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Measles virus expressed *Helicobacter pylori* neutrophil-activating protein significantly enhances the immunogenicity of poor immunogens

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ABSTRACT

Helicobacter pylori neutrophil-activating protein (NAP) is a toll-like receptor 2 (TLR2) agonist and potent immunomodulator inducing Th1-type immune response. Here we present data about characterization of the humoral immune response against NAP-tagged antigens, encoded by attenuated measles virus (MV) vector platform, in MV infection susceptible type I interferon receptor knockout and human CD46 transgenic (Ifnarko-CD46Ge) mice. Immunogenicity of MV expressing a full-length human immunoglobulin lambda light chain (MV-lambda) was compared to that of MV expressing lambda-NAP chimeric protein (MV-lambda-NAP). MV-lambda-NAP immunized Ifnarko-CD46Ge mice developed significantly higher (6–20-fold) anti-lambda ELISA titers as compared to the MV-lambda-immunized control animal group, indicating that covalently-linked NAP co-expression significantly enhanced lambda immunogenicity. In contrast, ELISA titers against MV antigens were not significantly different between the animals vaccinated with MV-lambda or MV-lambda-NAP. NAP-tagged antigen expression did not affect development of protective anti-measles immunity. Both MV-lambda and MV-lambda-NAP-immunized groups showed strong virus neutralization serum titers in plaque reduction microneutralization test. These results demonstrated that MV-encoded lambda-NAP is highly immunogenic as compared to the unmodified full-length lambda chain. Boost of immune response to poor immunogens using live vectors expressing NAP-tagged chimeric antigens is an attractive approach with potential application in immunoprophylaxis of infectious diseases and cancer immunotherapy.

1. Introduction

Induction of strong and long-lasting protective immunity is the ultimate goal of vaccine development. Immunogenicity is a key characteristic of vaccine preparations related to ability of their components to elicit immune response in vaccinees. Immunogenicity could be influenced by multiple factors however, including the antigen nature (protein or polysaccharide), form (purified antigen or crude preparations) and route of administration. Many pathogens evolve to evade or confuse the immune system by expression of superantigen or masking the protective epitopes. For example, immunodominant epitopes of the human immunodeficiency virus envelope protein are located in the hypervariable regions [1,2]. Common protective hemagglutinin epitopes of influenza A viruses can remain unrecognized by immune system, because the humoral response is directed against serotype specific immunodominant epitopes [3–5]. These findings indicate the need for advances in vaccine development based on synthetic antigens or engineering of chimeric molecules carrying the epitopes with desired specificity. Purified antigens are usually weak immunogens, which require formulation with adjuvants in order to stimulate efficiently the immune response [6,7]. Because of self-tolerance, tumor associated antigens (TAA) can escape host immune surveillance. Successful anti-cancer immunization could be achieved by co-administration of TAA with strong immunoadjuvants or co-expression with Th1 cytokines by viral or bacterial vector systems [8–11]. Genetically engineered antigens can be covalently linked to T-cell epitopes that can boost immunity to poorly immunogenic antigens [12]. Anti-idiotypic vaccination against lymphomas follows a similar approach using a chemical conjugation of unique surface lymphoma immunoglobulin determinants to keyhole limpet hemocyanin as carrier protein [13].

Bacterial cells and bacterial cell wall components are potent immunostimulators and are used as components of formulated vaccines or as live vectors. *Helicobacter pylori* neutrophil-activating protein (NAP) is a key virulence factor and one of the protective

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antigens against *Helicobacter* infection [14]. NAP is a small dodecameric structure forming protein composed of 144 amino acid residues [15]. It is a homolog of *Enterobacteriaceae* bacterioferritins with iron-binding and DNA protective function. NAP acts as a toll-like receptor 2 ligand and potent Th1 response immunomodulator by induction of interleukin-12 (IL-12) and IL-23 expression [16]. NAP can reverse Th2 polarization of the immune response and reduced IgE serum level and eosinophilia in models of allergic diseases [17,18]. Local treatment with purified NAP induced T-cell infiltration, reduced vascularization and inhibited bladder cancer growth in mice [19]. It has been established that immunostimulatory properties of NAP are mediated by the C-terminus of molecule and do not require dodecamer formation [20].

The potent immunostimulatory effect and the short length of the protein make NAP an attractive transgene insert capable to boost immunogenicity of virus vector vaccines. Recently, we generate recombinant measles virus (MV) expressing secretory NAP forms based on the Edmonston vaccine strain platform [21] and developed immunoassays for detection of the NAP transgene [22]. Immunization of measles infection permissive interferon receptor type I knockout and human CD46 transgenic (Ifnarko-CD46Ge) mice with these vectors triggered strong antibody and cell-mediated anti-NAP immunity. Biological activity of MV-encoded NAP was confirmed both in vitro and in vivo. Treatment with MV strains expressing secretory NAP induced local inflammatory cytokine release and significantly improved survival in mouse models of metastatic breast cancer [23].

Here, using human lambda immunoglobulin as an antigen model we demonstrate that MV-encoded NAP-tagged chimeric antigen can induce significantly stronger immune response than the control strain expressing lambda chain alone following single immunization of MV susceptible Ifnarko-CD46Ge transgenic mice.

2. Materials and methods

2.1. Cell lines, MV strains and MV propagation

African green monkey Vero cells (ATCC) were maintained in DMEM culture medium supplemented with 10% fetal bovine serum (Invitrogen). Generation and characterization of MV-lambda and MV-lambda-NAP has been described recently [21,24]. MV-lambda expresses a full-length human immunoglobulin lambda light chain transgene introduced upstream of nucleoprotein (N protein) gene in the genome of MV Edmonston vaccine strain. In MV-lambda-NAP a major part of the variable lambda-immunoglobulin domain was substituted by NAP of *H. pylori* strain 26695 (Fig. 1). The lambda-NAP transgene expressed the constant lambda domain and the immunoglobulin leader sequence that allowed extracellular secretion of the chimeric protein. Both viruses were grown and titrated on Vero cells as previously described [21,24]. Virus titer was determined by both plaque-forming units (PFU/ml) and tissue culture infectious doses 50% ($TCID_{50}$) [21]. Purified viral stock from MV strain expressing sodium iodide symporter (MV-NIS) [25] was used as antigen in antigen-mediated ELISA. MV-NIS growth and purification procedure were performed as previously described [26].

2.2. Animal experiments

Since rodents are naturally resistant to measles (innate immunity and absence of viral receptors), Ifnarko-CD46 transgenic mice [27] are suitable small animal model for studying pathogenesis and immune response mechanisms against MV infection [28–30]. Mice were maintained in the animal facilities of Mayo Clinic, Rochester MN. The study was reviewed and approved by Mayo Foundation Institutional Animal Care and Use Committee.

2.3. Immunization and immune response in MV susceptible mice

Female 6–8-week old Ifnarko-CD46Ge mice (9–10 mice per group) were immunized with 2×10^5 PFU of MV-lambda or MV-lambda-NAP by an intraperitoneal (i.p.) route. Mice were bled before MV injection and on day 18 and 32 of the study. Serum samples were heat inactivated at 56 °C for 30 min and tested for response against human lambda immunoglobulin antigen, MV neutralization titer and total anti-MV ELISA titer. The experiment was repeated twice with Ifnarko-CD46Ge mice of different age: 10–12-week and 16-week-old. Sera were collected four weeks and six months post-immunization.

2.4. Antigen-mediated enzyme-linked immunosorbent assay (ELISA)

For human lambda chain specific antibody response tests, ELISA 96-well plates (Nunc) were coated overnight with 0.3 μg/well of human IgG lambda (Bethyl Laboratories) in carbonate-bicarbonate buffer (CBB), pH 9.6. Plates were blocked with 1% bovine serum albumin (BSA) for 1 h. Serum dilutions (2-fold or 4-fold) were added and incubated for 1 h at room temperature. Then plates were washed in phosphate-buffered saline (PBS) with 0.05% Tween 20 (PBS/T) and incubated with the secondary anti-mouse polyvalent immunoglobulin (G, A, M) horseradish peroxidase (HRP) conjugated antibody (Sigma) or secondary HRP-conjugated (human protein pre-absorbed) goat anti-mouse IgG antibody (Santa Cruz Biotechnology). Secondary antibodies were diluted 1:2000–4000 in 1% BSA in PBS/T. After 1-h incubation plates were washed 5 times in PBS/T and reaction was developed using a TMB substrate (Bethyl Laboratories).

The titer of the different IgG isotypes against human lambda chain was determined using isotype specific anti-mouse IgG1, IgG2a, IgG2b and IgG3 HRP-conjugated secondary antibodies (Santa Cruz Biotechnology).

To determine the serum titer against whole MV antigen ELISA plates were coated with 2×10^4 $TCID_{50}$ per well of heat-inactivated (60 °C/1 h) MV-NIS resuspended in CBB. After overnight incubation plates were blocked in 1% BSA and assay followed the steps described above.

Serum endpoint titers were determined as the highest dilution with readings (absorbance >0.100 or >4 × SD) above that of the control samples. Sera collected on day 0 before immunization or from age-matched non-immunized animals were used as controls.

2.5. Serum antibody avidity test

Avidity of serum antibodies against human lambda immunoglobulin was determined in antigen-mediated ELISA using 6 M urea in PBS as dissociating agent [31,32]. ELISA plates were coated with 0.3 μg/well of human IgG lambda in CBB. Serum samples collected 6 months after immunization of the mice were diluted and incubated with the antigen as described for antigen-mediated ELISA. Then plates were washed once with PBS/T and incubated for 5 min with 6 M urea in PBS or PBS alone (for control wells). HRP-conjugated anti-mouse IgG (Santa Cruz Biotechnology) was used as a secondary antibody and reaction development followed the steps described above.

2.6. Capture ELISA for measurement of lambda chain transgene expression

Vero cell monolayers were inoculated either with MV-lambda-NAP or MV-lambda at multiplicity of infection (MOI) of 1.0 in Opti-MEM medium (Invitrogen). After 4-h incubation inoculum was replaced with fresh DMEM supplemented with 2% FBS.

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