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Rapid Communication

Impact of infection or vaccination on pre-existing serological memory

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ABSTRACT

Once established, serum antibody responses against a specific pathogen may last a lifetime. We describe a cohort of four subjects who received smallpox vaccination, and a single subject who received multiple vaccinations, with antibody levels to unrelated antigens monitored for 1–3 years. These immunizations provided the opportunity to determine if infection/vaccination and the resulting toll-like receptor stimulation would alter antigen-specific serological memory to other antigens, including bacterial toxins (tetanus, diphtheria, and pertussis) and viruses (yellow fever virus, measles, mumps, rubella, Epstein-Barr virus, and varicella-zoster virus). Our results indicate that serum IgG levels are remarkably stable and infection or vaccination are unlikely to increase or decrease pre-existing antigen-specific antibody responses.

1. Introduction

Maintenance of pathogen-specific serum antibody levels following infection or vaccination is important in establishing protective immunity. Long-term maintenance of T cell-dependent serum antibody responses to viral and vaccine antigens has been previously described [1], but the mechanisms that contribute to maintaining long-term humoral immunity remain unclear and controversial [2–4]. Three primary theories on the maintenance of long-term antibody responses have emerged [2–4]. The polyclonal stimulation model [3] is based on memory B cells (which do not produce immunoglobulins directly) becoming activated continuously or intermittently through toll-like receptor (TLR) engagement or bystander T cell activation to proliferate and differentiate into antibody-secreting daughter cells, which then repopulate a declining antibody-secreting plasma cell pool. The prediction from this model is that pre-existing antibody responses to unrelated antigens will increase following a defined vaccination or infection. The plasma cell displacement theory [4–6] is based on long-lived plasma cells surviving within a niche in the bone marrow, until the plasma cells are forced out following competition with newly arriving plasma cells, resulting in their release into the circulation and eventual decline. This model predicts that pre-existing antibody responses to unrelated antigens will decrease following sequential vaccinations or infections, and that pre-existing antibody responses will demonstrate a uniform rate of decline over time. The third model [2] suggests that plasma cells and memory B cells represent independently regulated populations and that plasma cell lifespan results from imprinting at the time of induction, and not due to repopulation by memory B cells or competition for an immunological niche. The prediction from this model is that pre-existing antibody responses will remain unaltered following repeated infection or vaccination.

To determine if vaccines or microbial infections either increase or decrease pre-existing antibody levels, we followed serum antibody responses against nine or more antigens in two different settings. In one cohort, antibody responses from four individuals were monitored using 40 serum samples collected over the course of a one-year period following smallpox vaccination, representing a defined infection with vaccinia virus. In a second case study, humoral responses in an individual subject receiving multiple vaccinations were monitored using 32 serum samples collected during a 3.4-year period of time.

2. Materials and methods

2.1. Human subjects

Adult subjects provided informed written consent and completed medical history questionnaires before participation in the study. All human subject research was approved by the Institutional Review Board for OHSU.

Abbreviations: DT, diphtheria toxin; EBV, Epstein-Barr virus; EU, ELISA units; HepA, hepatitis A virus; MV, measles virus; PT, pertussis toxin; RUBV, rubella virus; TLR, toll-like receptor; TT, tetanus toxin C-fragment; Typhoid, *Salmonella typhi* Ty21a; VV, vaccinia virus; VZV, varicella-zoster virus; YFV, yellow fever virus.

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2.2. Antigen specific ELISA

Antigen-specific IgG levels and total IgG were measured by ELISA as previously described [7,8]. Antigens included vaccinia-WR (VV, prepared in-house as previously described) [7], yellow fever 17D (YFV, prepared in-house), measles-Edmonston (MV, Biodesign, Saco, ME), mumps-Enders (Biodesign), rubella-HPV77 (RUBV, Viral Antigens, Inc, Memphis, TN), varicella-zoster virus-rod (VZV, Biodesign), Epstein-Barr virus-gp125 (EBV, Viral Antigens Inc.), tetanus toxin C-fragment and diphtheria toxin (TT and DT, EMD Biosciences, San Diego, CA), and pertussis toxin (PT, List Biological Laboratories, Campbell, CA). For total IgG ELISA, affinity purified goat α-human IgG (minimally cross-reactive to bovine, horse, and mouse serum proteins, JacksonImmuno Research Laboratories, West Grove, PA) was used to coat ELISA plates at 1 μg/ml. For determining the concentration of serum IgG (mg/ml), purified human IgG (ChromePure human IgG, JacksonImmuno Research Laboratories) was included as a standard. Each ELISA plate included an internal standard/positive control sample for normalization between plates and between experiments performed on different days. Serum antibody titers of >120 ELISA units (EU) were considered seropositive.

2.3. Statistical analysis

Statistical analysis was performed by two-tailed Student's *t*-test using StatPlus; mac (AnalystSoft Inc.) for Microsoft Excel. Significance thresholds were calculated using both the Bonferroni correction and the Holm procedure [9], based on an experimentwise α of 0.05.

3. Results

3.1. Limited effect of vaccinia virus infection on pre-existing antibody responses

We had previously investigated the stability of serum antibody responses in a longitudinal setting spanning up to 25 years in individual subjects [1]. Our results indicated that antibody responses to specific antigens were well maintained, with some responses persisting indefinitely. To better understand the role that vaccination or infection might play in increasing or decreasing these pre-existing antibody responses to unrelated antigens, we examined a subset of subjects that had received a single, defined live vaccination [1]. Four previously vaccinated subjects received a booster vaccination with the live smallpox vaccine (vaccinia virus) and were monitored closely (days 7, 14, 21, 33, 63) as well as long-term (months 4, 6, 9, 12) after booster vaccination (Fig. 1A). We chose to follow immune responses after booster vaccination with vaccinia virus since this stimulates pre-existing T cell help, in addition to causing an acute infection as evidenced by the formation of a Jennerian vesicle on all four subjects. The four vaccinees showed strong vaccine-induced antibody responses, with mean vaccinia-specific IgG titers increasing from 909 EU/ml at baseline to 21,615 EU/ml at the peak of the response 14 days after vaccination (range: 8- to 80-fold increase in antibody titers). Since pre-existing antibody titers vary considerably between individuals and even between different antigens within an individual [1], each antigen-specific antibody response was normalized to 100% at baseline prior to vaccinia booster immunization. This allowed the average percent change in each individual antigen-specific antibody response to be measured and compared among the four subjects regardless of the individual variation in absolute magnitude of the ELISA titer. As shown in Fig. 1A, booster vaccination had little influence on pre-existing antibody responses to other non-vaccinia antigens such as tetanus toxoid, diphtheria toxoid, measles, mumps, rubella, Epstein-Barr virus, or varicella-zoster virus. Small fluctuations in serum antibody levels were observed, but these were not statistically significant (Table 1). Based on analysis of 30 individual ELISA titers specific for up to 8 antigens examined at each time point, there was an apparent significant increase in antibody titers at day 14 and day 33 post-vaccination ($P = 0.03$ at day 14 and 33, unadjusted for multiple testing analysis). However, these are unlikely to be biologically meaningful differences since there was no statistically significant increase or decrease in the levels of pre-existing serum antibody levels at an earlier time point (day 7, $P = 0.18$), an intermediate time point (day 21, $P = 0.07$), or at a later time point (day 63, $P = 0.75$). Indeed, after correction for multiple testing analysis, no significant trends were observed (Table 1). Overall, these observations indicate that robust vaccine-specific immune responses following smallpox vaccination have no consistent or sustained impact on pre-existing antibody levels to other heterologous antigens.

3.2. Case study results examining multiple replicating or non-replicating vaccine antigens

The data in Fig. 1A is based on humoral immune responses elicited by multiple individuals to multiple antigens following administration of one vaccine. This represents an anamnestic response with only one potential form of polyclonal stimulation (vaccinia virus infection) and we did not observe any significant changes in pre-existing serum antibody levels. It is possible that a single infection or vaccination is not enough of an antigenic or inflammatory insult to induce a measurable increase or decrease in pre-existing antibody responses to other antigens. However, one might predict that multiple vaccinations/infections would augment a potential increase or decrease in pre-existing antibody responses if serological memory is amenable to manipulation by these factors. To further examine this question, we measured the humoral immunity of one subject who received multiple vaccinations over a relatively short period of time, including a tetanus/diphtheria booster vaccination, primary smallpox and yellow fever vaccinations, and vaccinations against hepatitis A (primary and booster vaccination), polio (booster vaccination) and *Salmonella typhi* (primary vaccination administered as four oral doses given 2 days apart). These vaccinations were either recommended or required for occupational risk or for international travel. Booster vaccination against tetanus (which includes diphtheria toxoid) was administered in a hospital setting due to an accidental puncture wound. This was an unexpected event that occurred 40 days after a scheduled blood sample had been drawn. For this reason, there was no serum sample obtained immediately prior to booster vaccination and it was 12 days following tetanus/diphtheria vaccination before the next serum sample was drawn (Fig. 1B). Tetanus/diphtheria booster immunization is likely to activate antigen-specific memory T cells and this was previously associated with a transient spike in circulating antibody-secreting cells shortly after vaccination [3]. To determine if tetanus/diphtheria vaccination would result in a measurable and sustained increase or decrease in pre-existing serum antibody levels, we closely monitored antibody responses to ten virus/vaccine antigens (Fig. 1B). Following tetanus/diphtheria vaccination, antibody titers against these two antigens increased by 200–400% at day 12 post-immunization, as expected during an antigen-specific humoral immune response (Fig. 1C). In contrast, there was no clear pattern of increased or decreased antibody responses to the other individual antigens tested and the combined pre-existing antibody responses to six nonspecific antigens (pertussis toxin, measles, mumps, rubella, EBV, and VZV) remained largely unchanged, (<2% difference from baseline, Fig. 1D). Similarly, immunization with the inactivated hepatitis A or polio