INFECTION AND IMMUNITY, Sept. 1978, p. 1027-1028
0019-9567/78/0021-1027$02.00/0

Vol. 21, No. 3
Printed in U.S.A.

Modulation of Immune Response by Killed *Brucella abortus* Organisms: Comparison of the Effects of Smooth and Rough Strains on T-Dependent Responses

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Received for publication 30 June 1978

Inactivated *Brucella abortus* organisms of the smooth (S) or rough (R) strain were tested comparatively on two T-dependent immune responses: mixed-lymphocyte reaction and delayed-type hypersensitivity. The intravenous injection of S organisms depressed the two tests, whereas R organisms increased mixed-lymphocyte reaction and did not alter delayed-type hypersensitivity significantly. This observation may be helpful in understanding the differences in adjuvant properties of S and R brucellae.

Heat-inactivated *Brucella abortus,* an antitumor agent in mice (2), can alter the antibody response against sheep erythrocyte (SRBC) antigens (7). Recently, smooth (S)- and rough (R)-phase cultured organisms were shown to affect differently the anti-SRBC response. In particular, R preparations, but not S, increased the number of antibody-forming spleen cells 4 days after intravenous (i.v.) injection in a mixture with SRBC (3). In the present work, it was questioned whether the different adjuvant properties of the two bacterial strains also applied to thymus-dependent immune reactions.

As previously reported (3), *B. abortus* strain B19 was used to prepare S (B19S) and R (B19R) heat-killed organisms. The bacterial suspensions were injected i.v. into ($C_{57}Bl_6/DBA_2$) F_1 hybrid, 6-week-old female mice (Centre National de la Recherche Scientifique, Orleans).The doses indicated in the figures are expressed in dry weight.

Mixed-lymphocyte-reaction (MLR) experiments were performed with spleen cells pooled from groups of four F_1 mice pretreated or not with B19S or B19R organisms. Cell suspensions at 1.5×10^7 per ml of culture medium (RPMI 1640 [Difco] supplemented with 10% fetal calf serum [Flow]) were added to equal quantities of mitomycin-treated spleen cells (mitomycin C [Sigma], 20 μg/ml, 37°C, 30 min). The mitomycin-treated cells were sampled either from normal Swiss mice for the stimulated cultures or from normal F_1 mice for the control cultures. Cultures were done in six replicates in Microtest II plates (Greiner), using 0.2 ml of cell suspension per well. After 3 days of incubation at 37°C in a 5% CO_2-air mixture, 1 μCi of [^{3}H]thymidine (TMM 48B, Saclay) was added in each well. After 24 h of additional incubation, the cells were removed with a multiple automatic sample harvester (Dynatech), and their radioactivity was counted. The MLR index was expressed as a ratio of mean radioactivity in stimulated cultures to that in control cultures.

The delayed-type hypersensitivity (DTH) to SRBC antigens was measured according to the technique of Lagrange et al. (1). Briefly, groups of 5 mice (15 for the controls) pretreated or not with *Brucella* preparations were sensitized by

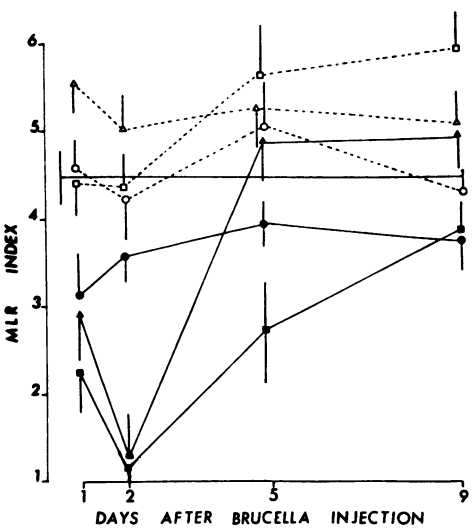

FIG. 1. *One-way MLR of spleen cells sampled 1 to 9 days after i.v. injection of B. abortus. Symbols:* (●) *50 μg of B19S;* (○) *50 μg of B19R;* (▲) *150 μg of B19S;* (△) *150 μg of B19R;* (■) *500 μg of B19S;* (□) *500 μg of B19R.*

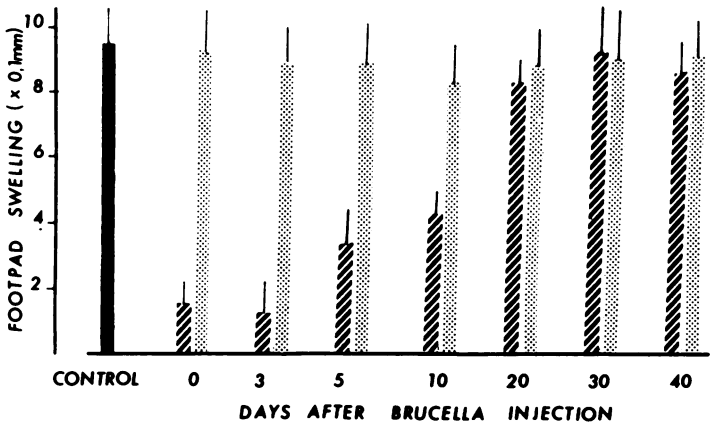

FIG. 2. *DTH to SRBC, injecting SRBC at different times after treatment with 500 μg of B. abortus B19S (hatched bar) or B19R (dotted bar).*

i.v. injection of 10^6 SRBC. Four days later they were challenged with 10^8 SRBC injected into one hind footpad. The following day the swelling provoked by the reaction was measured by the difference in thickness between hind footpads. The thymus dependency of this reaction has been well established (4).

The results of MLR experiments are shown in Fig. 1. Preinjection of B19S organisms clearly diminished the ability of spleen cells to mount a response. The effect, still attenuated with 50 μg of organisms, was very clear-cut, using injections of 150 and 500 μg. The diminution of reactivity reached its maximum on day 2 and lasted for a longer time with 500 than with 150 μg. In contrast, B19R-pretreated animals had a generally increased response.

DTH to SRBC antigens also was affected by prior treatment with *Brucella* organisms. The inoculation of 500 μg of B19S immediately provoked a diminution of the reaction, which was still apparent on day 10 and returned to normal on day 20 (Fig. 2). In contrast, B19R did not alter the response significantly.

Thus, the two tests were in agreement in showing that B19S depressed T-dependent reactions whereas B19R did not affect or increased them. These opposite effects may explain results previously obtained (3) on the anti-SRBC antibody response: B19S and B19R may have acted differently on the T-dependent control of the response, with B19S activating suppressor cells and B19R activating helper cells.

The depression of T-dependent responses seems to be related to the i.v. method of injection of B19S organisms. Indeed, after subcutaneous inoculation in a mixture with SRBC, B19S was shown to increase the DTH to the antigen strongly. Used with Freund incomplete adjuvant, it was very effective in inducing experimental allergic encephalitis (unpublished data). *Corynebacterium parvum* exhibits similar properties. Injected i.v., it was shown to depress MLR, phytohemagglutinin reactivity, and graft-versus-host reaction (6), whereas when used in Freund adjuvant it induced DTH (5).

The nondiminution of T-dependent responses after i.v. injection of B19R, a bacterium devoid of surface agglutinogens, suggests that the strong antigenicity of B19S plays an important part in the genesis of the depression of T-cell-mediated reactivity.

This work was supported by Institut National de la Santé et de la Recherche Médicale contracts.

LITERATURE CITED

1. **Lagrange, P., G. B. Mackaness, and T. E. Millet.** 1975. Effects of bacterial lipopolysaccharide on the induction and expression of cell mediated immunity. I. Depression of afferent arc. J. Immunol. **114**:442–446.
2. **Le Garrec, Y., D. Sabolovic, L. Toujas, J. Guelfi, and C. Pilet.** 1974. Activity of inactivated Brucella in anti-tumour prophylactic effect and combination with specific immunostimulation. Biomedicine **21**:40–44.
3. **Le Garrec, Y., L. Toujas, A. Martin, L. Dazord, and C. Pilet.** 1978. Influence of the antigenicity of *Brucella* preparations on modulation of the immune response to sheep erythrocytes. Infect. Immun. **20**:6–11.
4. **Miller, T. E., G. B. Mackaness, and P. Lagrange.** 1973. Immunopotentiation with BCG. II. Modulation of the response to sheep red cells. J. Natl. Cancer Inst. **51**:1669–1673.
5. **Neveu, T., A. Branellec, and G. Biozzi.** 1964. Propriétés adjuvantes de Corynebacterium parvum sur la production d'anticorps et sur l'induction de l'hypersensibilité retardée envers les protéines conjuguées. Ann. Inst. Pasteur Paris **106**:771–777.
6. **Scott, M. T.** 1972. Biological effects of the adjuvant corynebacterium parvum. I. Inhibition of PHA, mixed lymphocyte and GVH reactivity. Cell. Immunol. **5**:459–468.
7. **Toujas, L., D. Sabolovic, L. Dazord, Y. Le Garrec, J. P. Toujas, J. Guelfi, and C. Pilet.** 1972. The mechanism of immunostimulation induced by inactivated Brucella abortus. Rev. Eur. Etud. Clin. Biol. **17**:267–273.