6. V. F. Semenkov, Dokl. Akad. Nauk SSSR, 181, 1514 (1968).
7. M. I. Fedotova and Yu. I. Zimin, Byull. Éksp. Biol. Med., No. 6, 32 (1975).
8. H. Blomgren and B. Anderson, Cell. Immunol., 1, 545 (1971).
9. J. J. Cohen, J. Immunol., 108, 841 (1972).
10. J. J. Cohen, M. Fishbach, and H. N. Claman, J. Immunol., 105, 1446 (1970).
11. J. D. Stobo and W. E. Paul, J. Immunol., 110, 362 (1973).
12. R. E. Tigelaar and R. Asofsky, J. Immunol., 110, 567 (1973).

# TRANSFORMATION OF *Bacillus subtilis* IN MICE AFTER IMMUNOLOGICAL SUPPRESSION OF EXOGENOUS DEOXYRIBONUCLEASE 1 ACTIVITY

Academician N. N. Zhukov-Verezhnikov,* V. A. Drozhennikov, L. G. Stolyarova, E. B. Orlova, and O. S. Perevezentseva

UDC 576.851.513.083.23

Transformation of *Bacillus subtilis* was carried out intraperitoneally in mice. The frequency of transformation was considerably reduced by intraperitoneal injection of bovine deoxyribonuclease 1 (DNase 1) into the animals in a dose of 3-5 μg. Immune rabbit γ-globulins, containing antibodies against bovine DNase 1, inhibit the activity of DNase 1 in vivo, thus protecting the transforming DNA against the hydrolytic action of that enzyme. The model suggested can be used to search for ways of preserving a nucleic acid introduced into an animal for the purposes of genetic engineering.

KEY WORDS: *transformation in vivo; γ-globulins; DNase 1; inhibition in vivo.*

One of the main problems facing genetic engineering, a new field of investigation in molecular biology and genetics, is correction of defects of the genetic material of man and animals at the DNA level. However, whereas for prokaryotes and, in some cases, for eukaryotes also (in cell culture) the question of introducing foreign genetic information into a recipient cell is to some extent solved already [13, 14], with regard to the whole organism this problem still remains open. The reason is that simulation of the transmission of genetic material in higher animals by the nonsexual route is difficult because they possess a powerful system of protection, including the immune system, the system of interferon synthesis, and the nuclease barrier [3, 4]. The last of these merits the closest attention when experiments are carried out in genetic engineering, for nucleases and, in particular, deoxyribonucleases (DNases), enzymes specifically hydrolyzing DNA, are constantly present in all animal tissues [6] and are capable of destroying exogenous DNA introduced into the organism [4, 13]. Difficulties arise when this problem is studied in experiments involving transformation of eukaryote cells. Meanwhile, in bacterial models, where transformation can easily be achieved, factors inhibiting transformation in vivo are absent. The writers accordingly suggested a new model combining the technical simplicity and advantages of bacterial transformation with the introduction of conditions preventing transformation in vivo into the experimental system. For this purpose, experiments on bacterial transformation were carried out in animals. The use of such a model provides an approach to the development of methods of overcoming the obstacles existing in the intact organism in the way of introducing foreign DNA in the course of genetic engineering. Anti-DNase γ-globulins, specifically inhibiting the activity of these enzymes in experiments in vitro [2], were used as one of the factors protecting DNA against the destructive action of DNases.

*Academy of Medical Sciences of the USSR.

Research Laboratory of Experimental Immunobiology, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 84, No. 7, pp. 68-70, July, 1977. Original article submitted December 3, 1976.

TABLE 1. Frequency of Appearance of $trp^+$ and $leu^+$ Transformants during Transformation of *B. subtilis* in vitro and in vivo

| Serial No. | Ingredients | Number of experiments | Frequency of appearance of transformants | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | in vitro | | | | in vivo | | | |
| | | | $trp^+$ | | $leu^+$ | | $trp^+$ | | $leu^+$ | |
| | | | $(M \pm m) \cdot 10^{-2}$ | $P$ | $(M \pm m) \cdot 10^{-2}$ | $P$ | $(M \pm m) \cdot 10^{-2}$ | $P$ | $(M \pm m) \cdot 10^{-2}$ | $P$ |
| 1 | DNA + B. subtilis (control) | 5 | 1,20±0,02 | — | 1,20±0,02 | — | 0,60±0,09 | — | 0,60±0,02 | — |
| 2 | DNA + B. subtilis + DNase 1 | 5 | 0,13±0,04 | <0,001 | 0,070±0,008 | <0,001 | 0,040±0.003 | <0,01 | 0,040±0,001 | <0,01 |
| 3 | DNA + B. subtilis + C* γ-globulin + DNase 1 | 5 | 0,27±0,01 | <0.001 | 0,18±0,01 | <0,001 | 0,046±0,002 | <0,01 | 0,045±0,002 | <0,01 |
| 4 | DNA + B. subtilis + E* γ-globulin + DNase 1 | 5 | 1,30±0,03 | >0,1 | 1,20±0,01 | >0,1 | 1,50±0,05 | <0,05 | 1,30±0,12 | <0,05 |

Legend: C* and E* denote control and experimental (immune) γ-globulins, respectively. Values of P obtained by comparing levels of transformation of Nos. 2, 3, and 4 with No. 1 (control).

## EXPERIMENTAL METHOD

### Method of Transformation

Transformation was based on Spizizen's method [15]. Prototrophic strain *Bacillus subtilis* W23EMB was used as the donor strain, the source of transforming DNA. Auxotrophic strain *Bacillus subtilis* 168-2 ($trp^-$, $leu^-$) was used as the recipient. Transforming DNA was isolated by the method of Bresler et al. [1] using sodium dodecyl sulfate and phenol deproteinization [8]. The concentration of the DNA isolated was 0.2 mg/ml.

### Isolation of γ-Globulins

To obtain immune anti-DNase sera a full course of immunization of rabbits was carried out for three weeks. The preparation of DNase 1 was injected with Freund's complete adjuvant subcutaneously. The total dose of the injected preparation of DNase was 15 mg per animal.

The method of Cohn et al. [7] was used to isolate γ-globulins from the immune and control (intact animals) sera. The anti-DNase activity of the γ-globulins was determined by four tests: the CFT with 50% hemolysis [10], a viscosimetric test [12], a quantitative precipitation test [9], and a spectrophotometric method [11]. The last two methods were modified for determination of DNase 1 by the use of an ultramicrosystem of biochemical analysis as developed by Pokrovskii [5].

### Transformation of *B. subtilis* in vivo

Male DBA/2 mice weighing 20 g were given an intraperitoneal injection of 0.5 ml each of immune and nonimmune (control) γ-globulins. The same animals were given an intraperitoneal injection of a transforming mixture, consisting of a culture of the recipient strain of *B. subtilis* and transforming DNA, 60 min later. The animals were killed 90 min after injection of the transforming mixture and fluid from the peritoneal cavity was removed and seeded on appropriate selective media.

## EXPERIMENTAL RESULTS

In the experiments of series I the effect of immune and nonimmune γ-globulins was studied on activity of exogenous (bovine) DNase 1 in vivo. For this purpose, mice were given an intraperitoneal injection of DNase 1 and physiological saline (group 1), DNase 1 and nonimmune γ-globulins (group 2), and DNase 1 and immune γ-globulins (group 3). The animals were killed after 60 min, the peritoneal fluid was withdrawn, and activity of DNase 1 in it was determined. The results are given in Fig. 1. They show that the activity of DNase 1 is inhibited by immune γ-globulins. After injection of nonimmune γ-globulins, DNase 1 activity is not inhibited.

In the experiments of series II a model was developed to permit transfer of foreign genetic information in vivo under conditions of protection against the action of DNases.

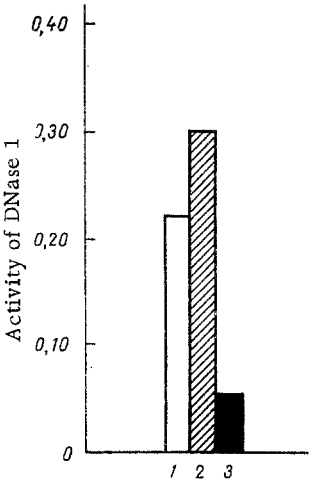


Fig. 1. Inhibition of DNase 1 activity by immune γ-globulins in experiments in vivo. 1) Injection of physiological saline (control); 2) of nonimmune γ-globulins; 3) of immune γ-globulins. Activity of DNase 1 was expressed in micromoles nucleoside monophosphates liberated as the result of incubation of 0.02 ml of the test solution with DNA for 60 min at 37°C. The number of micromoles corresponding to $A_{260}$ of the solutions was found from a calibration curve plotted for AMP.

The results of experiments to carry out transformation of *B. subtilis* in mice following inhibition of activity of exogenous DNase 1 by immune γ-globulins are given in Table 1. The same table gives the results of an in vitro experiment with the same ingredients. It will be clear from Table 1 that immune γ-globulins abolish the action of DNase on transforming DNA. Nonimmune γ-globulins had no such effect.

The results indicate that transforming DNA can be protected in the body of an animal against the hydrolytic action of DNases by means of γ-globulins containing antibodies against these enzymes. The proposed model for the transfer of foreign genetic information under conditions of suppression of DNase activity may be of great importance, in principle, for the search for ways of preserving DNA introduced into a recipient in the course of genetic engineering experiments on higher organisms. On the other hand, these results may point to the possibility of using DNases as a protective factor should undesirable genetic information, capable of transforming the bacterial flora of the recipient, enter the body of an animal or man.

## LITERATURE CITED

1. S. E. Bresler, V. L. Kalinina, and D. A. Perumov, Biokhimiya, No. 5, 907 (1963).
2. V. A. Drozhennikov, M. A. Guberniev, and A. A. Perelaznyi, in: Current Problems in Cellular Immunology and Immunogenetics [in Russian], Sofia (1973), p. 224.
3. M. B. Maksimovich, S. P. Lisovaya, and M. S. Parfenova, Zh. Mikrobiol., No. 11, 58 (1974).
4. C. Paoletti, C. Gosse, and J. B. Le Pecq, Biokhimiya, No. 4, 647 (1963).
5. A. A. Pokrovskii, in: Chemical Bases of Processes of Vital Activity [in Russian], Moscow (1962), p. 311.
6. V. S. Shapot, Nucleases [in Russian], Moscow (1968), p. 72.
7. E. J. Cohn, L. E. Strong, W. L. Hughes, et al., J. Am. Chem. Soc., 68, 459 (1946).
8. E. Ephrati-Elisur, P. Srinivasan, and S. Zamenhof, Proc. Nat. Acad. Sci. USA, 47, 56 (1961).
9. S. Fuchs, P. Cuatrecasas, D. A. Ontjes, et al., J. Biol. Chem., 244, 943 (1969).
10. E. A. Kabat and M. M. Mayer, Experimental Immunochemistry, Springfield (1958), p. 97.
11. M. Kunitz, J. Gen. Physiol., 33, 349 (1950).
12. M. McCarty, J. Exp. Med., 90, 543 (1949).
13. C. R. Merril and H. Stanbro, Z. Pflanzenphysiol., 72, 371 (1974).
14. J. F. Morrow, S. N. Cohen, A. C. Y. Chang, et al., Proc. Nat. Acad. Sci. USA, 71, 1743 (1974).
15. J. Spizizen, Proc. Nat. Acad. Sci. USA, 44, 1072 (1958).