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Effect of repeat dose of BCG vaccination on humoral response in mice model

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BCG is the only vaccine presently available against tuberculosis but it is estimated to prevent only 5% of the all potentially vaccine-preventable deaths due to Tuberculosis. Keeping these in view the present study has been undertaken to evaluate the efficacy of BCG and the effect of repeat dose of BCG on antimycobacterial humoral response in mouse model. To improve BCG immunogenicity, specific anti-mycobacterial immune responses (anti-BCG titre and total IgG level) were evaluated in mouse model using boost immunization protocols with the BCG vaccine. Mice induced with a repeat dose of BCG showed an increased anti mycobacterial humoral response, which gradually declined few weeks after single dose of BCG administration. The results suggest improved efficacy of BCG vaccine by giving repeat dose of BCG that can enhance the level of immunoprotection against tuberculosis as opposed to a single BCG dose.

Keywords: Anti-BCG titer, BCG, Tuberculosis

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* is the second leading cause of mortality worldwide, especially in Asia and Africa[1]. Bacillus Calmette Guerin (BCG) is the only vaccine presently available for mass immunization against tuberculosis and has been used over 80 years. This live, attenuated vaccine is derived from a virulent strain of *Mycobacterium bovis*, and its protective effect has been well appreciated especially against miliary tuberculosis and tuberculous meningitis in the childhood[2]. In 1974, BCG was included in WHO's expanded program on immunization (EPI) calendar with more than three billion people receiving this vaccine. Currently; BCG vaccination covers 85% of newborn infants all over the world[3].

Despite the widespread use, the protection afforded by the currently available tuberculosis vaccine, BCG is insufficient. Various clinical trials and case controlled studies have been carried out to test the efficacy of BCG vaccine leading to often diverse and controversial results. For example, a major trial in the United Kingdom showed >75% protection[4]; however, trials in south India and Malawi demonstrated that BCG failed to protect consistently against pulmonary TB[5,6], Therefore the worldwide control of tuberculosis thus needs development of new immunization strategies.

Currently WHO recommends the use of single dose of BCG vaccine against TB, given the lack of evidence supporting the use of additional doses[3]. However there are some countries which use repeated doses of BCG, based on the assumption that the protection provided by the vaccine decreases with time. For example, Turkey gives BCG immunization four times: during infancy at two months after birth, at 6-7 years of age, at 11-12 years of age, and 16-17 years of age[5]. Hungary and Russia administer multiple doses of BCG, whereas in Thailand and Japan school children who do not develop a scar receive a second vaccine[3].

During the last few years extensive work has been done to develop a better vaccine than BCG and more than 200 candidate vaccines have been evaluated using different animal models[1]. In spite of all these efforts, no new TB vaccine has been developed as an alternative to BCG. This suggests that instead of developing several new molecules, a focus can be placed on the improvement of the current BCG vaccination protocol. The aim of our present study is to evaluate the efficacy of BCG and the effect of repeat dose of BCG on antimycobacterial humoral response in mouse model.

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Materials and Methods

Animals—Mice (Swiss Albino) 3-6 weeks old, female, inbred strains, weighing 20-25 g were obtained from Shree Farms, Bhandara, (Nagpur District), India. All the mice were housed and maintained in pathogen free conditions in the Animal House facility developed at Nagpur Veterinary College.

All the protocols for animal experimentation were approved by Institutional Animal Ethics Committee of Central India Institute of Medical Sciences (CIIMS), Nagpur.

Experimental protocol—The mice were divided in two groups. Mice (n=16) of first group were vaccinated subcutaneously at the base of the tail with 0.1 ml dose (10^5 CFU) of BCG Moscow (obtained from Serum Institute of India, Pune and stored in 4-8°C until used). A control group of mice (n=4) without BCG vaccination was also maintained. Blood samples were collected (retro-orbital route) from each mouse prior to BCG immunization and every week after the BCG immunization to study humoral response (Anti-BCG level and Total IgG) by ELISA method. After 7th week (when both IgG level and Anti-BCG titre were found to decrease) booster doses of BCG (0.05ml) were administered to each group and to see whether humoral response in groups of mice increases. Detailed experimental protocol is given in Figure 1.

Analysis of humoral response—

(a) Anti-BCG titre: An in-house developed indirect enzyme linked immunosorbent assay (ELISA) method was employed using a BCG vaccine (Bacillus Calmette Guerin strain, Serum Institute of India Ltd, Pune, India) to estimate the Anti-BCG (IgG) titer/level. Briefly, the 96-well microtiter plates (MaxisorpImmunoplate, Nalge Nunc International, Naperville) were coated with 10 ng of BCG (diluted in sterile saline). After 3 h of incubation at 37°C, the plates were washed and blocked with 0.25% BSA in phosphate buffered saline (PBS) *p*H 7.4. After 60 min of incubation plates were washed once and kept overnight at 4°C. Next day plates were incubated with mouse serum samples (1:400 diluted) in PBS. After 45 min of incubation plates were washed and incubated with rabbit anti-mouse IgG, HRP conjugate (1:10,000) for 45 min. For colour development, substrate tetramethyl benzidine in hydrogen peroxide (TMB/H_2O_2) was added and incubated for 10 min. The reaction was stopped by adding 2.5 *N* H_2SO_4 and optical density of plates was read at 450 nm.

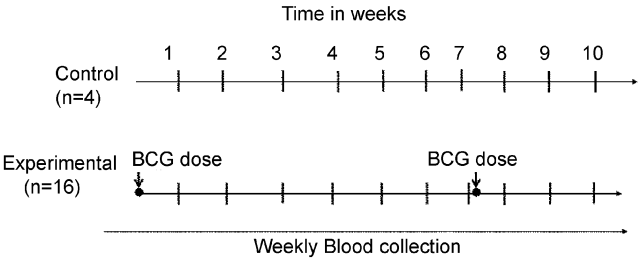

Fig. 1—Schematic representation of experimental protocol

(b) Total IgG estimation: Total IgG estimation was done using in house developed ELISA method. Briefly, the 96-well microtiter plates (MaxisorpImmunoplate, Nalge Nunc International, Naperville) were coated with samples (1:800 diluted) and incubated at 37°C for 90 min. After incubation plates were washed and blocked with 0.25% BSA in phosphate buffered saline (PBS) *p*H 7.4. After 60 min of incubation plates were washed and incubated with rabbit anti-mouse IgG, HRP conjugate (1:10,000) for 45 min. For colour development, substrate tetramethyl benzidine in hydrogen peroxide (TMB/H_2O_2) was added and incubated for 10 min. The reaction was stopped by adding 2.5 *N* H_2SO_4 and optical density of plates was read at 450 nm.

Results

An initial rise in anti-BCG titre was observed in samples each week after immunization which reached to its maximum at 4th week (Fig. 2a). After fourth week the anti-BCG titre gradually declined reaching its minimum at 7th week. However, administering booster dose of BCG on 7th week, the titre level again started to increase up till 9th week, beyond which the anti-BCG titre started to decrease.

The Total IgG level increased after immunization, but the trend observed was not as specific as compared to Anti-BCG titre; however the after few weeks decrease in IgG level was observed which again increased after administering the booster dose as in case of anti-BCG titre (Fig. 2 b).

Discussion

There is an urgent need to have an effective vaccine for the ultimate control of the current TB pandemic. WHO has estimated that more than 1 billion people will be infected with TB bacilli in the next 20 years[1]. At present BCG is the only available vaccine. During the two decades extensive work has

been done and more than 200 candidate vaccines have been evaluated using different animal models, but in spite of all these efforts not a single candidate vaccine has been developed in last 85 years[1]. Most of these new molecules being developed are based on one or a few antigens. However, being a very complex vaccine, BCG vaccine theoretically provides the immune system with more than 4000 different antigens and stimulates the immune system for prolonged periods of time. It is, therefore, not surprising that in general these novel candidate vaccines have had difficulty surpassing BCG's activity in animal models of TB[7-9].

Therefore in place of trying new molecules, study was focused on increasing immunogenicity of BCG vaccine by using boost immunization protocols with BCG in mouse model. Efficacy of BCG and effect of booster dose of BCG were evaluated on anti-mycobacterial humoral response (anti-BCG titre, total IgG estimation). The results suggest that groups of mice receiving booster doses of BCG showed rise in anti-mycobacterial humoral response which gradually declined few weeks after receiving single BCG dose.

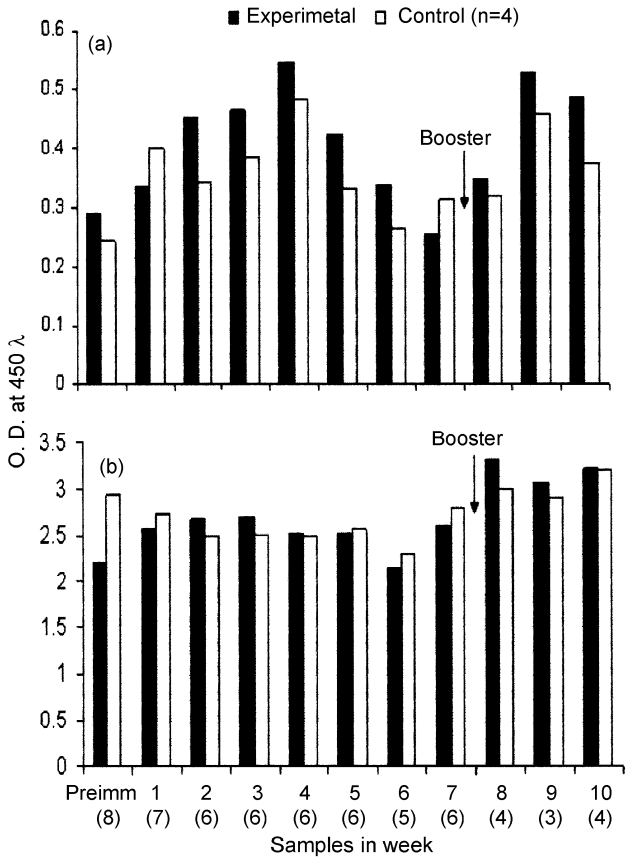

Fig. 2—Anti-BCG (a) and total IgG (b) in bloods samples of mice before vaccination and each week after vaccination and after booster dose of BCG vaccine. Figures in parentheses are no. of mice (1= 1st week, 2= 2nd week, 3= 3rd week, 4=4th week, 5= 5th week, 6=6th week, 7=7th week, 8=8th week, 9= 9th week, 10 = 10th week

Kashyap *et al.*[10] in an *in vitro* study using PBMC model have similarly shown that boosting with BCG increased specific anti-mycobacterial immune response (like Anti-BCG, IFN-g), and T cell activity-ADA, as opposed to single BCG dose and control. Theses studies indicate that efficacy of BCG increases with booster approach.

Currently only single dose of BCG vaccine is given in India that gives protection against meningeal and miliary forms of TB, but has failed to protect against pulmonary TB as reported in Chingleput trial[11]. It is postulated that efficacy of BCG vaccine may improve with booster doses. As stated earlier there are some countries where revaccination with BCG is a regular practice. Ultimately with all experimental evidences, India may start repeated immunization with BCG to reduce risk of TB in future generations.

An important question arises while discussing booster concept of BCG is whether booster dose of BCG causes TB or not. A careful perusal of literature did not reveal any such case of TB development in mouse model. Infact, Menzies *et al.*[12] have reported that repeated BCG immunization rarely causes TB. However whether revaccination with BCG causes TB or increases its efficacy can only be found out after extensive clinical trials.

Conclusion

The result of the present study in animal (mice) model suggests that repeat dose of BCG enhanced immune activation with respect to anti-BCG titre and total IgG value, suggesting that efficacy of BCG vaccine may improve if booster doses are given.

Acknowledgement

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