Immunotherapy of Friend Disease in Mice Employing Viable BCG Vaccine[1] (36534)

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Mice immunized with *Mycobacterium bovis* (BCG) vaccine (1) develop resistance to infection with Friend's disease virus (FDV). Sjögren and Ankerst (2), and Lemond and Clode (3, 4) immunized mice with BCG during the latent period of adenovirus infection and noted the sparing effect of immunization upon development of tumors. Mathé *et al.* (5, 6), Amiel (7), and Reif and Kim (8) found that BCG was effective in raising resistance to leukemia L1210 in mice. Schwartz *et al.* (9) and Zbar *et al.* (10, 11) studied the relationship of BCG vaccination to the occurrence of sarcomas due to the murine sarcoma virus of Moloney and to tumors in guinea pigs injected with ascites cells obtained from a line of chemically induced tumors. Maté *et al.* (12, 13) have employed BCG immunotherapy in the treatment of human leukemia and Morton *et al.* (14, 15) have used this form of therapy to treat malignant melanoma in man.

On the basis of the above evidence, immunotherapy was initiated in two groups of mice infected with FDV either 1 or 3 weeks prior to BCG immunization. In the first experiment, 79 mice were injected intravenously (iv) with 0.2 ml of a $10^{-2.3}$ dilution of FDV and 79 animals were retained as controls. Three weeks later, 39 of the infected mice and 40 of the normal mice were immunized by iv injection of BCG (4×10^6 vu). At intervals of 3, 4, and 5 weeks after immunization mice from each of the above groups were tested for delayed hypersensitivity by injection of 5.0 μg of PPD into the footpad of the right hind leg. Twenty-four hours later the reactions were recorded, the animals were killed, and the spleen and body weight determined. The results are shown in Table I. The mean spleen weight of immunized, infected mice was from 3.3 to 2.8 times that of the immunized, noninfected mice, and the mean spleen weight of infected, unimmunized mice was 11.2 to 16.5 times greater than that of normal, unimmunized mice. At the time of the last autopsy eight of the infected, unimmunized animals and 14 of the infected, immunized animals were alive. If those animals sacrificed for spleen data at 6 and 7 weeks are eliminated from consideration, the survival rate 8 weeks after challenge with FDV was 29% (8/27) for the infected, unimmunized mice and 67% (14/21) for the infected, immunized animals.

In the next experiment, groups of mice were infected iv with either 10^{-3} or 10^{-4} dilutions of FDV and vaccinated iv with viable BCG 7 days later and again on days 14 and 21 with BCG administered subcutaneously. Each inoculum contained 4×10^6 vu of BCG. The results of this study are summarized in Table II and Fig. 1. The data in Table II was obtained 28 days after infection. The spleens of the mice in groups A and B (infected but not vaccinated) were greatly enlarged as compared to those of the normal control mice (group F), but the spleens of mice in groups C and D (infected and vaccinated) were only slightly larger than those of the vaccinated but uninfected mice (group E). Plaque counts obtained by Plutznick's method (15) revealed fewer plaques in the spleens of vaccinated animals than in those of unvaccinated ones. The death rates depicted in Fig. 1 show 6–8 weeks longer are required to attain a 50% mortality

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TABLE I. Results Obtained from Mice Injected iv with Friend Disease, Vaccinated 3 Weeks Later with Viable BCG, and Autopsied at 6-, 7-, and 8-Week Intervals After Injection with Virus.

Group	FDV: 0.2 ml, iv, $10^{-2.3}$ dil, day 0	BCG: 4×10^7 vu, iv day 21	Time of autopsy (weeks)	No. of mice	Spleen data: Mean[a] SpW (g)	SpI[b]/SpC[c]	SpW[e]/BW (%)[d]	Increase SpW/BW	Skin tests (mm)
A	+	+	6	9	2.08	2.8×	7.6	3.3×	1.5
B	+	−	6	8	1.81	11.2×	7.7	15.4×	0
C	−	+	6	10	0.74		2.3		1.8
D	−	−	6	10	0.16		0.5		0
E	+	+	7	9	1.14	2.6×	5.2	3.5×	1.7
F	+	−	7	5	1.89	13.5×	6.9	17.2×	0
G	−	+	7	10	0.45		1.5		1.8
H	−	−	7	10	0.14		0.4		0
I	+	+	8	7	1.79	2.3×	6.2	2.3×	1.0
J	+	−	8	8	2.31	16.5×	7.8	19.5×	0
K	−	+	8	10	0.79		2.7		2.4
L	−	−	8	10	0.14		0.4		0

[a] SpW = mean spleen weight.
[b] SpI = mean spleen weight of infected mice.
[c] SpC = mean spleen weight of respective control mice.
[d] BW = mean body weight.
[e] $\frac{\text{SpW}}{\text{BW}} = \frac{\text{mean spleen weight}}{\text{mean body weight}} \times 100.$

TABLE II. Results Obtained from Mice Treated by iv Injection of BCG 1 Week After Challenge with Friend Disease Virus. Observations made 4 Weeks After Infection with FDV.

Group	No. of mice	Dilution, FDV: 0.2 ml, iv day 0	BCG: 4×10^7 vu iv, day 7	Mean[a] SpW (g)	SpI[b]/SpC[c]	SpW[e]/BW (%)[d]	Increase SpW/BW	Plaque counts: <100	1–100	0
A	10	10^{-3}	−	1.16	8.9×	4.93	12.8×	9	1	
B	10	10^{-4}	−	0.97	7.5×	3.88	9.9×	8	2	
C	10	10^{-3}	+	0.94	1.5×	3.95	1.8×	8		2
D	10	10^{-4}	+	0.75	1.2×	2.75	1.2×	2	2	6
E	5	None	+	0.64		2.22				5
F	5	None	−	0.13		0.39				5

[a] SpW = mean spleen weight.
[b] SpI = mean spleen weight of infected mice.
[c] SpC = mean spleen weight of respective control mice.
[d] BW = mean body weight.
[e] $\frac{\text{SpW}}{\text{BW}} = \frac{\text{mean spleen weight}}{\text{mean body weight}} \times 100.$

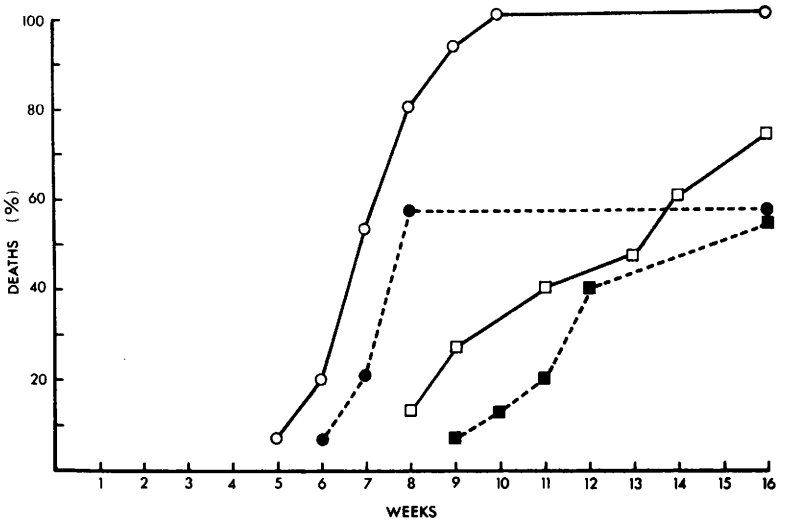

FIG. 1. Mortality rates of normal mice infected iv with either 10^{-2} or 10^{-3} dilutions of FDV, and of mice infected with FDV and immunized with BCG 7 days later. O—O, 10^{-3} FDV; ● - - - ●, 10^{-4} FDV; □—□, 10^{-3} FDV+BCG; ■ - - - ■, 10^{-4} FDV+BCG.

rate in vaccinated than in unvaccinated animals.

The course of Friend leukemia in mice is significantly modified if the animals are treated by iv injection of viable BCG vaccine 1 to 3 weeks after infection with FDV. It is of interest that in this infection, which requires a relatively long time to cause death of the recipients, the administration of viable BCG can both increase the survival time and decrease the splenic involvement in mice vaccinated after injection has occurred. Stimulation of nonspecific cellular immunity by immunotherapy during active infection appears to be a valuable method for control of this infection.

1. Larson, C. L., Ushijima, R. N., Florey, M. J., Baker, R. E., and Baker, M. B., Nature (London) New Biol. **229**, 243 (1971).

2. Sjögren, H. O., and Ankerst, J. Nature (London) **221**, 863 (1969).

3. Lemonde, P., and Clode, M., Proc. Soc. Exp. Biol. Med. **111**, 739 (1962).

4. Lemonde, P., and Clode, M., Cancer Res. **26**, 585 (1966).

5. Mathé, G., Rev. Franc. Études Clin. Biol. **13**, 881 (1960).

6. Mathé, G., Pouillart, P., and Lapeyraque, F., Brit. J. Cancer **23**, 814 (1969).

7. Amiel, J-L., Rev. Franc. Études Clin. Biol. **12**, 912 (1967).

8. Reif, A. E., and Kim, C. H., Cancer Res. **31**, 1606 (1971).

9. Schwartz, D. B., Zbar, B., Gibson, W. T., and Chirigos, M. A., Int. J. Cancer **8**, 320 (1971).

10. Zbar, B., Bernstein, I., Tanaka, T., and Rapp, H. J., Science **170**, 1217 (1970).

11. Zbar, B., Bernstein, I., and Rapp, H. J., J. Nat. Cancer Inst. **46**, 831 (1971).

12. Mathé, G., Brit. Med. J. **4**, 7 (1969).

13. Mathé, G., Amiel, J. L., Schwarzenberg, L., Schneider, M., Cattan, A., Schlumberger, J. R., Hayat, M., and deVassal, F., Lancet **2**, 697 (1969).

14. Morton, D. L., Eilber, F. R., Malmgren, R. A., and Wood, W. C., Surgery **68**, 158 (1970).

15. Morton, D. L., Holmes, E. C., Eilber, F. R., and Wood, W. C., Ann. Int. Med. **74**, 587 (1971).

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