Effect of Pyridoxine Deficiency on the Induction of Immune Tolerance in Mice[1] (34145)

ANTHONY C. TRAKATELLIS[2] AND A. E. AXELROD

Biochemistry Department, School of Medicine, University of Pittsburgh, Pennsylvania 15213

It is now well documented that induction of tolerance to tissue homografts can be achieved in adult mice by administration of an effective dose of viable splenic cells syngeneic with those of the skin donor (1–8). Previous experiments from this laboratory (9) demonstrated that a dose of splenic cells ineffective in inducing tolerance in adult mice can be rendered effective if the recipients are deficient in pyridoxine at the time of administration. In these experiments we utilized male mice of the CBA/J strain as recipients and male mice of the C3H/HeJ strain as splenic cell and skin donors. In all cases, skin was grafted while the host animals were receiving an adequate control diet. In the present investigation we have conducted similar experiments on the induction of immune tolerance in C57Bl/6J female mice to C57Bl/6J male skin isografts in order to ascertain in a more quantitative fashion the effect of a pyridoxine deficiency upon the dosage of splenic cells required. In this sex-linked histocompatibility system, a male skin isograft transplanted to a female behaves like a homograft and is rejected (10). Many experiments have substantiated the conclusion that the mechanism of this incompatibility resides in a Y-chromosome linkage of a male antigen foreign to the female (11–14).

Materials and Methods. Male and female mice of the C57Bl/6J strain, 4–5 weeks of age, were obtained from the Roscoe B. Jackson Memorial Laboratory. Compositions of the purified control and pyridoxine-deficient diets, preparation and method of injection of the pyridoxine antagonist, deoxypyridoxine, preparation and counting of the splenic cells, and skin grafting procedures have been described in detail in a previous publication (9). Female mice, employed as recipients of both splenic cells and skin grafts, were fed the purified diets whereas males, serving as donors of both splenic cells and skin grafts, received a commercial stock diet.[3] Splenic cells were administered intraperitoneally in a single injection.

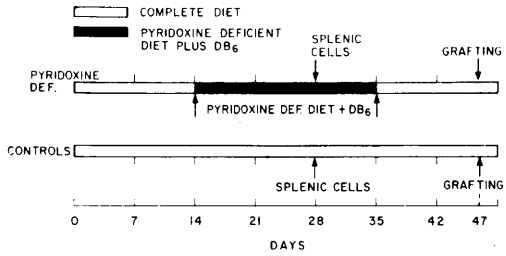

FIG. 1. Experimental design; DB_6 refers to deoxypyridoxine.

Experimental Methods and Results. The experimental design of the present study is shown in Fig. 1. Female control mice were fed the purified complete diet throughout the experiment and were grafted with skin of male mice at the indicated time (day 47). Three control groups received graded amounts of splenic cells derived from male donors on day 28 (Table I). The pyridoxine-deficient groups comprised of female mice were treated as follows. After a 2-week period during which the purified control diet was consumed, the animals were fed the pyridox-

[1] Supported in part by Public Health Service Research Grant no. A-727 from the National Institute of Arthritis and Metabolic Diseases and by Office of Naval Research Contract no. NOO14-66-CO-125.

[2] Present address: Department of Biochemistry, Mount Sinai School of Medicine, New York, New York.

[3] Purina laboratory chow, Ralston Purina Company, St. Louis.

TABLE I. Induction of Immune Tolerance in C57Bl/6J Females to C57Bl/6J Male Skin Isografts with Splenic Cells from C57Bl/6J Male Mice.

Group	Dose of splenic cells (no. of cells/animal)	No. of tolerant animals/no. grafted	Immune tolerance (%)
Controls	None	0/38	0
Pyridoxine deficient	None	1/25	4
Controls	5×10^7	6/13	46
Pyridoxine deficient	5×10^7	25/25	100
Controls	2.5×10^7	5/14	36
Pyridoxine deficient	2.5×10^7	20/22	91
Controls	1×10^7	2/20	10
Pyridoxine deficient	1×10^7	20/24	84

ine-deficient diet and given daily intraperitoneal injections of 100 μg of deoxypyridoxine/100 g of body weight for 3 weeks. Splenic cells derived from male donors were administered in graded amounts to three groups (Table I) on day 28. One week later, deoxypyridoxine treatment was discontinued and the animals resumed consumption of the purified control diet for the remainder of the experiment. Growth response was immediate and 12 days later (day 47) each mouse received a skin graft from a male donor. Growth and general appearance of the animals assumed the normal characteristics of mice fed the commercial stock diet.

Experimental results are presented in Table I. Animals were considered tolerant if their graft was in excellent condition 12 weeks postgrafting. Most of these grafts remained in excellent condition as long as 1 year postgrafting. Appearance of representative surviving grafts is shown in Fig. 2. It is evident that a very high percentage of tolerance (84-100%) was achieved in the pyridoxine-deficient groups with doses of splenic cells ranging from 1×10^7–5×10^7 whereas comparable doses of splenic cells in control animals produced a degree of tolerance ranging from 10–46%.

Discussion. Data presented in this paper clearly indicate that the dose of splenic cells required for induction of immune tolerance in C57Bl/6J female mice to male C57Bl/6J skin isografts can be markedly reduced—at least fivefold—if the splenic cells are administered to the recipient C57Bl/6J female mice while they are in a state of pyridoxine deficiency. It should be noted that the grafting procedure was performed *after* recovery with pyridoxine therapy and that the graft recipients continued to receive a complete diet providing adequate nutrition for the duration of the experiment. This observation bears particular relevance to the clinical aspects of the homotransplantation problem. If induction of immune tolerance is to be considered as a method for preventing rejection of homografts, it is very important to develop methods for the ready induction of such tolerance. Utilization of a pyridoxine deficiency in the manner described merits clinical attention since a readily reversible pyridoxine-deficient state can be produced in humans with the pyridoxine antagonist, deoxypyridoxine. Furthermore, the possibility exists that the effectiveness of various immunosuppressive agents, *e.g.*, actinomycin D, mitomycin C, 6-mercaptopurine, methotrexate, or X-irradiation could be enhanced by a concurrent pyridoxine-deficiency state.

The mechanism of induction of immune tolerance in these experiments remains a matter of speculation. The deleterious effects of a pyridoxine deficiency upon immune phenomena are very possibly related to the observation that this deficiency impairs nucleic acid synthesis (15, 16), decreases protein biosynthesis (17), and prohibits cellular proliferation subsequent to an antigenic stimulus (18). A combination of these effects could produce an impairment of immune response of the host against donor splenic cells leading

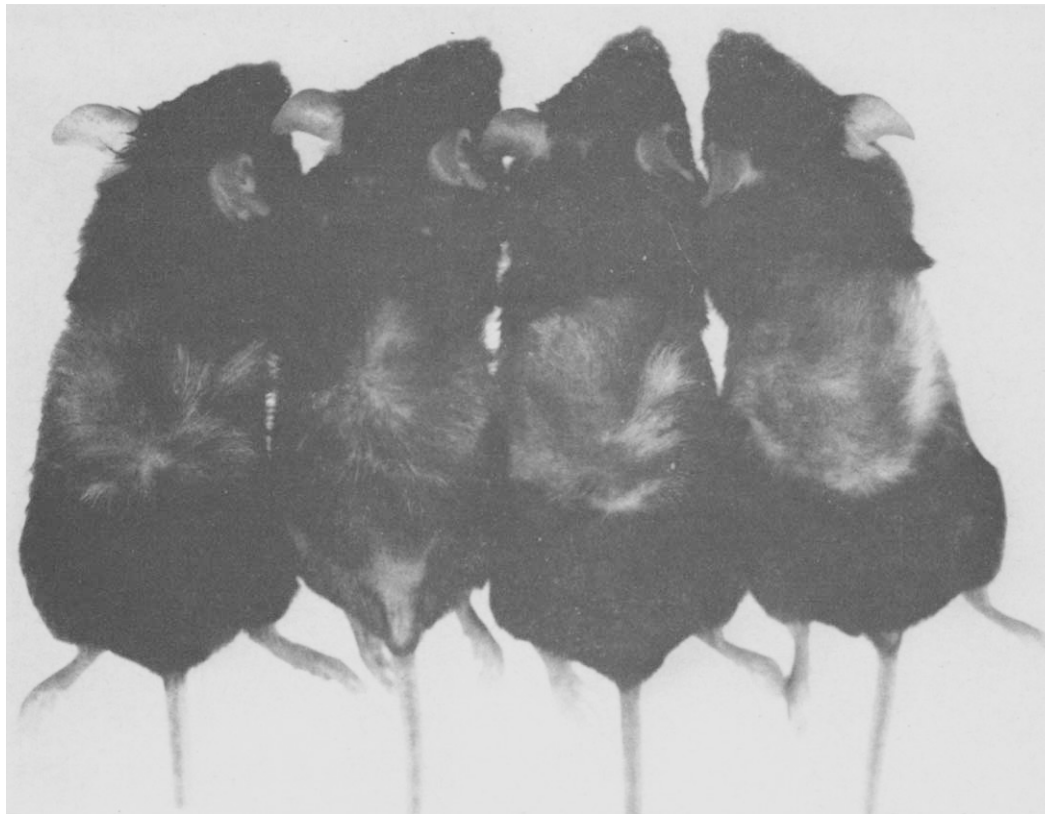

FIG. 2. Surviving grafts in C57B1/6J female mice treated with C57B1/6J male, splenic cells while in a state of pyridoxine deficiency and grafted with skin of C57B1/6J male mice subsequent to pyridoxine therapy.

to increased survival of such cells in the host with subsequent prolonged exposure of the host to donor antigens. This postulated ability of a pyridoxine deficiency to facilitate induction of a cellular chimeric state is analogous to a comparable effect of X-irradiation operating, perhaps, through a similar mechanism (4, 19–21).

Numerous authors have expressed the view that production of immune tolerance may be related to the capacity of donor antigens to overwhelm the immunological capacity of the host (21–25). In accordance with this concept, a diminution in the immunological potential of the host would reduce the dose of cells required to develop a tolerant state. Results of our experiments are consonant with this viewpoint.

Summary. A very high degree of immune tolerance of C57B1/6J female mice to skin grafts from C57B1/6J male mice has been achieved by injection of splenic cells derived from skin donors into prospective recipients while they are in a state of pyridoxine deficiency. Equal numbers of splenic cells are much less effective in the production of immune tolerance when injected into control animals than when administered to pyridoxine-deficient recipients.

1. Mariani, T., Martinez, C., Smith, J. M., and Good, R. A., Proc. Soc. Exptl. Biol. Med. **101**, 596 (1959).

2. Shapiro, F. Martinez, C., Smith, J. M., and Good, R. A., Proc. Soc. Exptl. Biol. Med. **106**, 472 (1961).

3. Lindner, O., Transplantation Bull. **28**, 134 (1961).

4. Lustgraaf, E. C., Fuson, R. B., and Eichwald, E. J., Transplantation Bull. **26**, 145 (1960).

5. Brent, L. and Gowland, G., Nature **196**, 1298 (1962).

6. McKhann, C. F., J. Immunol. **88**, 500 (1962).

7. Billingham, W. E. and Silvers, W. K., J. Cellular Comp. Physiol. **60**, 183 (1962).

8. Guttmann, R. D. and Aust, J. B., Nature **192**, 564 (1961).

9. Axelrod, A. E. and Trakatellis, A. C., Proc. Soc. Exptl. Biol. Med. **116**, 206 (1964).

10. Eichwald, E. J. and Lustgraaf, E. C., J. Natl. Cancer Inst. **26**, 1395 (1961).

11. Hauschka, T. S., Transplantation Bull. **2**, 154 (1955).

12. Eichwald, E. J., Silmser, C. R., and Wheeler, N., Ann. N. Y. Acad. Sci. **64**, 737 (1957).

13. Hauschka, T. S., Grinnell, S. T., Meagher, M., and Amos, D. B., *in* "Genetics and Cancer," Jr. 271. Texas University Press, Austin, Texas (1959).

14. Eichwald, E. J., Silmser, C. R., and Weissman, I., J. Natl. Cancer Inst. **20**, 563 (1958).

15. Trakatellis, A. C. and Axelrod, A. E., Biochem. J. **95**, 344 (1965).

16. Montjar, M., Axelrod, A. E., and Trakatellis, A. C., J. Nutr. **85**, 45 (1965).

17. Trakatellis, A. C. and Axelrod, A. E., J. Nutr. **82**, 483 (1964).

18. Kumar, M. and Axelrod, A. E., J. Nutr. **96**, 53 (1968).

19. Main, J. M. and Prehn, R. T., J. Natl. Cancer Inst. **19**, 1053 (1957).

20. Michie, D. and Woodruff, M. F. A., Proc. Roy. Soc. (London) **156**, 280 (1962).

21. Fefer, A. and Davis, W. C., Transplantation **1**, 75 (1963).

22. Argyris, B. F., J. Immunol. **92**, 630 (1964).

23. Martinez, C., Smith, J. M., Blaese, M., and Good, R. A., J. Exptl. Med. **118**, 743 (1963).

24. Kelly, W. D., Smith, J. M., Martinez, C., and Good, R. A., Proc. Soc. Exptl. Biol. Med. **115**, 8 (1964).

25. Miller, J., Martinez, C., and Good, R. A., J. Immunol. **93**, 331 (1964).

Received May 1, 1969, P.S.E.B.M., 1969, Vol. 132.