Evaluation of oral vaccination against mouse-typhoid in a new animal model

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Summary. In a simple, new animal model the spread of mouse-typhoid within a mouse-colony was studied and oral vaccination against this disease was evaluated. Live vaccine was superior to inactivated vaccine.

In animal experimental studies mouse-typhoid caused by *Salmonella typhimurium* has been considered to resemble human typhoid, although the pathomechanism of the animal disease is somewhat different. Mouse-typhoid is very suitable for studying the effects of vaccination. In most studies the challenge organisms after immunization were given in heavy doses either by the parenteral route[1-4] or using the oral route[5,6]. Neither way parallels the natural infection, which occurs with small doses of infective organisms over a certain period of time. We have developed a simple animal model which comes closer to the natural route of infection. Using this model, oral vaccination against mouse-typhoid has been studied.

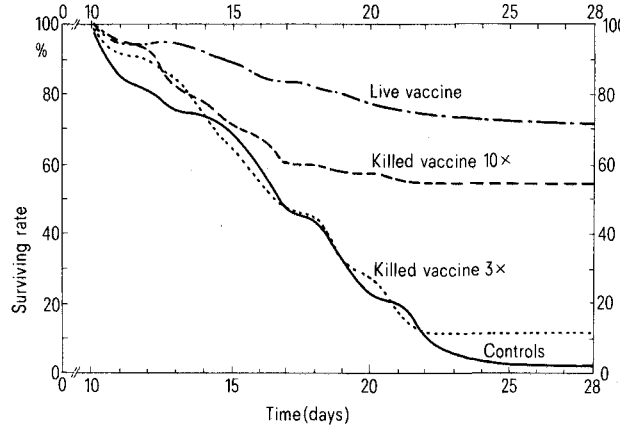
Material and methods. Animals: NMRI-mice, females, 18-22 g; Balb/C-mice, females, 16-18 g; C57BL/6J-mice, females, 12-14 g. All animals were kept under conventional conditions.

Vaccines (bacterial strains). 2 Galactose-epimerase-deficient mutants of *Salmonella typhimurium* were used: strain 10249 (kindly provided by Dr G. Schmidt, Freiburg i. Br.) and strain GE 7 (kindly supplied by Prof. R. Germanier, Bern/Switzerland). The strains were grown overnight on standard-I-nutrient agar (E. Merck, Darmstadt). The surface of each plate was washed with saline, the yield centrifuged and resuspended. Bacterial counts were performed by the pour-plate method. Killed vaccines were obtained by heating the suspension of 3 min at 100 °C[7]; 0.1 ml was streaked on agar to ensure sterility. In another series of experiments the suspension was inactivated for 1 h at 56 °C. For challenge infection strain 2816 of *S. typhimurium* was used; this was highly virulent for all the animals used (kindly provided by Dr. Baljer, Munich).

Statistical analysis. The analysis was performed with the regression model of Cox. It clarifies whether the hazard-rate for dying at the time t depends on some co-variables, e.g. different forms of vaccination[8].

Experimental procedure. Balb-C-mice of C57-mice were immunized by administering strain 10249 or strain GE 7 into the stomach using a blunt needle on a syringe. The doses are given in the table. The animals received either killed vaccine given on 3 or 10 consecutive days or live vaccine; 3 doses given at intervals of 1 week. 4 weeks after the last vaccination 20 mice were grouped together with NMIR-mice which had been newly infected with 10^{10} cells of the virulent strain of *S. typhimurium* 2816. The 2 groups were kept together for 1 week. Whenever NMRI-mice died, they were replaced to keep the number of infected animals to 10. The Balb/C or C-57-mice were observed over 28 days or until they died, after having the contact with the infected animals. In animals which died the liver and spleen were removed, homogenized and plated on endo-agar.

Results. Unvaccinated Balb/C and C-57-mice contracted mouse-typhoid, if they were placed together with NMRI-mice, which had been infected with virulent *S. typhimurium*. The mortality among the Balb/C and C-57-mice was almost 100%. If the animals were immunized with inacti-



Survival of Balb/C and C-57-black mice after immunization and subsequent exposure to *Salmonella typhimurium*. As there was no difference between the 2 strains, results from both were pooled.

Oral immunization of Balb/C respective C-57-black mice with a gal-E-mutant of *Salmonella typhimurium;* subsequent challenge through exposure to NMRI-mice in the same colony, which had been infected with 10^{10} cells of a virulent strain of *S. typhimurium*

Number	Strain (immunized animals)	Immunisation	Infected animals	Survival (immunized animals)
I. Inactivated vaccine				
20	Balb/C	-	10 NMRI	0/20
20	Balb/C	3×10^{10} cells	10 NMRI	4/20
20	Balb/C	10×10^{10} cells	10 NMRI	12/20
20	C 57	-	10 NMRI	1/20
20	C 57	3×10^{10} cells	10 NMRI	1/20
20	C 57	10×10^{10} cells	10 NMRI	10/20
II. Live vaccine				
20	Balb/C	-	10 NMRI	0/20
20	Balb/C	3×10^{4} cells	10 NMRI	15/20
20	C 57	-	10 NMRI	1/20
20	C 57	3×10^{4} cells	10 NMRI	14/20

vated vaccine no significant protection could be achieved, unless high doses (10^{10} cells) were given on 10 consecutive days. Vaccination with inactivated vaccine on 3 consecutive days yielded no success. After receiving the live vaccine a significant number of animals survived (p 0.001) (table, fig.). No difference was found in the immunogenic effect between the 2 strains of Gal E mutants of *S. typhimurium*. Regarding the inactivated vaccine there was no difference between a vaccine inactivated at 100 °C and the one inactivated at 56 °C. In the table therefore only the term inactivated vaccine is used. 10% of the Balb/C- and C-57-mice, who had died, could by examined for *S. typhimurium:* in 2/3 of these animals Salmonellae could be isolated from liver and spleen.

Discussion. Our results confirm previous findings that a live oral vaccine of *S. typhimurium* protects mice against a subsequent challenge with virulent germs[5,9–12]. Live vaccine is superior to inactivated vaccine, unless the latter one is given in high doses on 10 consecutive days[4,6]. Our animal model is a simple method to study the spread of infections among a given population. Vaccinations can be tested under more natural conditions. The individual animal is not submitted to a single heavy dose of the challenging agent but exposed continuously to the infective agent. This model has been applied with equally good results to other infections among rabbit (rabbit-pox) and mice (mouse-pox)[13].

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Suppression of PHA-induced lymphocyte blastogenesis by concomitant presence of PPD in the culture[1]

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Summary. PHA-induced lymphocyte blastogenic response was remarkably suppressed by the simultaneous presence of PPD in cultures of lymphocytes derived from individuals sensitized to PPD, but not affected by the presence of PPD when the cultures contained lymphocytes from an individual not sensitized to the protein. This double stimulation blastogenesis study with PHA and a specific antigen is feasible as a simple and rapid test to measure cell-mediated immunity to the antigen.

It has recently been demonstrated[2,3] that suppressor cells are generated during in vitro culture of peripheral mononuclear cells (PMC) with purified tuberculoprotein derivative (PPD), leading to a marked regulation of the final expression of PPD-induced blastogenic response in the culture. The generation of such PPD-induced suppressor cells is limited to cultures employing PMC derived from individuals sensitized to the protein. Dose-dependent elevation of suppressor activity following PPD stimulation is in parallel to the blastogenic response to PPD in the same culture. Our previous work revealed[2] that suppressor cell generation in PPD-stimulated PMC cultures is measurable very early – within 40 h, or even after 24 h of incubation; and that suppressor cells once generated act nonspecifically to reduce the blastogenic response of PMC to phytohemagglutinin-P (PHA) as well as PPD. It was therefore naturally expected that DNA synthesis in a PHA-driven PMC culture would be considerably affected by the concomitant presence of PPD in the cultures. Such antigen-activated suppressor activity in the PPD and PHA double stimulation cultures would be most evident when DNA synthesis was measured at day 3 of culture, considering maximal blastogenic response to PHA, and in contrast, the negligible contribution of PPD-induced DNA synthesis to the total response in the cultures at this time. The present study was undertaken to confirm the early inhibitory effects exerted by the concomitant presence of PPD (specific antigen) on PHA-induced lymphocyte blastogenic response. If such a double stimulation assay can detect the early generation of suppressor cells associated with antigen specific lymphocyte responses, it might offer another simple and rapid test to measure cell-mediated immunity.

Materials and methods. 3 subjects who were differently sensitized to PPD, as assessed by tuberculin skin reaction, were examined for the present study. Subject A and subject B showed strong and weak skin test responses, respectively, whereas subject C gave a negative response. Conventional in vitro blastogenesis studies using an optimal concentration of PPD (2.5 µg/ml) as described previously[2] gave stimulation indices of 24.0, 3.5 and 0.9 for subjects A, B and C, respectively, at day 7 of culture. PMC were separated on Ficoll-Paque (Pharmacia) from heparinized blood and cultured in F-10 culture medium (GIBCO) supplemented with 10% fetal calf serum (GIBCO) at 37 °C in 5% CO_2 in moist air. The PMC were cultured in 6–8 multiplicates in microculture plates (Falcon), each well containing 8×10^4 cells in 0.2 ml of culture medium with or without mitogens. As mitogens, PPD (Parke-Davis) and PHA (Difco) were used. Optimal dose levels for maximal blastogenic response in the present culture system were 2.5 µg/ml for PPD and a 1:100 dilution for PHA[2]. For the double-stimulation studies, various doses of PPD ranging 0.0025 µg/ml to 2.5 µg/ml and a constant suboptimal dose of PHA at a final dilution 1:1000 were used as shown in table 1. The advantage of using a suboptimal dose of PHA to appreciate minimal generation of suppressor activity in PMC cultures was described previously[4]. PHA was added to the cultures simultaneously with PPD, or 20 h after addition of PPD. In every case, DNA synthesis was measured exactly 72 h after the addition of PHA. The cells were pulsed with ^{125}I-