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Surface plasmon resonance measurements of plasma antibody avidity during primary and secondary responses to anthrax protective antigen

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ABSTRACT

Establishment of humoral immunity against pathogens is dependent on events that occur in the germinal center and the subsequent induction of high-affinity neutralizing antibodies. Quantitative assays that allow monitoring of affinity maturation and duration of antibody responses can provide useful information regarding the efficacy of vaccines and adjuvants. Using an anthrax protective antigen (rPA) and alum model antigen/adjuvant system, we describe a methodology for monitoring antigen-specific serum antibody concentration and avidity by surface plasmon resonance during primary and secondary immune responses. Our analyses showed that following a priming dose in mice, rPA-specific antibody concentration and avidity increases over time and reaches a maximal response in about six weeks, but gradually declines in the absence of antigenic boost. Germinal center reactions were observed early with maximal development achieved during the primary response, which coincided with peak antibody avidity responses to primary immunization. Boosting with antigen resulted in a rapid increase in rPA-specific antibody concentration and five-fold increase in avidity, which was not dependent on sustained GC development. The described methodology couples surface plasmon resonance-based plasma avidity measurements with germinal center analysis and provides a novel way to monitor humoral responses that can play a role in facilitating vaccine and adjuvant development.

1. Introduction

Generation of a high-avidity antigen-specific antibody response is crucial for efficacy of many vaccines (Pulendran, 2004). Success of a vaccine is dependent on the strength and duration of elicited protective immunity. Protective humoral immunity against many pathogens is dependent on the establishment of long-lived plasma cells that secrete high-affinity antibodies, which are a result of B cell selection events that occur in germinal centers (GC) within B cell follicles of reactive lymphoid tissues (Kelsoe, 1996; Allen et al., 2007a,b; Cyster and Schwab, 2012; Victora and Nussenzweig, 2012). Development of germinal centers in response to antigens (Nieuwenhuis and Opstelten, 1984) occurs over a period of days with the formation of clusters of antigen-specific B cells that undergo proliferation and somatic hypermutation of the immunoglobulin V gene and thus give rise to memory and plasma B cells that secrete high-affinity antibodies (Eisen and Siskind, 1964; MacLennan, 1994). Within GCs, B cells undergo a selection process that involves clonal expansion, somatic

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hypermutation and class switching (Klein and Dalla-Favera, 2008). Development and maturation of high affinity antibody responses occurs in lymphoid tissue germinal centers, where high-affinity mature B cells are positively selected, proliferate, and differentiate into memory B cells or long-lived plasma cells (McHeyzer-Williams and McHeyzer-Williams, 2005). These developments within GCs give rise to memory B cells with high-avidity B cell receptors (BCR) and plasma cells that secrete high-avidity antibodies, maintain long-term antibody production, and protect the host during secondary challenge. Thus, for a vaccine that provides protective humoral immunity, it is critical to monitor the progressive development of the affinity maturation process and to quantitate the avidity of induced antibody responses following both a single or multiple (i.e. boosting) antigen immunization regimen.

The model antigen utilized in this study was recombinant *Bacillus anthracis* protective antigen (rPA), the predominant immunogenic component of the anthrax vaccine. Anthrax pathogenesis is mediated by two *B. anthracis* toxins: edema toxin and lethal toxin. Function of both toxins requires complex formation with PA. The current vaccine for anthrax, Anthrax Vaccine Adsorbed (AVA), is a cell-free filtrate of an attenuated *B. anthracis* culture adsorbed to alum. AVA contains PA as well as the other functional components of edema and lethal toxins (Friedlander et al., 2002), which may account for frequently reported adverse injection site reactions (Pittman et al., 2001; Wasserman et al., 2003; Sever et al., 2004). In addition to the occurrence of adverse reactions, anthrax vaccination traditionally required an inconvenient administration regimen of six doses over eighteen months followed by yearly boosters. A recent study in rhesus macaques, however, indicated that a 3-dose IM injection can induce sustained responses and long-term protection against inhalation anthrax (Quinn et al., 2012). Continued development of optimally effective vaccine/adjuvants and administration regimens are vital for protection from anthrax and other infectious agents.

Successful vaccination regimens result in antibody responses that are robust in both quantity and quality. Avidity is an assessment of antibody quality that is influenced by antibody valency and affinity of antibody–antigen binding. High-avidity antibody responses to vaccination, measured by traditional avidity ELISA or surface plasmon resonance (SPR), correlate with improved antibody function, as assessed by in vitro neutralizing activity (Kasturi et al., 2011; Mouquet et al., 2012) or by protection from challenge in an in vivo model (Kasturi et al., 2011). Thus, antigen-specific antibody avidity following vaccination is a critical surrogate of protection that must be monitored in experimental vaccine studies (e.g. animal models and humans).

In the present study we have demonstrated that SPR technology can be readily used to measure antibody avidity and concentration in a large number of individual (not pooled) longitudinal murine serum samples using a small sample volume (1–10 μL). By simultaneously measuring plasma antibody avidity and histologically assessing germinal center development in draining lymph nodes, we have described a methodology for the evaluation of the antigen-specific response to experimental vaccines and adjuvants.

2. Materials and methods

2.1. Immunizations and serum isolation

Groups of eighteen (18) female C57Bl/6 (National Cancer Institute/Charles River Laboratories, Wilmington, MA) mice at 8–12 weeks of age were subcutaneously immunized with saline, 5 μg recombinant anthrax protective antigen (rPA; List Biological Laboratories, Inc., Campbell, CA) alone or with 1.3 mg alum (Alhydrogel; Sigma, St. Louis, MO). On day 71 post-immunization, three mice from each group were given a boost of rPA (no adjuvant) at the same dose as the primary immunization (see Fig. 1). All animal studies were performed in accordance with approved Duke IACUC protocols in the AAALAC-certified Duke Division of Laboratory Animal Resources vivarium (Durham, NC).

Blood samples were collected from three mice from each group by submandibular puncture at indicated time points. Samples were coagulated at room temperature for 1–2 h. Serum was collected after a 10 minute centrifugation at 3000 rpm in a microcentrifuge and stored at −80 °C until analysis. All serum samples were analyzed individually and were not pooled.

Four draining lymph nodes (brachial and inguinal) were harvested from two mice from each group at the indicated time points for immunohistochemistry. Mice were sacrificed according to approved Duke IACUC protocols.

2.2. SPR binding measurements

Surface plasmon resonance binding and kinetic measurements were carried out on a BIAcore™ 3000 instrument. Data analysis was done using BIAevaluation version 4.1 software (BIAcore/GE Healthcare, Pittsburgh, PA). Assays were performed in the Duke Human Vaccine Institute Biomolecular Interaction Analysis Shared Resource Facility (Durham, NC).

CM5 sensor chip (BIAcore/GE Healthcare, Pittsburgh, PA) immobilization was carried out on the BIAcore 3000 in manual mode at a flow rate of 5 μL/min with 0.22 μM filtered, degassed, sterile 1X PBS. Using amine coupling chemistry, flow cell (Fc) 1 was activated with a 50 μL solution of N-Hydroxysuccinimide/N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide (NHS/EDC), followed by an injection of approximately 10 μL recombinant anthrax lethal factor (rLF; List Biological Laboratories) at a concentration of 10 μg/mL in 10 mM sodium acetate buffer, pH 4.5. Flow cell 2 was activated in the same way, followed by a 35 μL injection of rPA at 10 μg/mL in sodium acetate, pH 5.0. This process was repeated for flow cells 3 and 4. After immobilization, 40 μL Ethanolamine-HCl was injected over all surfaces to block any remaining active sites on the sensor chip surface. Post-ethanolamine response for all flow cells was approximately 3500 response units (RU).

A method was written in the BIAcore control software to run all monoclonal antibody and serum samples. The flow rate was set to 10 μL/min over all flow cells. For each cycle, 20 μL of sample was KINjected, optimized injection for kinetic measurements. Report points were taken 5 s before injection start to establish baseline, 15 s after injection end for an early stability measurement, and 595 s later for a late stability measurement. After 600 s of dissociation time to monitor bound complex, the flow rate was changed to 50 μL/min. A 5 μL QUICKinject of 25 mM NaOH was run to