557. Co-Administration of an Adenovirus Encoding the B Cell Stimulating Factor BAFF with Heat-Inactivated *Pseudomonas aeruginosa* Leads to Increased Anti-*pseudomonal* Humoral Immunity

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Pseudomonas aeruginosa is an important pathogen in conditions such as severe burns and cystic fibrosis, causing significant morbidity and mortality. Despite many efforts, no clinically effective vaccine against *P. aeruginosa* is available to date especially in CF patients. In order to optimize an antipseudomonal vaccine and enhance mucosal immunity, we focused our study on the enhancement of the B cell response. Stimulating B cells through B cell activating molecules during immunization against *P. aeruginosa* may increase the efficiency of a vaccine. B cell activating factor (BAFF), a 34kD single chain TNF-family member, is produced by activated antigen presenting cells and stimulates B cells. Similar to other members of the TNF family, BAFF is secreted as a trimer by cleavage of the protein from the membrane. Following binding to its receptors on B cells BAFF prolongs the life-span of activated B cells by preventing apoptosis and induces T cell independent immunoglobulin class switch *in vitro* and is crucial for B cell development and for the generation of humoral immune responses *in vivo*. Based on these findings, we hypothesized that the strong B cell stimulatory properties of BAFF can be exploited for the induction of immunity against *P. aeruginosa* and that the transient overexpression of BAFF during immunization will favor the development of a rapid, strong humoral immune response. To evaluate this concept, AdmBAFF, an E1-E3- adenovirus expressing full-length murine BAFF under control of a CMV promotor, was constructed. After infection of A549 cells with AdmBAFF, expression of full-length BAFF could be seen in the cell lysate by Western analysis, and a cleaved form of BAFF was detected in the supernatant, demonstrating that the overexpressed BAFF was properly shed from the membrane. To assess the potency of AdmBAFF to induce humoral immunity against *P. aeruginosa in vivo*, C57Bl/6 mice were injected subcutaneously with $7.5x10^{10}$ particle units of AdmBAFF together with 10^5 cfu of heat-inactivated *P. aeruginosa* strain PAO1. Mice injected with AdNull or PBS plus PAO1 served as controls. Serum binding antibodies against PAO1 were evaluated by ELISA 1, 2, 3, and 4 wk following immunization. Mice injected with PAO1 + AdmBAFF showed higher levels of PAO1-specific IgM titers 1 and 2 wk after immunization (1175 ± 506 and 304 ± 118, respectively) compared to mice immunized with PAO1 + AdNull (280 ± 105 and 73 ± 48) or PAO1 + PBS (305 ± 91 and 61 ± 17, $p<0.02$ for both comparisons at both timepoints). Similarly, higher PAO1-specific total IgG levels were observed 2, 3 and 4 wk following immunization with PAO1 + AdmBAFF (373±206, 158±35 and 164±99, respectively) compared to mice immunized with PAO1 + AdNull (79±70, 47 ± 45 and 38 ± 32) or PAO1 + PBS (63 ± 41, 30 ± 9 and 40 ± 15, $p<0.05$ for both comparisons at all 3 time points). These data indicate that coadministration of AdmBAFF and heat-inactivated *P. aeruginosa* leads to an increased humoral immune response against *P. aeruginosa*, and thus overexpression of BAFF may be useful for the development of a genetic vaccine against *P. aeruginosa*.

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558. Anti-SARS Humoral and Cellular Immunity Evoked by an Adenovirus Vector Expressing Spike Glycoprotein from SARS Coronavirus

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Severe acute respiratory syndrome (SARS) is a newly described disease caused by SARS-CoV, a novel coronavirus. Due to the limited treatment options and concern over consequences of SARS re-emergence, we have focused on using adenovirus (Ad)-based gene transfer vectors encoding SARS-CoV specific antigens to develop an effective vaccine. We tested the hypothesis that expression of the SARS-CoV spike glycoprotein (S), or its component parts S1 and S2, in E1-E3- Ad serotype 5 vectors can elicit cellular and humoral immune responses against S in immunized mice. A synthetic spike gene was made by an overlapping PCR strategy with codon optimization for mammalian cells. An Ad vector expressing S driven by a CMV promoter was constructed and protein expression was confirmed by Western analysis. To evaluate the immune responses stimulated by this vaccine, 10^{10} particle units (pu) of the vector was administered by intravenous, intramuscular or subcutaneous routes to C57Bl/6 mice and serum neutralizing antibody titers were measured in an assay assessing the neutralization of SARS-CoV *in vitro*. Subcutaneous immunization induced anti-SARS-CoV neutralizing antibodies at 2 wk (reciprocal neutralizing titer 32±15) and at 5 wk (690±470) post-administration. Mice immunized by either intramuscular or intravenous routes also developed neutralizing titers (380±96 and 450±78 respectively) by 5 wk post-administration. Immunization by all routes resulted in statistically similar titers ($p>0.3$ all pairwise comparisons). At a dose of 10^{11} pu, anti-SARS-CoV neutralizing titers were also measurable (700±160, intravenous; 130±20 intravenous; 450±210 subcutaneous). In contrast, naive mice and mice immunized with AdNull (an Ad with no transgene) had no detectable neutralizing antibody titers at any time point. Cellular immunity stimulated by immunization was evaluated in BALB/c mice injected intravenously with AdnS or AdNull at a dose of 10^{11} pu. After 2 wk, splenic CD8+ T cells were isolated and exposed to syngeneic target cells stably expressing S. Accumulation of intracellular IFNγ was observed in 8% of CD8+ cells as opposed to 2.5% of cells exposed to syngeneic cells not expressing S. Similar results were observed in C57Bl/6 mice, with 11.5% of CD8+ cells accumulating intracellular IFNγ in response to syngeneic cells expressing S, as opposed to 3.3% of CD8+ cells exposed to syngeneic cell lines not expressing S. The AdNull vaccinated controls did not stimulate antigen-specific IFNγ accumulation in the splenic CD8+ cells. To determine the location of the major cellular epitopes in the spike glycoprotein, Ad vectors and target syngeneic cell lines expressing the N-terminal domain (S1) or the C-terminal domain (S2) of spike were constructed. The specific accumulation of IFNγ in immunized BALB/c mice at 2 wk post-administration was more dependent on the S2 domain than the S1 domain (19% specific intracellular IFNγ accumulation using the S2 domain *vs* 6.3% with S1). We conclude that immunization with an Ad vaccine vector expressing the SARS-CoV S elicits high titers of SARS-CoV-neutralizing antibodies and that the S2 domain of spike contains the immunodominant CD8+ T-cell epitopes.