Extended Abstract

Annals of Clinical Trials and Vaccines Research

EVALUATION OF IMMUNE RESPONSE AGAINST LIVE RECOMBINANT MYCOBACTERIUM SMEGMATIS EXPRESSING MYCOBACTERIUM TUBERCULOSIS PROTEINS IN MICE

Hanady Amoudy

Kuwait University, College of Medicine

Abstract :

Tuberculosis remains a serious ill health liable for 8-10 million new cases and 1.3 million deaths annually. Until today, the only approved vaccine is BCG which has been used since 1921 and provides variable efficacy in adults around the world probably because it is void of some immunodominant key antigens of Mycobacterium tuberculosis. Development of non-pathogenic recombinant builds up bear Mycobacterium tuberculosis-specific proteins antigens provides the prospect to guage candidates to be included in diagnostic tools and preventive vaccines. PE35, esxA, esxB and Rv3619 are among the main antigenic proteins of tubercle bacillus with vaccine potentials. For this conclude we are introducing these tubercle bacillus genes in Mycobacterium smegmatis and evaluating the immune reaction against these recombinant constructs in mice. Cellular mediated immune response of Th1 type, characterized by production of INF-γ cytokine, is associated with protection against the infection. On the other hand, Th2 type of immune response characterized by production of IL-5, Th17 characterized by production of IL-17 and Treg characterized by production of IL-10 are associated with progressive pathological tuberculosis infection. As this, mice were vaccinate with Mycobacterium smegmatis combining constructs and their splenocytes were cultured and evaluation for various cytokines to research the sort of immune reaction provoked against the introduced antigens. Results from our experiments showed an elevated ratio of Th1:Th2 response in the case of Rv3619 only and not the others.

Introduction

Mycobacterium bovis Bacille Calmette-Guerin (BCG), a live, attenuated mycobacterial strain first utilized in humans in 1921 remains currently the sole vaccine available against tuberculosis (TB) but its protection is extremely variable. While effective against the severe sorts of the disease in children, BCG displays limited effects on adult pulmonary TB and transmission of the causative agent, tubercle bacillus (MTB) . Hence, improved vaccines against TB are desperately needed. Mycobacterium smegmatis may be a rapidly growing saprophyte, ready to propagate one generation every 1–3 h. It is non-pathogenic and commensal in humans and can act as a powerful cellular immune adjuvant . M. smegmatis also has a number of properties that renders it an effective vaccine vector. This fast-growing Mycobacterium ishelpless to capture phagolysosome maturation and cannot evade intracellular killing. Moreover, its rapid clearance by the host differs from that of M. tuberculosis or even the vaccine strain BCG M. smegmatis can activate dendritic cells and induce CD8-mediated immune responses, and immunization with recombinant M. smegmatis has been shown to get more durable memory T cells as compared to intramuscular DNA vaccination . These observations encourage further development of mycobacteria as efficient recombinant vaccine delivery vectors.

Aside from having an efficient delivery vector, the choice of an immunogenic target antigen is also important for developing a successful vaccine. The heparin-binding hemagglutinin (HBHA) may be a mycobacterial cell surface protein that mediates adhesion to epithelial cells which has been implicated within the dissemination of M. tuberculosis from the site of primary infection [11]. The lymphocytes from healthy human individuals infected with M. tuberculosis produce high levels of HBHA-specific interferon-γ (IFN-γ). Protective immunity convinced by methylated HBHA is like that afforded by vaccination with BCG, and DNA vaccination with the HBHA gene has resulted in both HBHA-specific antibodies and IFN-γ production [12], [13]. Recombinant HBHA which has no methylation produced in Escherichia coli isn't immunogenic. Methylation of HBHA is required for the complete immunological properties of the protein. It has been proved that HBHA produced in recombinant M. smegmatis (rMS) can express the immunogenic methylated sort of HBHA

Mycobacterial infections cause the activation of natural immunity , followed by the induction of the Th1 T cell subset, which is assumed to be influenced by IL-12 in an antigen-specific fashion. IL-12 may be a novel potential cytokine immunotherapy for the treatment of tubercle bacillus infection. It has been proved IL-12 cound stimulate lymphocytes to supply Th1 cytokines and enhance both innate and cellular immunity in some ways against intracellular pathogens . Okada M [18] reported that DNA vaccine expressing mycobacterial heat shock protein 65 and IL-12 exerted strong therapeutic efficacy (100% survival and augmentation of immune responses) within the TB-infected monkeys.

Methoods:

Mycobacterium smegmatis (MS) is a rapidly growing non-pathogenic environmental species that can function as a strong cellular immune adjuvant,13 with deficiency in arresting phagolysosomal maturation or evading intracellular killing in macrophages.14,15 Recombinant MS (rMS) has been demonstrated to stimulate T-lymphocyte proliferation, initiate Th1-type immune responses, promote the secretion of various cytokines, such as IFN-γ, IL-2, and IL-12, and enhance the phagocytosis and killing of invading pathogens

RESULTS:

we clearly detected a 40-kDa band that is equivalent to the sum of the molecular weight of Ag85B and ESAT6 by western blotting analysis. This suggests that the fusion protein was successfully expressed in the AE–rMS strain. Moreover, similar growth patterns between the parental and the AE–rMS strains were observed. Both strains entered the plateau phase growth at the same time and did not show significant differences in proliferation rates. Humoral immune responses were determined by measuring total IgG in sera collected from the immunized mice. The specific IgG levels in the sera of immunized mice were increased continuously along with the immune duration and reached the highest levels at week 8 after first immunization. The antibody titers of AE–rMS in AE group were significantly higher than MS group at both week 6 and 8 ($P < 0.05$).

Discussion

Recent attention has been drawn to immunotherapy against drug-resistant TB, as this type of therapy has shown the potential for improved treatment for MDR and latent TB which received relatively low cure rates by conventional therapies.22 RUTI as therapeutic vaccines reduced significantly the bacterial loads and showed effectiveness for the persistent MTB with slow replication rates and drug sensitivity.23 However, immunotherapy also brought side effects for patients who lack intact immune system, such as tissue damages and certain harmful responses involving exacerbated release of TNF-α and the activation of downstream pro-inflammatory cytokines.24 Moreover, previous studies have also indicated limited effectiveness of therapeutic adjuncts .