

霊長類を用いた粘膜免疫誘導型ワクチンの開発

保富 康宏 (医薬基盤研究所霊長類医科学研究センター)

Development of vaccine inducing mucosal immune responses

Yasuhiro YASUTOMI, National Institute of Biomedical Innovation

1. Matsuo K, Yasutomi Y. (2011)

***Mycobacterium Bovis* bacilli Calmette-Guérin as a vaccine vector for global infectious disease control.**

Tuberculosis Res Treat, in press.

Mycobacterium bovis bacille Calmette-Guérin (BCG) is the only available vaccine for tuberculosis (TB). Although this vaccine is effective in controlling infantile TB, BCG-induced protective effects against pulmonary diseases in adults have not been clearly demonstrated. Recombinant BCG (rBCG) technology has been extensively applied to obtain more potent immunogenicity of this vaccine and several candidate TB vaccines have currently reached human clinical trials. On the other hand, recent progress in the improvement of the BCG vector, such as the codon optimization strategy and combination with viral vector boost, allows us to utilize this bacterium in HIV vaccine development. In this article, we review recent progress in rBCG-based vaccine studies that may have implications in the development of novel vaccines for controlling global infectious diseases in the near future.

2. Mori H, Yamanaka K, Matsuo K, Yasutomi Y, Mizutani H. (2009)

Administration of Ag85B showed therapeutic effects to Th2-type cytokine-mediated acute phase atopic dermatitis by inducing regulatory T cells.

Arch Dermatol Res, 301: 151-157.

Increase in the number of patients with atopic dermatitis (AD) has been recently reported. T helper (Th) cells that infiltrate AD skin lesions are Th2-type dominant; reduced exposure to environmental Th1-cytokine-inducing microbes is believed to contribute to the increased number of AD patients. Regulatory type immune responses have been also associated with the occurrence of AD. It has been reported that antigen 85B (Ag85B) purified from mycobacteria is a potent inducer of Th1-type immune response in mice as well as in humans. In this study, we have examined the effect of plasmid DNA encoding Ag85B derived from *Mycobacterium kansasii* on AD skin lesions induced by oxazolone (OX) application. Th2-cytokine mediated mouse AD model with immediate type response followed by a late phase reaction was developed by repeated applications of low-dose OX to sensitized mice. Mice were immunized with plasmid DNA encoding cDNA of Ag85B before OX sensitization or during repeated elicitation phase. Both therapies were associated with significant suppression of immediate type response, clinical appearance, dermal cell infiltration, reduced IL-4 production, and augmented IFN-gamma mRNA expression compared to placebo-treated mice. Additionally, increased number of Foxp3(+) regulatory T cells were observed in the skin sections in Ag85B treated mice. The results of this study suggest that Ag85B DNA vaccine is a potential therapy for Th2 type dermatitis.

3. Takamura S, Matsuo K, Takebe Y, Yasutomi Y. (2005)

Ag85B of mycobacteria elicits effective CTL responses through activation of robust Th1 immunity as a novel adjuvant in DNA vaccine.

J Immunol, 175: 2541-2547.

CD4+ T cells play a crucial role in CTL generation in a DNA vaccination strategy. Several studies have demonstrated the requirement of CD4+ T cells for the induction of a sufficient immune response by coadministering DNAs. In the present study we investigated the effectiveness of Ag85B of mycobacteria, which is known to be one of the immunogenic proteins for Th1 development, as an adjuvant of a DNA vaccine. HIV gp120 DNA vaccine mixed with Ag85B DNA as an adjuvant induced HIV gp120-specific Th1 responses, as shown by delayed-type hypersensitivity, cytokine secretion, and increasing HIV-specific CTL responses. Moreover, these responses were enhanced in mice primed with *Mycobacterium bovis* bacillus Calmette-Guérin before immunization of HIV DNA vaccine mixed with Ag85B DNA. Furthermore, these immunized mice showed substantial reduction of HIV gp120-expressing recombinant vaccinia virus titers compared with the titers in other experimental mice after recombinant vaccinia virus challenge. Because most humans have been sensitized by

spontaneous infection or by vaccination with mycobacteria, these findings indicate that Ag85B is a promising adjuvant for enhancing CTL responses in a DNA vaccination strategy.

4. Kuromatsu I, Matsuo K, Takamura S, Kim G, Takebe Y, Kawamura J, Yasutomi Y. (2001)
Induction of effective antitumor immune responses by using DNA of an α Ag from mycobacteria.

Cancer Gene Ther, 8: 483-490.

One of the main objectives of cancer immunotherapy is the activation and increase in number of antitumor effector cells. Recently, genetically modified tumor cell vaccines have been proposed for elicitation of antitumor effector cells. Native alpha antigen (alpha Ag) (also known as MPT59 and antigen 85B) of mycobacteria, which cross-reacts among mycobacteria species, may play an important biological role in host-pathogen interaction because it elicits various helper T-cell type 1 immune responses. To assess the induction of antitumor immune responses by alpha Ag, mouse tumor cell lines transfected with cDNA of alpha Ag from *Mycobacterium kansasii* were established, and the possibility of producing a tumor cell vaccine for induction of antitumor effects was explored. Transfection of tumor cell lines with an alpha Ag gene lead to primary tumor rejection and the establishment of protective immunity to nontransfected original tumor cell lines in *Mycobacterium bovis* bacillus Calmette-Gurin (BCG)-primed and unprimed mice. Mice immunized with tumor cell lines transfected with the alpha Ag gene showed delayed-type hypersensitivity responses in vivo and proliferative responses together with induction of interferon-gamma of spleen cells against nontransfected wild-type tumor cell lines in in vitro experiments. Moreover, immunization of mice with alpha Ag-expressing tumor cells elicited tumor-specific and cytotoxic T lymphocyte (CTL) epitope peptide-specific CD8⁺ CTLs. The results of this study provided evidence of the potential usefulness of alpha Ag in tumor cell vaccines.

5. Kawano M, Kaito M, Kozuka Y, Komada H, Noda N, Nanba K, Tsurudome M, Ito M, Nishio M, Ito Y. (2001)

Recovery of infectious human parainfluenza type 2 virus from cDNA clones and properties of the defective virus without V-specific cysteine-rich domain.

Virology, 284: 99-112.

A full-length cDNA clone was constructed from the genome of the human parainfluenza type 2 virus (hPIV2). First, Vero cells were infected with recombinant vaccinia virus expressing T7 RNA polymerase, and then the plasmid encoding the antigenome sequence was transfected into Vero cells together with polymerase unit plasmids, NP, P, and L, which were under control of the T7 polymerase promoter. Subsequently, the transfected cells were cocultured with fresh Vero cells. Rescue of recombinant hPIV2 (rPIV2) from cDNA clone was demonstrated by finding the introduced genetic tag. As an application of reverse genetics, we introduced one nucleotide change (UCU to ACU) to immediate downstream of the RNA-editing site of the V gene in the full-length hPIV2 cDNA and were able to obtain infectious viruses [rPIV2V(-)] from the cDNA. The rPIV2V(-) possessed a defective V protein that did not have the unique cysteine-rich domain in its carboxyl terminus (the V-protein-specific domain). The rPIV2V(-) showed no growth in CV-1 and FL cells. Replication of the rPIV2V(-) in these cells, however, was partially recovered by adding anti-interferon (IFN)-beta antibody into the culture medium, showing that the rPIV2V(-) is highly sensitive against IFN and that no growth of rPIV2V(-) in CV-1 and FL cells is mainly due to its hypersensitivity to endogenously produced IFN. These findings indicate that the V-protein-specific domain of hPIV2 is related to IFN resistance. On the other hand, the rPIV2V(-) efficiently replicated in Vero cells, which are known as a IFN-non-producers. However, the virus yields of rPIV2V(-) in Vero cells were 10- to 100-fold lower than those of control rPIV2, although syntheses of the viral-specific proteins and their mRNAs in rPIV2V(-)-infected Vero cells were augmented up to 48 p.i. in comparison with those of rPIV2. Furthermore, the rPIV2V(-) virions showed anomalous in size as compared with rPIV2 virions. These results suggest that the V protein plays an important role in the hPIV2 assembly, maturation, and morphogenesis.