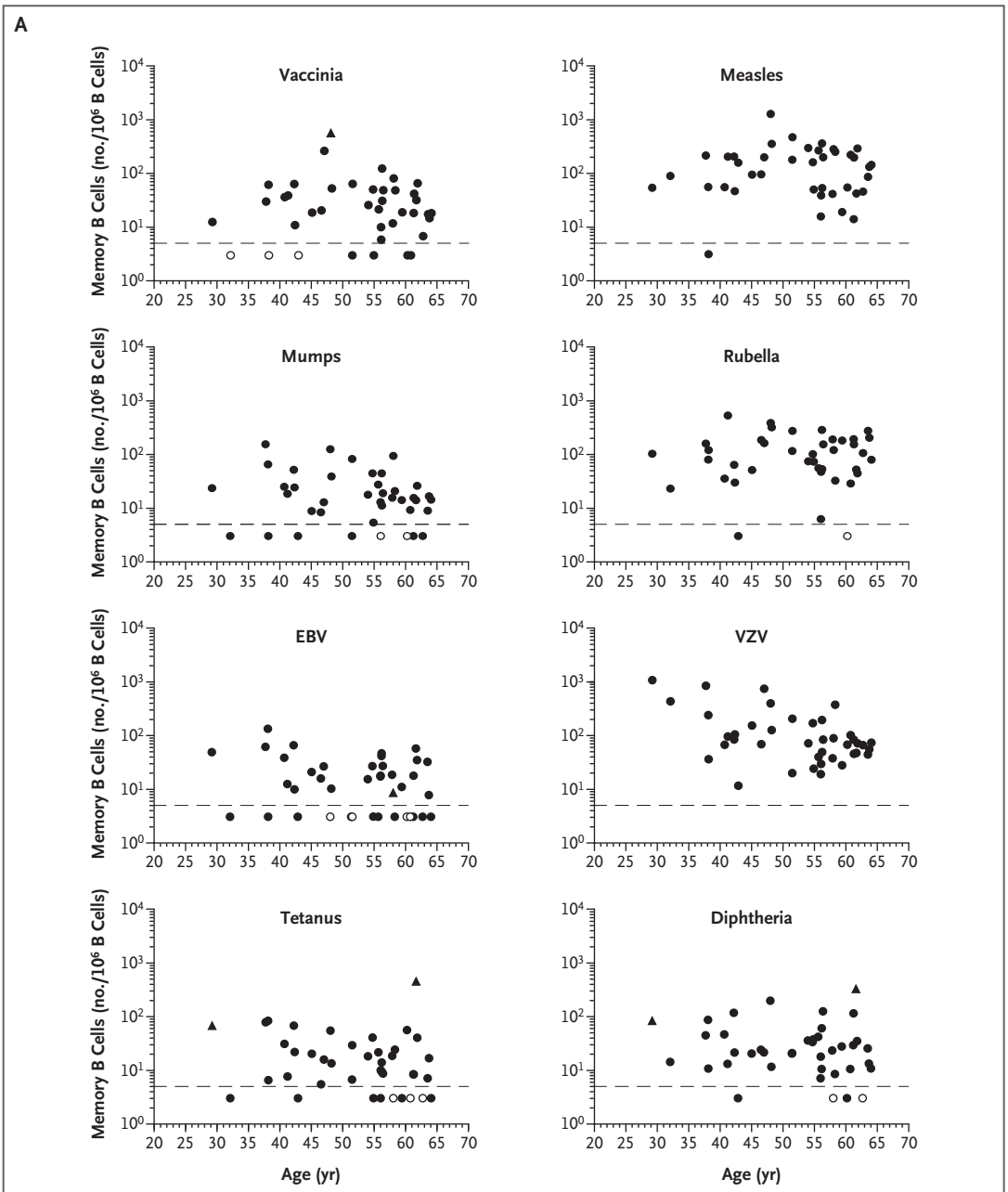

Figure 3 (and facing page). Relationship between the Number of Memory B Cells, Age, and Serum Antibody Levels. Panel A shows the results of an antigen-specific limiting-dilution assay performed for single time points for 40 subjects with available peripheral-blood mononuclear cells to determine memory B-cell frequencies for vaccinia, measles, mumps, rubella, varicella–zoster virus, Epstein–Barr virus, tetanus, and diphtheria. Solid circles represent samples with positive antibody titers (>200 ELISA units [EU]) or B-cell memory (>5 memory B cells per 10^6 B cells). Samples that scored below the level of detection for both antibody titer and B-cell memory are shown as open circles. Samples obtained during the acute phase (<1 year after exposure or a spike in the antibody titer) of an antigen-specific immune response are indicated as triangles. Dashed lines indicate the limit of detection. Panel B shows memory B-cell frequencies as compared with serum antibody titers from the corresponding blood sample. Seronegative and equivocal samples (<200 EU and <5 memory B cells per 10^6 B cells) and samples from subjects undergoing an acute immune response (<1 year after an antibody spike) were excluded before data analysis in order to focus on conditions involving long-term immunologic memory. The results of linear regression analysis are shown with the associated correlation coefficients for each comparison. EBV denotes Epstein–Barr virus, and VZV varicella–zoster virus.

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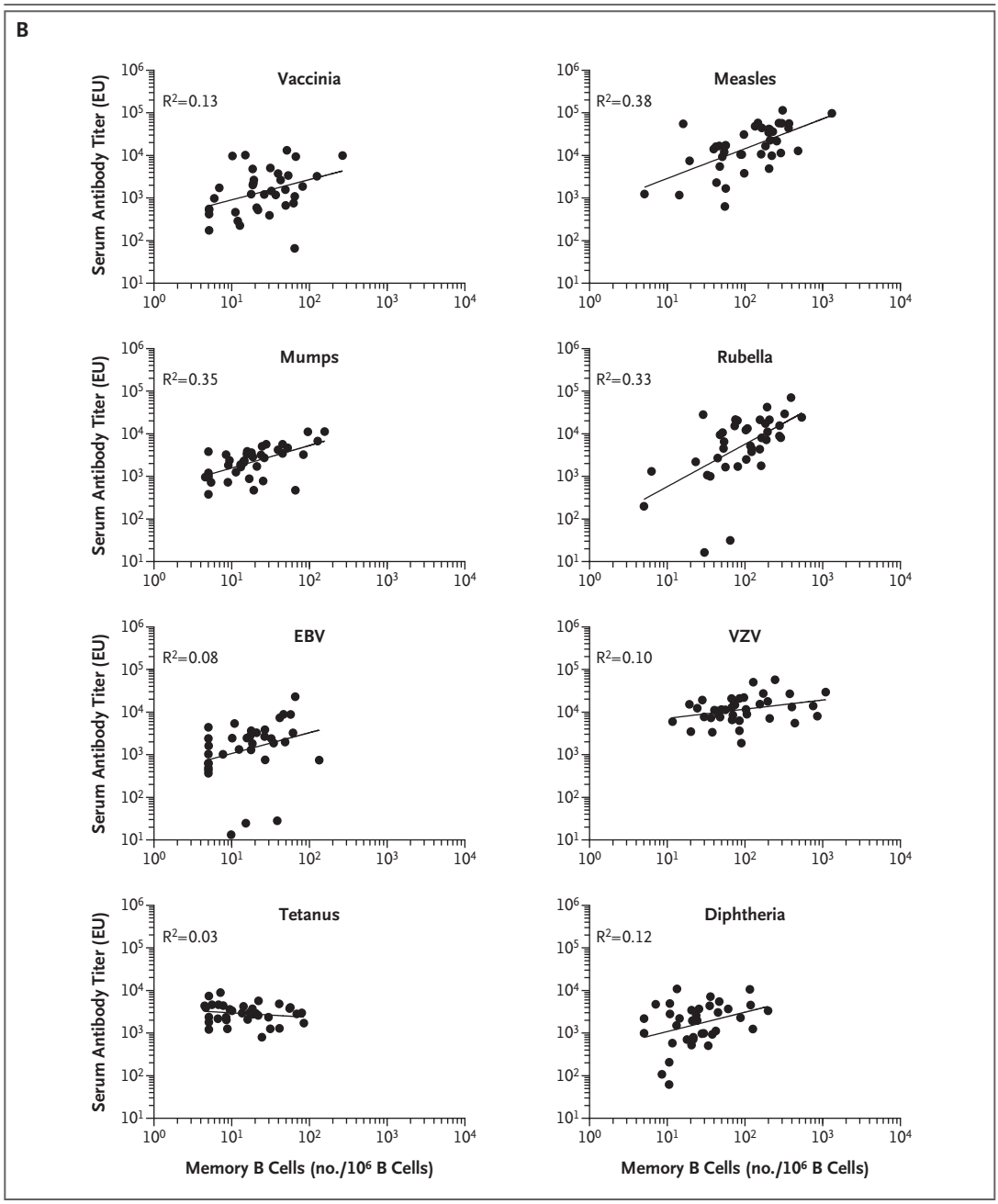

long-term humoral immunity have been proposed: memory B-cell–dependent antibody production by short-lived plasma cells and memory B-cell–independent antibody production by long-lived plasma cells.[3-10] In memory B-cell–dependent models, memory B cells divide and differentiate into plasma cells after stimulation by persistent antigen, reinfection, cross-reactive antigens, or non–antigen-specific polyclonal activation. If polyclonal memory B-cell activation is the key to antibody maintenance,[29] then the duration of antibody production should be the same, regardless of antigen specificity.

Moreover, hypotheses of memory B-cell dependence require that after each infection, a correlation between memory B cells — or at least peripheral memory B cells[29] — and antibodies be maintained. This correlation has been noted in some instances[35,36] but not in others.[37,38] We found an association between memory B-cell levels and antibody levels for measles, mumps, and rubella but not for vaccinia, varicella–zoster virus,

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Table 3. Comparison of Memory B-Cell Frequencies and Serum Antibody Titers.*

Antigen	No. of Subjects†	Regression Slope‡	P Value§	Significance Threshold¶
Measles	40	0.70±0.15	<0.001	0.006
Mumps	38	0.53±0.12	<0.001	0.007
Rubella	39	0.99±0.23	<0.001	0.008
Vaccinia‖	36	0.47±0.21	0.03	0.010
Diphtheria‖	36	0.45±0.21	0.04	0.013
VZV‖	40	0.21±0.10	0.05	0.017
EBV‖	34	0.49±0.29	0.10	0.025
Tetanus‖	35	–0.10±0.10	0.31	0.050

* Plus–minus values are estimated slope values ±SE. VZV denotes varicella–zoster virus, and EBV Epstein–Barr virus.

† Peripheral-blood mononuclear cells for the measurement of memory B-cell frequencies were available for these subjects. Samples were excluded from analysis if a subject was seronegative or had equivocal results (antibody titers <200 ELISA units and <5 memory B cells per 10^6 B cells), or if a subject was undergoing an antigen-specific, acute-phase immune response (<1 year after exposure) when the peripheral-blood mononuclear cells were acquired.

‡ Least-squares linear regression slopes were calculated to estimate the magnitude of the observed associations between memory B-cell frequencies and serum antibody levels. The magnitude of the slopes showed little consistency between antigen types, indicating overall that memory B-cell frequency was a poor predictor of serum antibody levels.

§ P values were calculated with the use of least-squares regression analysis.

¶ Since independent analyses were collectively performed to test a single hypothesis (i.e., whether memory B-cell frequencies correlate with serum antibody titers from the same blood sample), multiple-test correction was required.[34] Significance thresholds for multiple tests were calculated with the use of the Holm procedure and are based on an experimentwise α of 0.05. A significant value after a Bonferroni correction would be a P value of <0.006.

‖ For these antigens, memory B-cell frequencies were not significantly correlated with serum antibody titers as determined by means of either the Holm procedure or the Bonferroni correction.

Epstein–Barr virus, tetanus, or diphtheria (Table 3). The associations with these first three viruses may reflect an epiphenomenon in which serum antibody levels and memory B cells are equally stable but independently maintained, without representing a direct cause-and-effect relationship.

If plasma-cell maintenance is memory B-cell–dependent, then booster vaccination or reinfection may reveal whether a cause-and-effect relationship is involved, because the association will continue to be maintained. However, if memory B cells and plasma cells are independently regulated, then multiple reexposures to antigens may cause divergence between memory B-cell levels and antibody levels. In support of this second hypothesis, antigens with the highest rates of boosting through vaccination or latent viral infection coincidentally showed the weakest association between memory B-cell titers and antibody titers (Table 3). Similarly, another study showed that multiple tetanus immunizations lead to a sustained increase in memory B cells without a concomitant increase in long-term antibody titers, indicating that memory B cells and antibody production are independently regulated.[38]

Long-lived plasma cells are another mechanism for maintaining serum antibodies. The existence of long-lived plasma cells was first shown in mice,[30-32] and preliminary studies in human subjects depleted of CD20+ B cells provide further support for the long-lived plasma-cell hypothesis.[39,40]

Our cohort had seroprevalence rates of 82 to 100%, depending on the antigen being tested. All subjects had a response to at least five of the eight antigens tested; thus, no subjects had an overt inability to mount long-lasting antibody responses. Participants in this study were in good health overall, although certain persons had coexisting conditions such as high blood pressure or asthma (Table 1 in the Supplementary Appendix). Unrecognized confounders, including immune deficiencies and ablative treatments such as chemotherapy, may affect humoral immunity, resulting in more rapidly decaying antibody responses than those that we observed. Likewise, our findings are based on T-cell–dependent antibody responses, and the mechanisms involved in antibody persistence may differ for humoral immunity against T-cell–independent antigens, which is often short-lived. Greater insight into the factors that determine the duration of specific anti-

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body responses will be important for future vaccine design, as well as for determining the timing of booster vaccinations required to sustain protective levels of immunity.

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No potential conflict of interest relevant to this article was reported.

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Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Amanna IJ, Carlson NE, Slifka MK. Duration of humoral immunity to common viral and vaccine antigens. N Engl J Med 2007;357:1903-15.

Supplementary Appendix

Duration of humoral immunity to common virus and vaccine antigens

Ian J. Amanna, Nichole E. Carlson, and Mark K. Slifka.

Subject Demographics and Medical History

A total of 630 serum samples from 45 subjects were drawn for this study or retrieved from the Oregon National Primate Research Center (ONPRC) serum bank. The study began when the first subject was recruited on 10/28/02 and ended on 3/20/06 when the last blood sample was taken for analysis. Overall, our cohort showed seroprevalence rates of 42% for cytomegalovirus, 82% for Epstein-Barr virus, and 100% for varicella-Zoster virus, comparable to results from similarly aged subjects in other epidemiological studies[1, 2]. Additionally, for those subjects born prior to 1972, the year routine vaccination was ended in the U.S., 39/43 subjects (91%) were seropositive for vaccinia, similar to a prior study based on greater than 300 subjects[3]. Together, these seroprevalence rates suggest that this cohort is broadly representative of the general population. No subjects were excluded for health reasons and Supplementary Table 1 summarizes the medical history of the study population.

Supplementary Table 1. Summary of reported subject coexisting conditions*

Symptoms	Have Not	Have Now	Had In Past	Total With Condition
glaucoma	44	0	1	1 (2%)
asthma/emphysema/tuberculosis†	36	3	6	9 (20%)
heart attack/heart disease	45	0	0	0 (0%)
high blood pressure	40	4	1	5 (11%)
kidney disease/difficulty urinating‡	42	2	1	3 (7%)
stroke/paralysis	45	0	0	0 (0%)
liver disease/hepatitis/cirrhosis§	43	0	2	2 (4%)
cancer/tumors‖	41	1	3	4 (9%)
diabetes	44	1	0	1 (2%)
epilepsy/seizures	45	0	0	0 (0%)
neurologic disease (such as MS)¶	44	1	0	1 (2%)
symptoms of menopause**	15	2	7	9 (38%)
prostate disease††	20	1	0	1 (5%)
immune problems (susceptibility to infection)	45	0	0	0 (0%)
Other (describe in the space below)‡‡	41	3	1	4 (9%)

*This table summarizes the results of a comprehensive medical history questionnaire that each subject completed prior to enrollment in the study. Nine subjects (20%) listed multiple symptoms.

†Seven subjects specified asthma (*n*=4 Had In Past, *n*=3 Have Now). Two subjects did not specify (*n*=2 Had In Past).

‡Subjects listed kidney stone (*n*=1 Have Now), benign prostatic hyperplasia (*n*=1 Have Now), or unspecified (*n*=1 Had In Past).

§One subject specified hepatitis. The other subject did not specify.

‖Of the subjects who listed Had in Past, 2 specified bladder tumors and 1 subject did not specify. Another subject (Have Now) did not list cancer at the time of study (clinically asymptomatic) but succumbed to an unspecified form of cancer approximately 4 months after the last blood sample was drawn.

¶The neurological disease was not specified.

**Only female subjects ($n = 24$) were considered for this category.

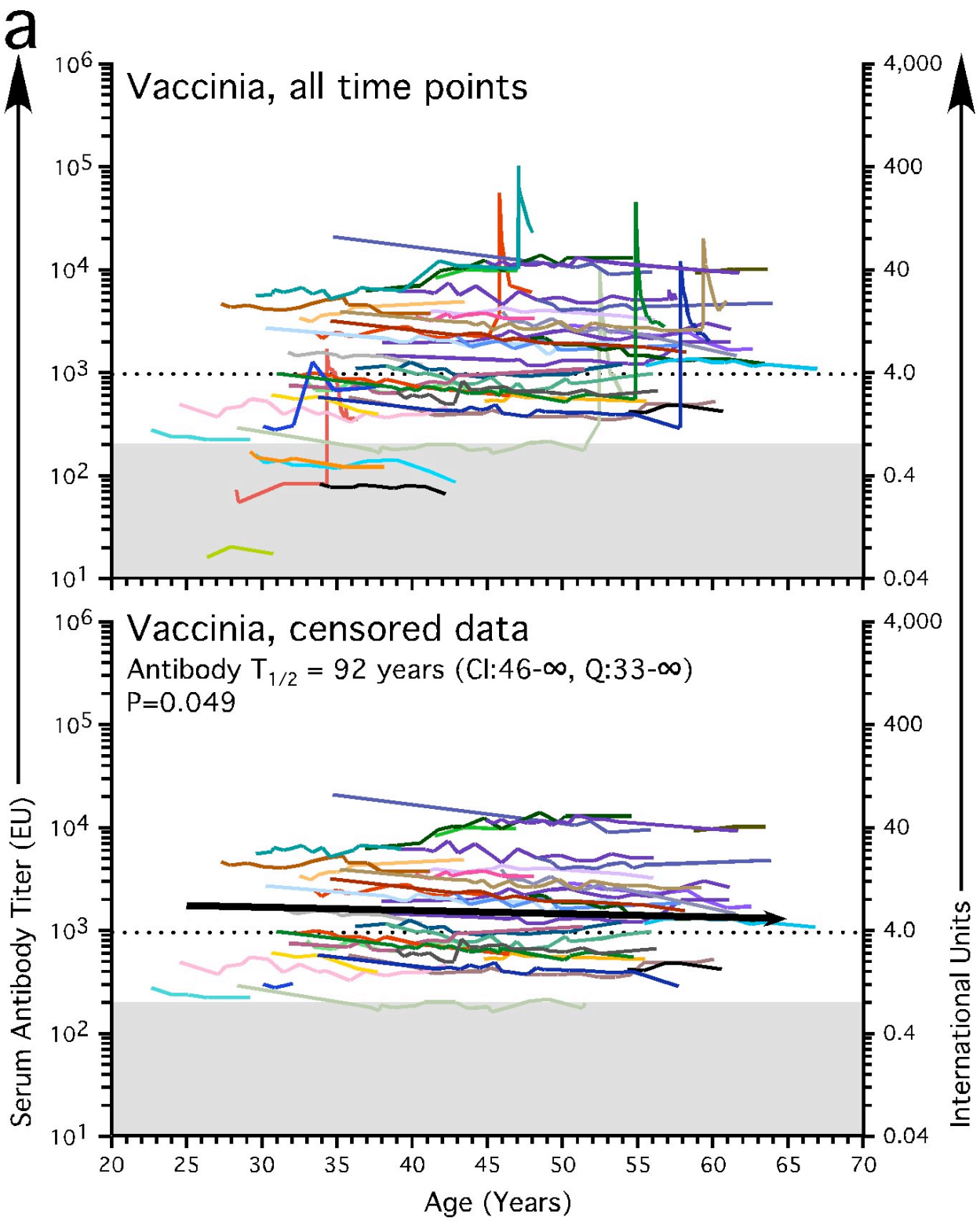
††Only male subjects ($n = 21$) were considered for this category.

‡‡Subjects listed hypoglycemia (Have Now), Crohn's disease (Have Now), ulcerative colitis (Have Now) and shingles (Had In Past).

Identification of distinct antibody responses of varying duration

Serum antibody responses to eight antigens were followed in our cohort of 45 subjects for up to 26 years. The uncensored data shown in Supplementary Figure 1 illustrates several interesting epidemiological points. For vaccinia, eight subjects were revaccinated due to occupational risk, with seven of eight subjects exhibiting antibody spikes following vaccination. To determine antibody half-life during the maintenance phase of the immune response, data was censored by removing seronegative/equivocal samples and time points at <3 years after recent serum rises of 2-fold or more. After censoring the data, vaccinia responses were found to be long-lived, with $T_{1/2}$ = 92 years. For mumps and rubella antigens, spikes in antibody titers were infrequent, suggesting that asymptomatic reboosting of immunity by circulating natural or wild type viruses is relatively rare. One notable exception was observed for measles, when four subjects unknowingly contracted a cross-reactive but uncharacterized paramyxovirus infection from exposure to diseased non-human primates during a 1999 primate center outbreak. However, after censoring the data neither measles-, mumps-, nor rubella-specific antibody showed significant decline and all responses are likely to be maintained for life. Antibody responses to two herpesviruses, Epstein-Barr virus (EBV) and Varicella Zoster virus (VZV) were also examined. Despite being a latent herpesvirus, EBV showed relatively few reactivation/reexposure events, and was maintained without significant decay. In contrast, VZV showed evidence of frequent reactivation/reexposure events (22% of subjects) and also exhibited the most rapid antibody decline of the viruses we examined ($T_{1/2}$=50 years). Finally, antibody responses against two protein antigens, tetanus and diphtheria, were also examined. As shown in Supplementary Figure 1, spikes in serum antibody responses to these vaccine antigens were frequent, as expected due to multiple booster vaccinations. In spite of frequent vaccination, the antibody half-life was significantly less than that observed for the viral antigens, suggesting that these protein antigens elicit a less durable antibody response.

Supplementary Figure 1. Antibody responses following viral infection or vaccination with non-replicating protein antigens. Longitudinal analysis of serum antibody titers was performed on 45 subjects against 8 antigens including (a) vaccinia, (b) measles, (c) mumps, (d) rubella, (e) EBV, (f) VZV, (g) tetanus, and (h) diphtheria. ELISA units (EU) are shown on the left side of each graph, with international units (IU) on the right side (if available). Estimated antibody $T_{1/2}$, 95% confidence intervals (CI), interquartile range (Q), and associated P-values were obtained using a mixed effects model of longitudinal analysis. Eight subjects were successfully vaccinated against smallpox in 2003 due to occupational risk associated with orthopoxvirus research at the ONPRC. Vaccinia infection following smallpox vaccination was confirmed by swabbing the inoculation site (data not shown) but viral load measurements were not performed for the other viral infections. There were no serious adverse events reported following smallpox vaccination. Subjects that were seronegative for EBV are each shown as a single data point of <200 EU. The shaded regions represent the cutoff between seropositive and seronegative serum titers by ELISA and the dotted lines indicate the putative protective levels of antibody, if known.


a
Vaccinia, all time points
Vaccinia, censored data
Antibody $T_{1/2}$ = 92 years (CI:46-∞, Q:33-∞)
P=0.049
Serum Antibody Titer (EU)
International Units
Age (Years)
10^6
10^5
10^4
10^3
10^2
10^1
4,000
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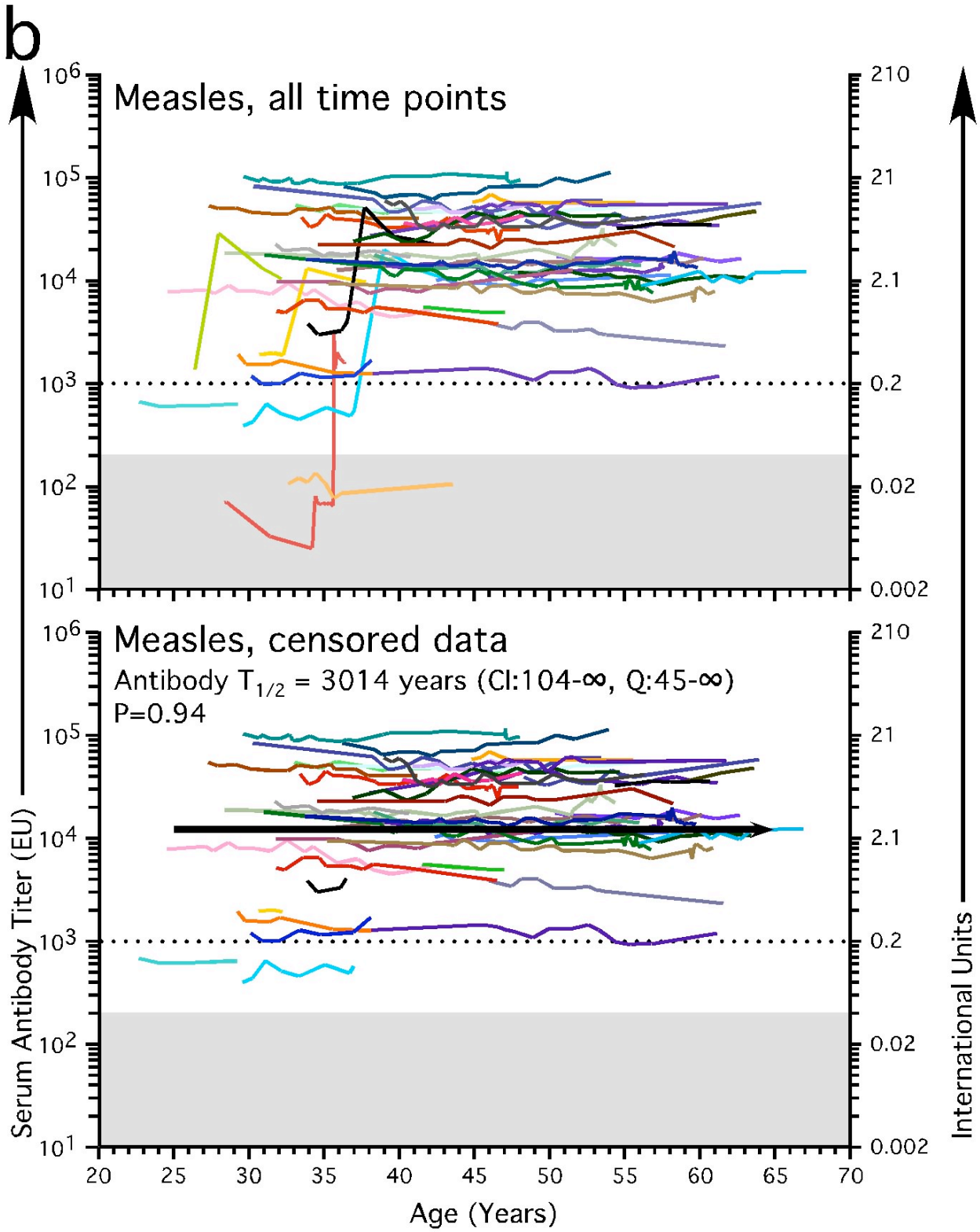
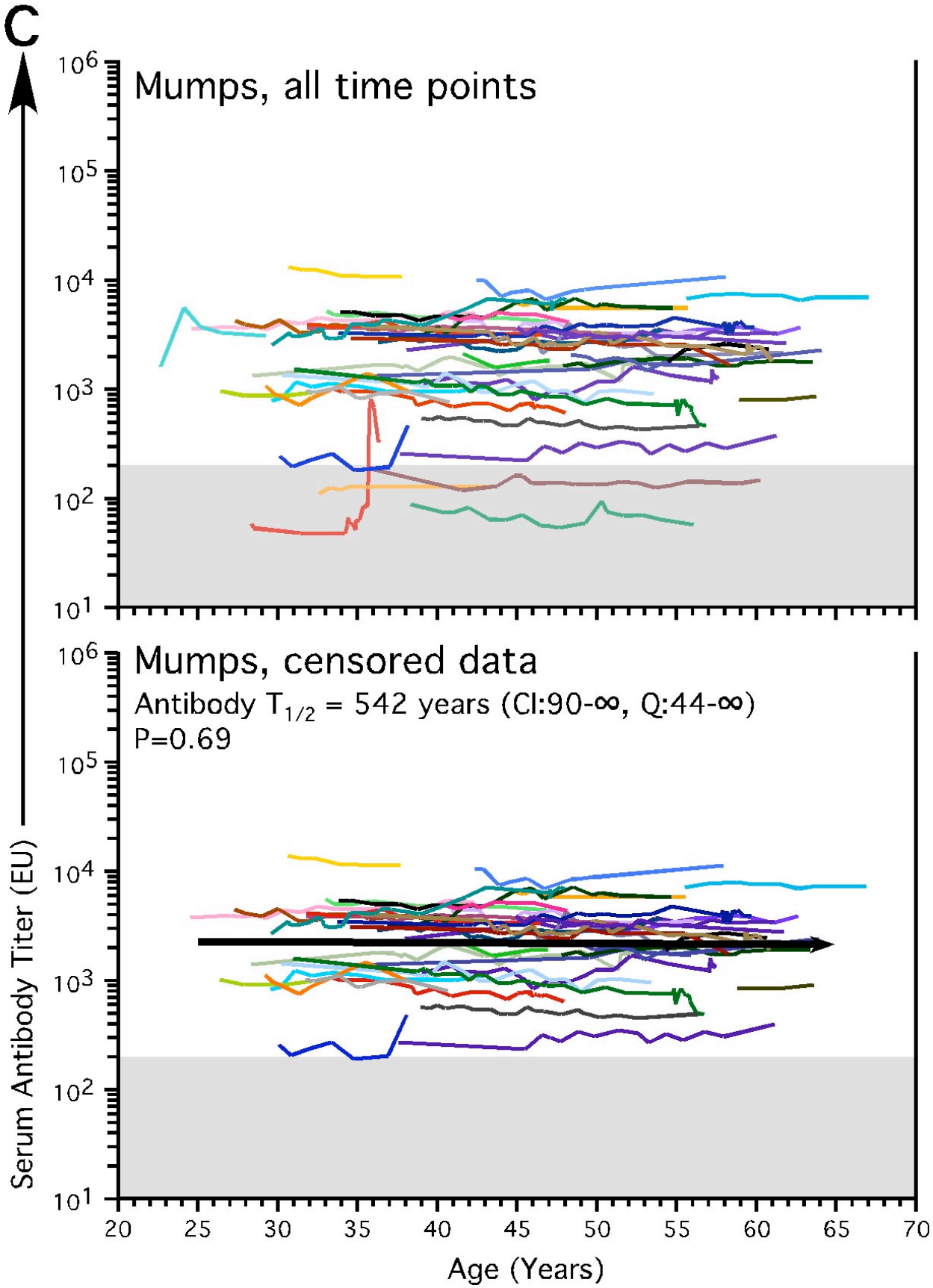
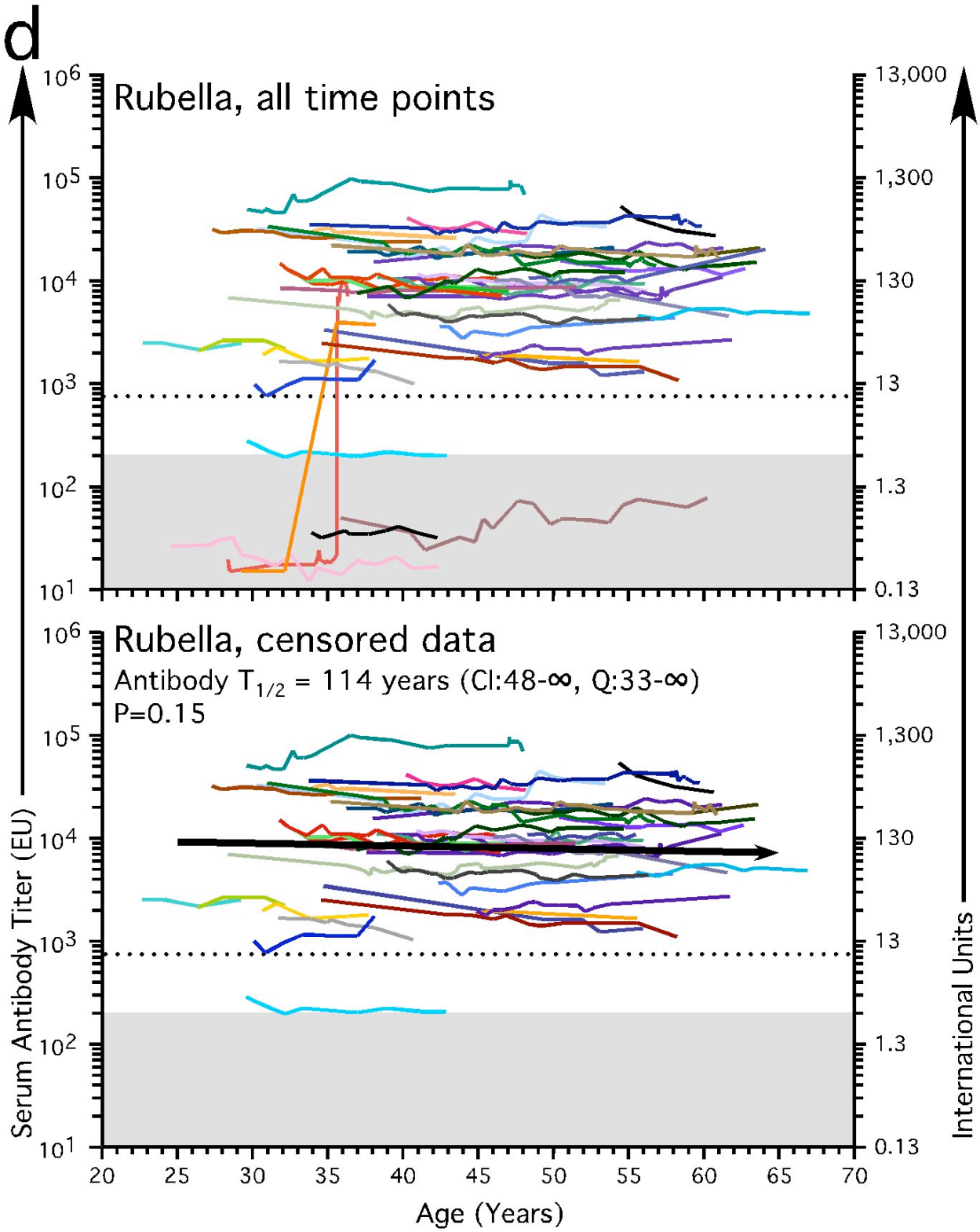
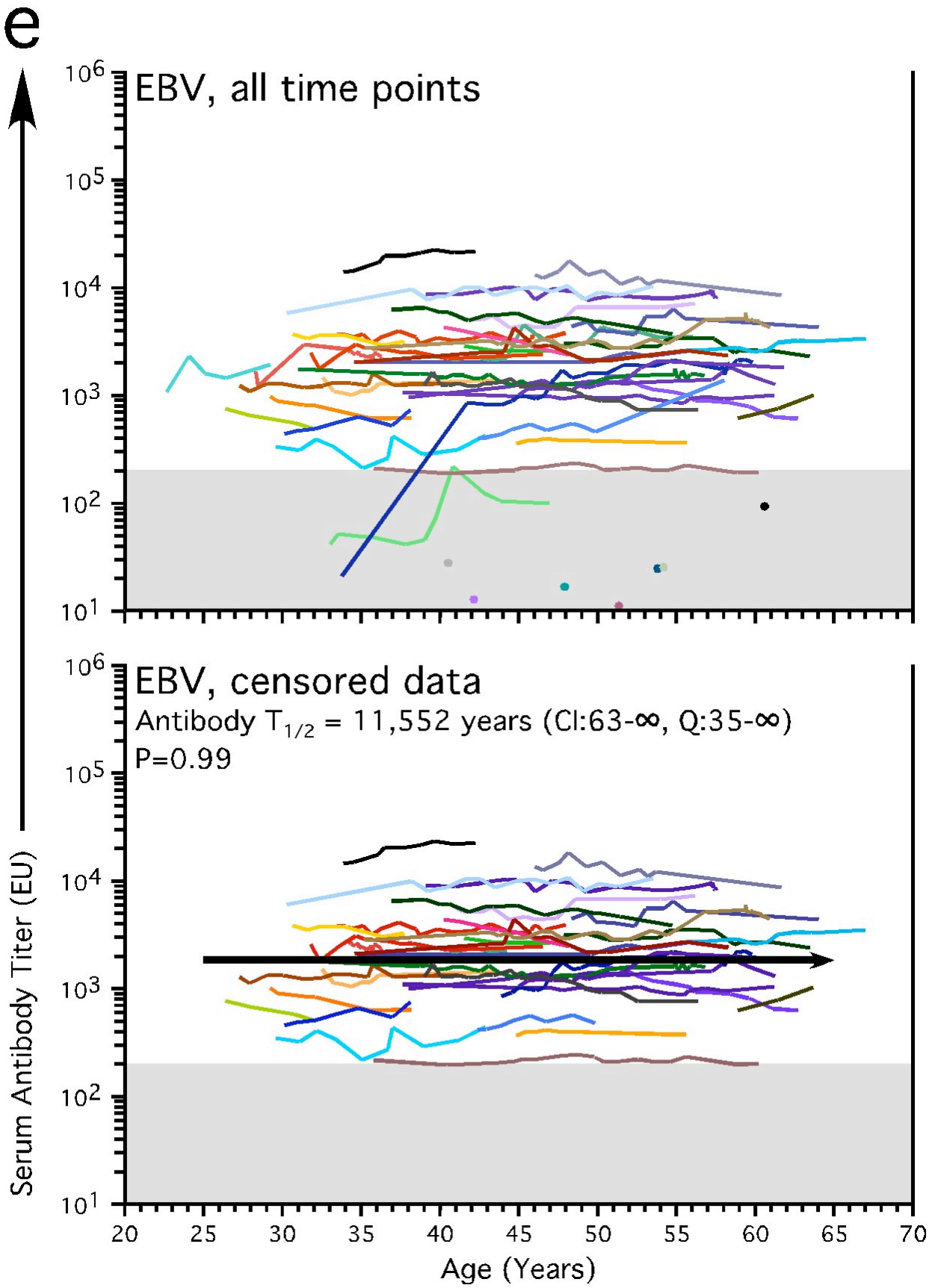
4.0
0.4
0.04
20
25
30
35
40
45
50
55
60
65
70

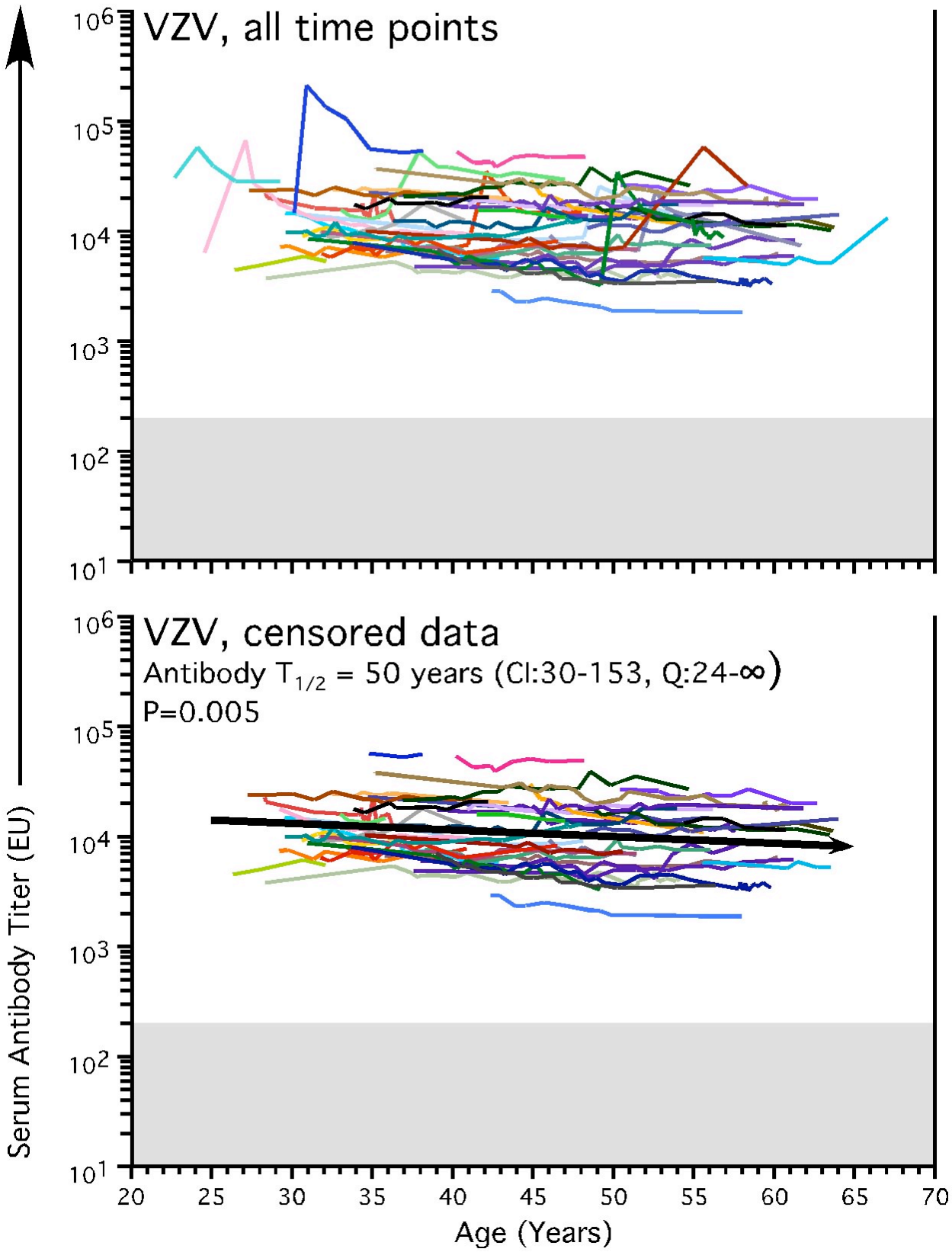

b
Measles, all time points
Measles, censored data
Antibody $T_{1/2}$ = 3014 years (CI:104-∞, Q:45-∞)
P=0.94
Serum Antibody Titer (EU)
International Units
Age (Years)
10^6
10^5
10^4
10^3
10^2
10^1
210
21
2.1
0.2
0.02
0.002
20
25
30
35
40
45
50
55
60
65
70

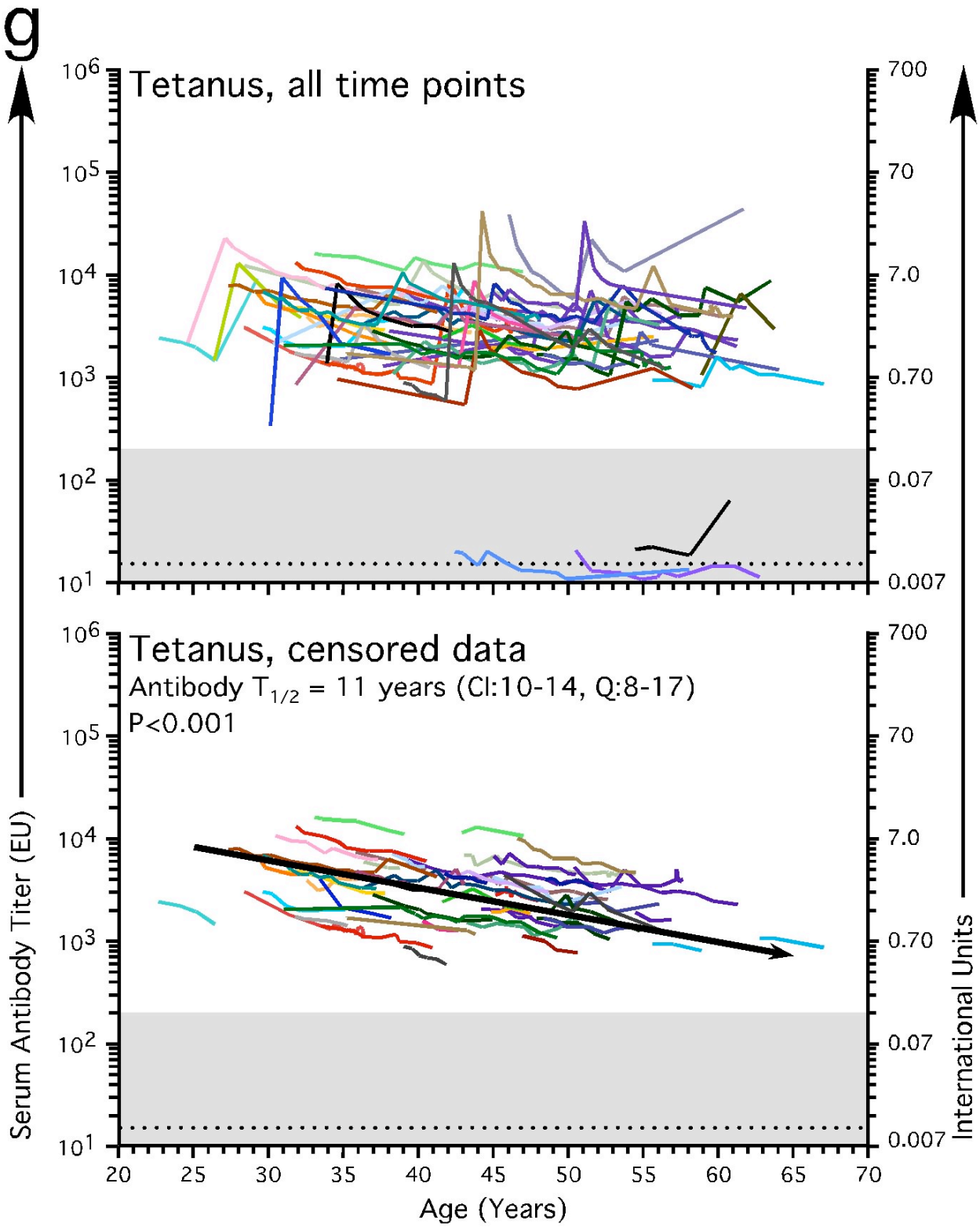

C
Mumps, all time points
Mumps, censored data
Antibody $T_{1/2}$ = 542 years (CI:90-∞, Q:44-∞)
P=0.69
Serum Antibody Titer (EU)
Age (Years)
10^1
10^2
10^3
10^4
10^5
10^6
20
25
30
35
40
45
50
55
60
65
70


d
Rubella, all time points
Rubella, censored data
Antibody $T_{1/2}$ = 114 years (CI:48-∞, Q:33-∞)
P=0.15
Serum Antibody Titer (EU)
International Units
Age (Years)
10^6
10^5
10^4
10^3
10^2
10^1
13,000
1,300
130
13
1.3
0.13
20
25
30
35
40
45
50
55
60
65
70


e
EBV, all time points
EBV, censored data
Antibody $T_{1/2}$ = 11,552 years (CI:63-∞, Q:35-∞)
P=0.99
Serum Antibody Titer (EU)
Age (Years)
10^1
10^2
10^3
10^4
10^5
10^6
20
25
30
35
40
45
50
55
60
65
70

f


VZV, all time points
VZV, censored data
Antibody $T_{1/2}$ = 50 years (CI:30-153, Q:24-∞)
P=0.005
Serum Antibody Titer (EU)
Age (Years)
10^1
10^2
10^3
10^4
10^5
10^6
20
25
30
35
40
45
50
55
60
65
70


g
Tetanus, all time points
Tetanus, censored data
Antibody $T_{1/2}$ = 11 years (CI:10-14, Q:8-17)
P<0.001
Serum Antibody Titer (EU)
International Units
Age (Years)
10^6
10^5
10^4
10^3
10^2
10^1
700
70
7.0
0.70
0.07
0.007
20
25
30
35
40
45
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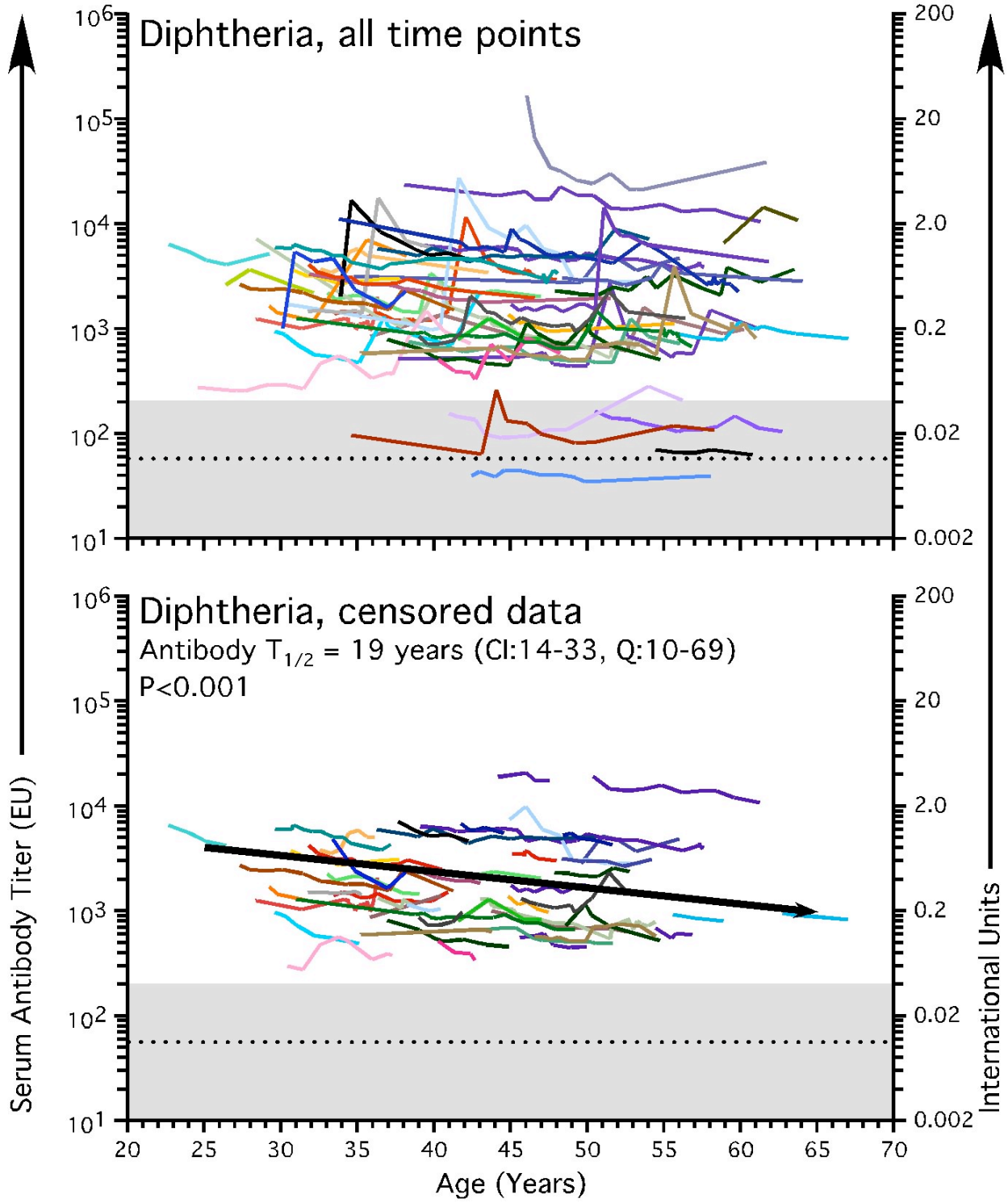
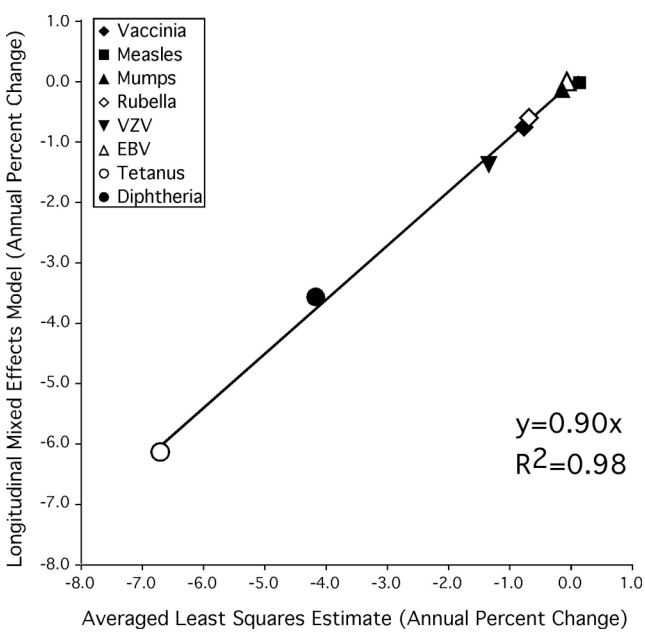
55
60
65
70

h


Diphtheria, all time points
Diphtheria, censored data
Antibody $T_{1/2}$ = 19 years (CI:14-33, Q:10-69)
P<0.001
Serum Antibody Titer (EU)
International Units
Age (Years)
10^6
10^5
10^4
10^3
10^2
10^1
200
20
2.0
0.2
0.02
0.002
20
25
30
35
40
45
50
55
60
65
70

Validation of mixed effects statistical model

In addition to the mixed effects model of longitudinal analysis ($\ln(Y_{t-1}) = \beta_0 + \beta_1 age_t + e$), we fitted a slightly different mixed effects model with the log of the ratio of time t and t-1 as the outcome ($\ln(Y_{t-1}/Y_t) = \beta_1(age_{t-1} - age_t) + e$). This is related to the model presented in the manuscript with the exception of a different error term. Both result from a single exponential decay model. The scientific conclusions from this model were the same as the model presented in the main text, which added confidence to the conclusions drawn from the model presented. Moreover, as an important check of a mixed effects model, we fitted individual models to each subject and checked that the individual specific estimates in the mixed effects model were similar to the estimates obtained from a least squares regression model fitted on each subject (Supplementary Figure 2). The average decay rate from the least squares model was similar to the population decay rate from the mixed effect model (data not shown). We also performed cross-validation to ensure that any one subject was not influencing (or causing) the presented results. To do this, we ran the full analysis repeatedly, wherein at each step one of the 45 subjects were removed from the analysis. The results from the full analysis and the analysis with each subject deleted were compared. We did not detect any outliers or influential subjects; therefore, the results are not being determined based on any one subject.

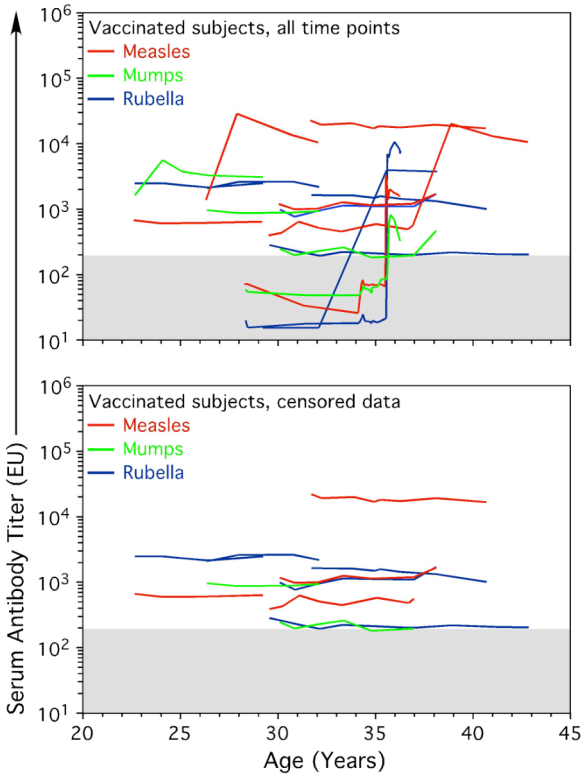


Supplementary Figure 2. The averaged least-squares estimates and longitudinal mixed effects model yield similar annual percent change for multiple antigens.
The average annual change in serum antibody levels for the cohort was determined using a longitudinal mixed effects model (see Methods) or by averaging the simple least squares slope for each individual subject following logarithmic transformation. Both analyses were performed on the same data set, which was censored as described in the Methods section prior to analysis. The resulting least squares linear equation, and associated correlation coefficient, are shown. This validation analysis illustrates that the longitudinal mixed effects model agreed closely with the simple average of the population behavior.

Analysis of vaccine-induced immunity vs. natural infection by measles, mumps, and rubella

The majority of subjects who participated in this study had contracted natural measles, mumps, and/or rubella infections. However, a subgroup of the cohort did not recall natural infection by these viruses and instead had developed antiviral immunity following vaccination. Exclusion of serum samples from vaccinated subjects did not substantially alter the estimated duration of antibody responses calculated from subjects who contracted natural infections (Supplementary Table 2). Subjects with vaccine-induced immunity against measles (n = 6), mumps (n = 4), or rubella (n = 7) are shown in Supplementary Figure 3. Following data censoring to remove equivocal samples and time points at <3 years after a serum spike of >2-fold or more, the number of subjects in each vaccinated group included n = 4 for measles, n = 2 for mumps and n = 5 for rubella. Vaccination against these viruses is achieved by infection with live attenuated virus strains and although the absolute titers of antiviral antibody are somewhat lower than that

observed after natural infection, the durability of the ensuing antibody response may be similarly long-lived. For instance, the range in estimated antibody $T_{1/2}$ = 31-∞ years for measles, 25-187 years for mumps, and 14-∞ years for rubella. However, it is important to note that this is based on a very small sample size (n = 2-5 subjects) and more studies on a larger group of vaccinated individuals will be necessary to determine statistically significant antibody half-life measurements.



Supplementary Figure 3. Subjects with live-attenuated viral vaccinations show long-term maintenance of serum antibody titers. Vaccination status for measles, mumps, or rubella viruses was determined through medical history questionnaires as well as subject birth date. These subjects recalled specific vaccination events, but reported no history of natural infection. Additionally, the birthdates of these subjects (birth date range = 1962-1974) are consistent with the time frame in which routine vaccination against these viruses was initiated and naturally occurring outbreaks decreased. One subject, previously naïve for all three viruses, received an MMR vaccination during the period of observation. A second subject, previously naïve for the rubella virus (but having contracted natural measles and mumps infections in childhood), also received the MMR vaccination during the study period. Two previously vaccinated subjects unknowingly contracted a measles cross-reactive paramyxovirus infection from exposure to diseased non-human primates during a 1999 outbreak at the ONPRC. Two other subjects showed serum rises of >2-fold in mumps-specific antibody titers, which may represent exposure to naturally occurring mumps virus. The panels show serum antibody titers (ELISA units, EU) with the shaded region representing the cutoff value between seropositive and seronegative serum titers. In the lower panel, data was censored to remove seronegative/equivocal samples and time points at <3 years after recent serum rises of 2-fold or more. The duration in observed antibody titers for all three vaccine antigens appears similar to the respective natural infections (Figure 1, main text), and considerably longer than that measured for non-replicating protein antigens such as tetanus and diphtheria (Figure 1, main text).

Supplementary Table 2. Serum antibody maintenance following natural infection.

Antigen	n	Antibody $T_{1/2}$ Total population*		n	Antibody $T_{1/2}$ Natural infection only†	
Measles	42	3014 years	CI: 104-∞ Q: 45-∞	34	2039 years	CI: 102-∞ Q: 53-∞
Mumps	40	542 years	CI: 90-∞ Q: 44-∞	36	2476 years	CI: 100-∞ Q: 45-∞
Rubella	40	114 years	CI: 48-∞ Q: 33-∞	33	108 years	CI: 47-∞ Q: 33-∞

*Antibody $T_{1/2}$ (half-life) was determined for the entire cohort population through a longitudinal mixed effects model as described in the Methods and shown in Figure 1 and Table 2 of the accompanying text. The 95% confidence intervals (CI) and interquartile range (Q), are shown.

†Antibody $T_{1/2}$ calculations were performed on the subject cohort after removing individuals who listed receiving the specified vaccinations and who did not recall contracting natural infection, or whose vaccination history was undetermined or uncertain based on the medical history questionnaire.

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