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Macrophage-derived insulin-like growth factor-1 affects influenza vaccine efficacy through the regulation of immune cell homeostasis

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ABSTRACT

The level of antibody production induced by a vaccine involves a variety of host factors. One of these, insulin-like growth factor-1 (IGF-1), plays an important role in lymphocyte maturation and antibody expression. Here, we investigated the role of macrophage-derived IGF-1 in the induction of influenza vaccine-specific antibodies using macrophage-derived IGF-1 gene knockout (MIKO) mice. The titers of vaccine-specific total immunoglobulin G (IgG) and IgG1 after immunization were about two- to fourfold lower in MIKO mice than in WT mice. Moreover, MIKO mice showed a relatively weak booster effect of repeated immunization. In contrast, antigen-nonspecific total IgG was about threefold higher in MIKO mice than in WT mice. After viral challenge, the viral titer and the pathological damage in lungs of MIKO mice were higher than those in WT mice despite vaccination. Interestingly, the proportions of proinflammatory immune cells including M1 macrophages, Th1 and Th17 cells was higher in unvaccinated MIKO mice than in unvaccinated WT mice. This suggests that nonspecific activation of immune cells may paradoxically impair the response to the vaccine. In addition, although the proportions of T follicular helper (Tfh) cells and GL-7$^+$ germinal center (GC) B cells were higher in MIKO mice than in WT mice, the population of CD138$^+$B220$^+$ antibody-secreting plasmablasts was lower in MIKO mice, which may be a cause of the low influenza-specific antibody titer in MIKO mice. Taken together, these results suggest that macrophage-derived IGF-1 might play an important role in the vaccine-triggered immune response by regulating immune cell homeostasis.

1. Introduction

Influenza virus belongs to the family *Orthomyxoviridae* and is an enveloped virus with a single-stranded negative-sense RNA segmented genome [1]. It induces respiratory illness and every year causes severe symptoms in some 3 to 5 million people and leads to 250,000 to 500,000 deaths worldwide [2]. In 1918, 50 million people died from influenza virus infection, the worst pandemic on record [3]. The available inactivated and attenuated influenza virus vaccines are an effective strategy for preventing virus infection and limiting spread of the disease [4,5]. However, the influenza virus continuously evolves: because of its segmented single-stranded RNA genome, genetic drift occurs as the result of point mutations, and genetic shift occurs as the result of reassortment of genes from two different virus strains in coinfected cells [6]. Thus, annual vaccination is important as the primary strategy for prevention of influenza virus infection [7].

Effective immunization with vaccines requires the induction of effector and memory cells in response to the vaccine [8]. These responses depend on the interactions of various immune cells. Antigen-presenting cells (APCs) such as macrophages and dendritic cells (DCs) bind to the vaccine and process it for presentation on the cell surface by major histocompatibility complex (MHC) molecules [9,10]. . The presented antigen triggers both B and T lymphocytes for antibody production and T cell responses. During these vaccine-induced immune responses, antibodies capable of specifically binding to the pathogen are produced by B lymphocytes [11], while effector cytotoxic CD8$^+$ T lymphocytes (CTL) limit virus spread by recognizing and killing infected cells. Activation, generation, and maintenance of both B and CD8$^+$ lymphocytes are assisted by diverse growth factors and cytokines produced by CD4$^+$ helper T lymphocytes (Th) and APCs [12,13]. Therefore, the maintenance and appropriate balance of different types of immune

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cells after vaccination is necessary for successful induction of vaccine-specific antibodies and CTL development.

Insulin-like growth factor-1 (IGF-1) is a 70-amino-acid peptide that is structurally similar to insulin. It is essential for cell growth and survival, and for embryonic and postnatal development [14,15]. IGF-1 is secreted by many tissues, including liver and macrophages [16,17], with the liver reported as the main source [16]. IGF-1 plays a crucial role in the cellular metabolism and function of cells of both the innate and acquired immune systems, including influences on hematopoiesis and direct effector functions through autocrine, paracrine, and systemic endocrine effects [18]. In addition, IGF-1 promotes diverse elements of bone marrow function including lymphocyte maturation [19] and can influence antibody production by plasma cells. For example, administration of IGF-1 in mice elevated the overall levels of serum antibodies [20]. Recently, we also reported that macrophage-secreted IGF-1 contributes to hyperplasia and hypertrophy in adipose tissue by increasing angiogenesis [17].

However, although IGF-1 has various known functions in biological homeostasis and immunological responses, the role of macrophage-derived IGF-1 in vaccine-induced immune responses is not clear. In this study, we investigated the role of IGF-1 in the production of vaccine-specific antibody and the activation and maintenance of vaccine-triggered immune cells using the influenza virus vaccine and macrophage-derived IGF-1 gene knockout (MIKO) mice. Interestingly, MIKO mice showed low levels of influenza vaccine-specific antibody and antibody-secreting plasmablasts, but high nonspecific T/B cell activation, and were not effectively protected against virus challenge. These results suggest that macrophage-derived IGF-1 may play an important role in the vaccine-induced immune responses of B and T cells.

2. Methods

2.1. Animals and maintenance

The WT mice were 12-week-old male C57BL/6 mice purchased from DBL Korea (Chungcheongbuk-do, Korea). The MIKO mice were provided by Dr. Anthony W. Ferrante Jr. (College of Physicians and Surgeons, Columbia, New York City, NY, USA). MIKO mouse was generated by mouse carrying a *Cre*-recombinase inserted into the *Lysozyme2* locus on the C57BL/6 J background. The detailed protocol describing MIKO mouse development was previously reported [21]. In the Catholic University of Korea, homozygous MIKO mice were generated by mating the donated heterozygous MIKO mouse pair. Mouse experiments were performed in accordance with the relevant ethical guidelines and regulations established by the Korean Association for Laboratory Animals [22]. All mice were housed in specific-pathogen-free conditions with a standard light cycle (12 h light/dark) and maintained according to protocols approved by the Institutional Animal Care and Use Committee, Sungsim Campus, Catholic University of Korea. All mice were fed a normal fat (5%) diet (Harlan Laboratories, Livermore, CA, USA) and sterile water. Four to five animals per group were used for the experiments.

2.2. Vaccination

The WT control and the MIKO mice were injected intramuscularly with two doses of 4.5 μg per mouse of the influenza virus vaccine (Sky Cell Flu Prefilled Syringe, from SK Chemical, Gyeonggi-do, Korea) given 3 weeks apart, which is quadrivalent (two influenza A strains, H1N1 and H3N2, and two influenza B strains, Yamagata and Victoria) and cell culture based vaccine. It is referred to as a subunit vaccine.

2.3. Virus infection

Each mouse was infected with 10 LD50 dose of the influenza virus (A/H1N1/California/04/09) by intranasal injection. Influenza viruses were provided by Professor Baik-lin Seong (Yonsei University, Seoul, Korea).

2.4. Serum collection

The mouse sera were collected at 0, 2, 4, 6, 8, and 10 weeks after the first vaccine injection. The sera were collected from the facial vein of mice using an 18G syringe. The samples were stored at −80 °C until use.

2.5. Indirect and sandwich enzyme-linked immunosorbent assays (ELISAs)

An indirect ELISA was performed to test for vaccine-specific antibodies in mouse sera. The influenza virus vaccine in coating buffer (200 ng/100 μl/well, BioLegend, San Diego, CA, USA) was incubated with 96-well plates overnight at 4 °C. After blocking (BioLegend), the coated plates were incubated with mouse serum samples (1:800 dilution for total IgG and 1:400 dilution for IgG1, these dilutions were determined by pre-experiments) for 2 h at room temperature. Then, plates were incubated with 100 μl/well of a 1:1000 dilution of HRP-conjugated goat anti-mouse IgG and IgG1 for 1 h at room temperature. After extensive washing, tetramethyl benzidine substrate (BD Biosciences, San Diego, CA, USA) was added at room temperature, the reaction was stopped with 2 N H_2SO_4, and absorbance was read at 450 nm. To analyze IFN-γ production by splenocytes, the splenocytes were cultured in a 48-well plate and treated with 0.5 μg per well of anti-mouse CD3 (BD Biosciences) and anti-mouse CD28 (BD Biosciences) for 48 h. The released IFN-γ in the splenocyte culture supernatant was measured using a Mouse IFN-γ ELISA Ready-SET-Go! Kit (eBioscience, San Diego, CA, USA). The IGF-1 protein in the mouse sera was measured using a Mouse IGF-1 ELISA Kit (Abcam, Cambridge, MA, USA). The total IgG antibody titer in the sera was measured using a Mouse total IgG Kit (eBioscience).

2.6. Hemagglutination inhibition (HI) test

To determine the HI units, sera taken from mice 6 weeks after the first vaccination were used. Mouse sera were incubated overnight with 3 × volume of Receptor Destroying Enzyme (Sigma-Aldrich, St. Louis, MO, USA) at 37 °C. After 1 day, the same volume of PBS was added, and sample mixtures were heat inactivated at 56 °C for 30 min. Inactivated samples were stored at 4 °C until use. Twofold serial dilutions of serum samples were incubated with influenza virus (of known hemagglutinin titer) in a 96-well V-bottom plate at 37 °C for 1 h. Then, the same volume of 1% chicken red blood cells was added, and samples were incubated at 4 °C for 30 min. The results were evaluated and calculated as HI units.

2.7. Reverse transcription quantitative PCR (RT-qPCR)

The peritoneal macrophages were isolated from the peritoneal cavity of 8-week-old WT and MIKO mice. Five milliliters of PBS was perfused into the peritoneal cavity of mice using a syringe with a 25-gauge needle and recollected together with the peritoneal macrophages. The mRNA was extracted from the peritoneal macrophages and livers of mice using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). For the cDNA template, a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA, USA) was used. To detect expression of the *IGF-1* gene, the following pri-

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