J. Hyg., Camb. (1975), **75**, 215
Printed in Great Britain

Experimental model of oral antityphoid vaccination with live streptomycin-dependent *Salmonella typhimurium* in C57BL/6 mice

By I. R. VLADOIANU* and F. DUBINI
Institute of Pharmacology, University of Milan, Medical School, Milan, Italy

(*Received* 12 *February* 1975)

SUMMARY

The present experimental model offered the opportunity to study particular aspects concerning the avirulence stability of live streptomycin-dependent (Sm D) *Salmonella* vaccines, under conditions resembling human enteric fever. The results seem to indicate that, in practice, the risk of reversion to a virulent form during oral antityphoid vaccination with Sm D strains remains slight even after the interruption of concomitant streptomycin administration.

INTRODUCTION

In a previous paper (Vladoianu, Dubini & Bolloli, 1975) we studied the recovery of virulence by means of streptomycin-independent (Sm I) revertants of a live streptomycin-dependent (Sm D) *S. typhimurium* vaccine in CD-1 mice. The results of this study suggest that during oral antityphoid vaccination with Sm D strains, favourable conditions for the removal of the risk of reversion to virulence seem to exist. This is due to the presence of streptomycin concomitantly administered with the Sm D strains.

However, one problem still remains: if a revertant selection would have been hindered during oral immunization (by the presence of the antibiotic in the bowel), it should have become possible at the end of the immunization period, i.e. when the antibiotic administration ceases and large numbers of Sm D cells find conditions similar to those which *in vitro* favour the process of recovery of virulence (Vladoianu, Dubini & Bolloli, 1975). In order to tackle this problem, an adequate experimental model of oral antityphoid vaccination in mice was necessary. Unfortunately, the CD-1 mice proved highly resistant to experimental oral infection and consequently were not suitable for this purpose. In the hope of finding a favourable solution to this problem, we then tested the behaviour of other strains of mice towards oral infection with *S. typhimurium*. It was observed that C57BL/6 mice were very susceptible, which enabled us to attempt the desired experiment in these mice. This investigation forms the basis of the present paper.

* Present address: Institute of Medical Microbiology, University of Geneva, 22, Quai E. Ansermet, 1211 Geneva 4, Switzerland.

MATERIALS AND METHODS

Organisms

(1) The *S. tym* 779 C–Sm D strain employed was the same as that used in the preceding paper (Vladoianu, Dubini & Bolloli, 1975), namely, a one-step streptomycin-dependent mutant derived from a virulent (oral LD 50, 3×10^3) streptomycin-sensitive *S. typhimurium* strain. This mutant grows abundantly in the presence of streptomycin; it requires at least 50 μg. of streptomycin-base/ml. of medium (Difco tryptose-agar) for growth and tolerates at least 20,000 μg. of antibiotic/ml. of medium. The frequency of reversion to streptomycin-independence (on plain Difco tryptose-agar) of this strain was $2{\cdot}8 \times 10^{-8}$.

(2) *S. tym* 779 C–Sm I revertants with partial recovery of virulence were obtained under conditions described earlier (Vladoianu *et al.* 1975). The minimum lethal dose by oral route, in C57BL/6 mice, of these mutants was about 10^7 organisms.

Experimental animals

Female specific pathogen-free C57BL/6 mice (weighing 18 g.) were obtained from Charles River Breeding Laboratories and maintained under the conditions described earlier (Vladoianu *et al.* 1975).

Experimental model of oral antityphoid vaccination

A lot of 20 C57BL/6 mice was used. Each animal received orally 5 separate doses (0·5 ml.) of about 10^9 *S. tym* 779 C–Sm D organisms/dose, at intervals of 48 hr. Each dose was introduced directly into the stomach by means of a bent metal tube attached to a 2 ml. tuberculin syringe. The vaccine was harvested from 18 hr. cultures at 37° C. on Difco tryptose-agar containing 1000 μg. of streptomycin/ml. After turbidity determination, an appropriate dilution was made in saline to obtain the desired vaccine dose, and the number of viable cells was always controlled by a pour plate technique. During the whole period of vaccination and 36 hr. both before and after this period (11 days in all) animals received streptomycin permanently in the drinking water (10 mg./ml.).

In order to follow up the growth of *S. tym* 779 C–Sm D organisms in the bowel of vaccinated animals and to detect an eventual selection of Sm I revertants *in vivo* under vaccination conditions, daily quantitative faeces cultures were performed for 20 days beginning 24 hr. before drug administration in the drinking water. To obtain faeces for culture each mouse was immobilized, with the left hand, in a vertical position head up until a fresh pellet was excreted into a small sterile container of known weight. The pellets of faeces from 10 mice were collected in the same container so that finally two pools of faeces were obtained. A 0·1 g./ml. suspension in saline of each pool was made. The number of living organisms was determined by inoculating 0·1 ml. of the faeces suspensions and of each of their serial 10-fold dilutions on Difco EMB-agar and Difco SS-agar both with and without 1000 μg. streptomycin/ml. The identity of the developed colonies was verified as *S. typhimurium* by slide agglutination, using specific antisera.

Fifteen days after the last culture, the mice were killed and their spleens removed and cultured under the conditions described earlier (Vladoianu *et al.* 1975).

RESULTS

Twenty-four hours after the beginning of streptomycin administration in drinking water, faeces cultures failed to reveal the normal intestinal flora of mice. On plain media, however, the normal flora reappeared 24 hr. after the beginning of oral administration of the vaccine strain. Only the Sm D strain grew, at a concentration of about 10^9 cells/g. of faeces, on media containing streptomycin. During the remaining days of oral administration of vaccine, colonies of the normal intestinal flora appeared in progressively larger numbers on EMB agar with or without streptomycin, while the Sm D strain continued to appear, at the same concentration as before, on the two media containing streptomycin. On the final day of oral vaccine administration, colonies of the normal intestinal flora also appeared on SS agar with streptomycin. After the administration of streptomycin in drinking water ceased, the numbers of Sm D organisms/g. of faeces diminished gradually, and by the sixth day none were isolated.

It should be emphasized that similar results were obtained from the two pools of faeces examined.

At the beginning of oral administration of vaccine, Sm I revertants were twice detected in numbers similar to those already observed *in vitro*, i.e. 2 colonies per 10^8 dependent cells. After the administration of streptomycin ceased, no Sm I revertants were detected.

During the whole period of oral vaccination the mice showed no symptoms. At post-mortem examination *S. typhimurium* was not isolated from the spleens of vaccinated mice.

DISCUSSION

After a short interval, having noticed the increased susceptibility of the C57BL/6 mice to oral *S. typhimurium* infection, we read the publication of Collins (1972) showing that specific pathogen-free mice of the C57BL strain are much more sensitive than CD-1 mice to challenge by *S. enteritidis*. This author feels that the increased susceptibility of the C57BL mice to oral *S. enteritidis* infection permits the development of a laboratory test model more closely resembling human enteric fever than heretofore. Our observations concerning the behaviour of C57BL/6 mice towards oral infection with *S. typhimurium* are completely in agreement with this assessment.

Within the framework of our experimental model developed in this paper, it is worth emphasizing the fact that even after the interruption of streptomycin administration, the Sm I revertants could not be detected in any instance. This correlates with the absence of pathological reactions observed under similar circumstances in vaccinated volunteers (Dupont *et al.* 1970).

It seems that in this system a real danger would result only if the number of

revertants reached 10^7, which implies the presence in the bowels of mice, at the end of vaccinated period, of a total Sm D cell population of about 10^{14}. The extreme improbability of such a figure may explain the benign results observed. However, as we have already noticed (Vladoianu *et al.* 1975) this type of system must not be considered as a general rule. In fact, cases are cited (Baron & Formal, 1960; Shuster *et al.* 1971) in which a complete recovery of virulence was observed, but only at a relatively slow rate. Although such circumstances are theoretically possible, in practice it seems that the risk of reversion to a virulent form during oral antityphoid vaccination with Sm D strains remains very slight even after the interruption of concomitant streptomycin administration.

In further studies we propose to adopt the model developed in these experiments, in investigating the efficiency of oral antityphoid vaccination with Sm D strains as well.

The authors express their gratitude to Prof. E. Trabucchi, the Director of the Institute of Pharmacology, University of Milan, Medical School, Milan, Italy, for his support in the accomplishment of this work.

REFERENCES

BARON, L. S. & FORMAL, S. B. (1960). Immunization studies with living vaccine of *Salmonella typhimurium*. *Proceedings of the Society for Experimental Biology and Medicine* **104**, 565–7.

COLLINS, F. M. (1972). Salmonellosis in orally infected specific pathogen-free C57B1 mice. *Infection and Immunity* **5**, 191–8.

DUPONT, H. L., HORNICK, R. B., SNYDER, M. J., LIBONATI, J. P. & WOODWARD, T. E. (1970). Immunity to typhoid fever: evaluation of live streptomycin-dependent vaccine. *Antimicrobial Agents and Chemotherapy* **10**, 236–9.

SHUSTER, B. IU., SERGEEV, V. V., ELKINA, S. I., LIMAREV, V. A. & LIKHODED, V. G. (1971). Virulent properties of revertants of streptomycin-dependent salmonella mutants. *Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii* **48**, 29–32.

VLADOIANU, I. R., DUBINI, F. & BOLLOLI, ADELE (1975). Contribution to the study of live streptomycin-dependent *Salmonella* vaccines: the problem of reversion to a virulent form. *Journal of Hygiene* **75**, 203.