

P1 Differential Induction of IL-8 and MIP1-beta by BCG.
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BCG has been extensively used as a vaccine against tuberculosis, but it has shown variable levels of protection because of strain as well as host dependent differences. Macrophages present BCG antigens to T cells for effective generation of memory. Chemokines regulate the recruitment and activation of T cells and therefore may determine vaccine efficacy. Using infected human macrophage (THP1) cultures and cytokine Elisas, we demonstrate that four BCG vaccines (Pasteur, Japan, Glaxo and Copenhagen) induced an early IL-8 (2000 pg/ml up to 48 h post infection) and MIP1-beta (3200 pg/ml up to 48 h post infection) responses, which declined by day 7 post- infection. This correlated with an initial survival but later decline in the viability of intracellular organisms. In contrast, MTB strains (Erdman, H37Rv) induced a sustained IL-8 (>2000 pg/ml) and MIP1-beta (>3000 pg/ml) secretion under identical culture conditions, correlating with an increase in their number over 7 days of culture. IL-8 can recruit and activate T cells while MIP1-beta can recruit both monocytes and CD4+ T cells. Consistent with these effects, human blood macrophages infected with BCG induced more gIFN and IL-2 release from autologous T cells 24 h post infection than those infected 7 days earlier. The differential induction of T cell stimulating chemokines may therefore be one mechanism by which vaccine efficacy of BCG may be affected.

P3 A LARGE COMMUNITY TRIAL TO EVALUATE THE EFFICACY
OF A SECOND BCG DOSE IN SCHOOLCHILDREN AGAINST
TUBERCULOSIS AND LEPROSY IN BRAZIL

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Background: A second BCG dose in school aged children has been used in some countries. However, there are not in the scientific literature evidences that this intervention gives substantial additional protection against leprosy or tuberculosis. **Objective:** To estimate the efficacy of a second dose of BCG vaccination in preventing tuberculosis and leprosy in a brazilian population of schoolchildren with high coverage of neonatal BCG. **Methods:** This is a randomized controlled trial. The study population consists of school children, who belongs to 826 schools, residing in two cities: Salvador and Manaus. The schools have been allocated at random in two groups. In one the children received a dose of BCG in the other none. History of previous BCG vaccination was registered. Cases are identified through the surveillance routine system, checked against the database and reviewed to confirmation. **Results:** 122502 have received a BCG dose and a similar number are controls. Cases follow up started at the end of 1996, in Salvador and from January 1999, in Manaus. **Conclusions:** The intervention has been completed and the follow up is in course. So far, 50 cases of tuberculosis has been identified in the study population. This experience has shown that in a third world context: 1- is it possible to perform well designed large community trial 2- to associate this trial with the surveillance routine system has decreased its cost and, at the same time, has contributed to increase the quality of the system and the care of the patients.

P2 CLONING, EXPRESSION AND IMMUNE RESPONSES TO THE
65KILODALTON HEAT SHOCK PROTEIN OF M.AVIUM
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Immune responses to mycobacteria are believed to be directed towards proteins secreted intracellularly by the live organism. The 65 kilodalton heat shock protein (Hsp65) has been shown to be immunodominant in *M. leprae* and *M. tuberculosis* infections. Hsp65 is highly homologous across the mycobacterial species and also conserved in evolution, sharing upto 50% homology with the mammalian Hsp65. Heat shock proteins have been implicated in autoimmune disorders and the use of Hsp65 as an experimental vaccine depends on the identification of protective Th1 epitopes. Using *M.avium* infection in mice as our experimental model, we are examining the immune responses to Hsp65, prior to identification of Th1 epitopes.

The gene encoding Hsp65 was amplified from *M.avium* genomic DNA. The sequence shows 95-99% similarity to the sequences of the other mycobacterial species. The gene was expressed as a GST-fusion protein. Purified Hsp65 reacts with serum and stimulates proliferation of lymphocytes from *M. avium* infected Balb/c mice. Lymphocytes from mice immunised with Hsp65 in IFA proliferate and recall interferon gamma on in-vitro stimulation with the antigen.

Studies are underway to further characterise the immune response to Hsp65 in infected and immunised mice.

P4 A Nasal Vaccine Induced Better Antibody Responses when
Given as Repeated Small Doses rather than One Single
Large Dose
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A vaccine consisting of outer membrane vesicles from *Neisseria meningitidis* group B was given intranasally without any additional mucosal adjuvant to groups of mice, either as four weekly doses or as one single dose equivalent to the sum of the four. Two dose levels (100 and 1000 µg) were used, and the immunizations were repeated two months later. Both the primary and secondary IgA antibody responses in saliva and extracts of feces were significantly better (P<0.001) when the vaccine had been given in repeated small doses than after one single large dose. The corresponding serum IgG antibody responses to the primary immunizations were also better with the repeated dosage schedules, but the differences from the responses with single large doses were significant (P<0.002) only after the second immunization round. The results indicate that a good effect of nasal vaccines depend on either repeated booster immunizations, or on a formulation that will increase the time of contact with the mucosal surfaces.