INFECTION AND IMMUNITY, Mar. 1977, p. 1004-1007

Vol. 15, No. 3
Printed in U.S.A.

Antibody to *Corynebacterium parvum* in Normal Human and Animal Sera

G. WOLBERG, G. S. DUNCAN, C. ADLAM,* AND J. K. WHISNANT

*Wellcome Research Laboratories, Research Triangle Park, North Carolina 27709, and Beckenham, England**

Received for publication 23 September 1976

Using a microtiter bacterial agglutination test, we have estimated antibodies to *Corynebacterium parvum* in "normal" human and "normal" and immune animal sera. Widely differing levels of *C. parvum* antibodies were found in the normal human sera. The median titer for all 310 human sera was 1:128, whereas that for the 1- to 17-year and 18- to 50-year subgroups was 1:64 and 1:512, respectively. Antibody titers in the various animal species were generally much lower.

Killed suspensions of *Corynebacterium parvum* (*Propionibacterium acnes*) cause major alterations in reticuloendothelial function and are used widely in animal models for prevention and/or therapy of tumors. Details regarding experimental and some clinical studies are available in the proceedings of a 1974 *C. parvum* international meeting (4). Recent reviews on tumor models and immunological alterations are also available (9, 11). The effects of *C. parvum* administration on human malignancy and its therapeutic modalities are also under study (1, 3, 6). These continuing programs require information on specific immunity to *C. parvum* since immunotherapeutic response and/or side effects may be influenced by levels of anti-*C. parvum* antibody.

C. parvum is the commonly used designation for species of *P. acnes* (2), but the name *C. parvum* is retained for convenience since a major portion of experimental and clinical studies use a "*C. parvum*" vaccine from the Wellcome culture collection (culture no. 6134). We have designed a simple agglutination test for antibody to this organism. The methodology, reference to standard hyperimmune sera, and levels of activity in normal sera are reported. The assay devised for these preliminary studies is simple, reproducible, and applicable to human and animal sera. This technique itself differs from that described by Woodruff et al. (16) and James et al. (7), but ranges of titers obtained are comparable.

Serum agglutination titrations were carried out in U-type well microtiter plates (Linbro Scientific Inc., New Haven, Conn.) by incubation of 0.05-ml volumes of antiserum (in twofold dilution steps) and 0.05-ml volumes of *C. parvum* suspension. The plates were incubated at 37°C for 1 h and then overnight at 2 to 5°C. Patterns were compared to control titrations of fetal calf serum and to reference-positive antisera. The diluent for titrating the sera was made by mixing 9 parts of 0.15 M Na_2HPO_4, 1 part of 0.15 M KH_2PO_4, and 10 parts of 0.85% NaCl solution. Fetal calf serum was added at a final concentration of 2% (final pH is 7.6). The bacterial suspension was prepared from the Wellcome *C. parvum* vaccine used for animal and clinical studies (Burroughs Wellcome Co., Research Triangle Park, N. C.). This organism is a *P. acnes* serogroup I. The formalin-killed suspension was washed free of media and formalin and resuspended in 0.9% saline with 0.01% thimerosal. The vaccine suspension was centrifuged at 3,000 × *g* for 10 min, the supernatant was removed, and the pellet of cells was resuspended and washed two times in physiological saline solution followed by centrifugation. After the final wash the cells were lyophilized. This bacterial preparation was suspended in 0.9% saline at a concentration of 0.5 mg/ml (optical density at 600 nm was approximately 1.30) and used for titration within 5 h. A typical titration is shown in Fig. 1.

Sera were chosen from samples stored at −20 to −4°C for as long as 6 years. Normal blood bank adult donors and adults from family studies on other infections comprised a major portion of the human sera (10). Sera from children attending a pediatric allergy diagnostic clinic were provided by Susan C. Dees at Duke University. Both sexes were approximately equally represented, and no patients with known malignancy were included.

Table 1 shows the range and percent positivity for all 310 human sera and for the subset of 152 adults (≥18 years). All sera were positive at

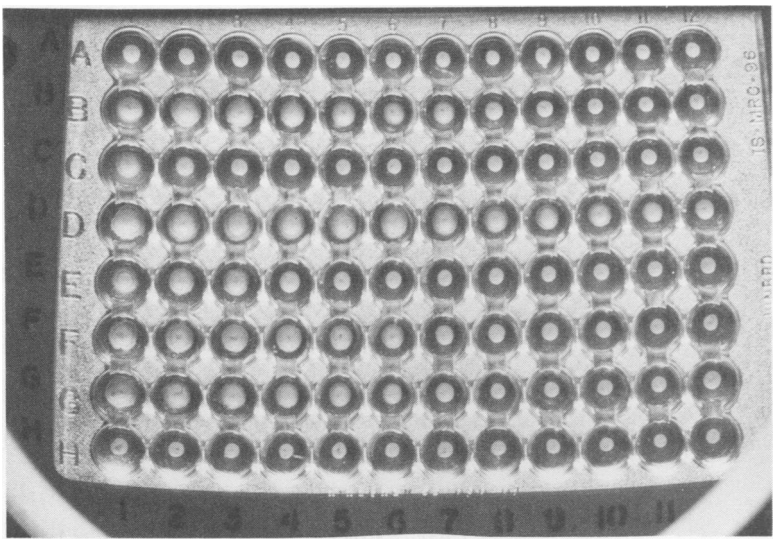

FIG. 1. *Microtiter plate showing typical agglutination patterns. Sera titrated, with end point for each row, are: (A) fetal calf, <1; (B) mouse anti-C. parvum, 8; (C) normal mouse, 2; (D) ovine anti-C. parvum, >12; (E) human, 4; (F) human, 8; (G) human, 7; (H) feltal calf, <1.*

TABLE 1. *Antibody to C. parvum: titration of normal human sera*

C. parvum titer	No. of sera (% of total)	Adult[a] sera (% of adults)
1:8	11 (3.5)	0 (0)
1:16	30 (9.7)	1 (0.6)
1:32	33 (10.7)	8 (5.2)
1:64	56 (18.1)	17 (11.2)
1:128	58 (18.7)	24 (15.8)
1:256	37 (11.9)	21 (13.8)
1:512	41 (13.2)	38 (25.0)
1:1,024	23 (7.4)	22 (14.5)
1:2,048	14 (4.5)	14 (9.2)
1:4,096	5 (1.6)	5 (3.2)
1:8,192	2 (0.6)	2 (1.3)

[a] 18 to 50 years of age.

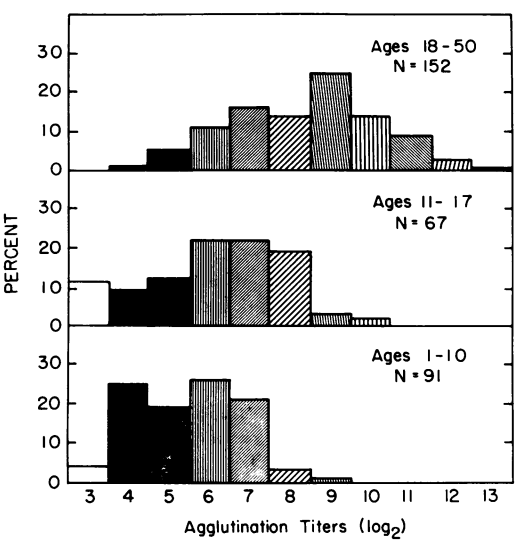

FIG. 2. *Anti-C. parvum agglutination titers of 310 normal human sera.*

the lowest dilutions tested, indicating substantial antibody by this relatively insensitive assay method. The median titers for the whole group and for adults only were 1:128 and 1:512, respectively; a very wide range of titers is apparent. Age-specific antibody positivity was also documented, as shown in Fig. 2. The median titer for children and adolescents is 1:64, and 30% of children under 10 years had a titer $\leq$1:16. Statistical analysis of age-specific antibody levels shows clustering of titers in children above and below age 9. Continuous age regression is not possible on this discontinuous distribution.

Hyperimmune animal sera have been produced for control purposes and for comparison with levels of naturally occurring antibody. Injection of killed *C. parvum* induces strong serological response, as expected. These sera were prepared by injection of the Wellcome *C. parvum* vaccine. CD-1 mice were given two 700-μg (dry weight) intraperitoneal injections 1 week apart. Sera collected and pooled 12 days after the second injection had a titer of 1:1,280. New Zealand white rabbits received six 7-mg intravenous injections over a 23-month period, and the serum was collected 14 days after the last

TABLE 2. *Antibody to C. parvum: titration of normal animal sera*

Serum	Source	Range of titers (no. of samples tested)
Swiss albino rabbit	Franklin Rabbitry Wake Forest, N.C.	1:8–1:128 (7)
Porcine	Miles Labs Elkhart, Ind.	1:512 (1)[a]
Piglet (colostrum fed)	J. G. Lecce N.C. State Univ. Raleigh, N.C.	1:32–1:256 (22)
Piglet (nonfed)	J. G. Lecce N.C. State Univ. Raleigh, N.C.	<1:16 (2)
Guinea pig	Dutchland Lab. Animals Denver, Pa.	1:32–1:64 (5)
Beagle dog	Marshall Res. Animals North Rose, N.J.	1:16–1:32 (8)
ARS/Sprague-Dawley rats	Sprague Dawley Madison, Wis.	1:4–1:16 (7)
CD-1 mice	Charles River Wilmington, Mass.	1:4–1:16 (5)[a]
CAF-1 mice	Charles River Wilmington, Mass.	1:4–1:16 (3)[a]
A/HEJ mice	Jackson Labs Bar Harbor, Me.	1:4–1:8 (3)[a]
ICR mice	Blue Spruce Altomont, N.Y.	1:16 (1)[a]
A/JAX mice	Jackson Labs Bar Harbor, Me.	1:4–1:32 (2)[a]
CBA mice	Jackson Labs Bar Harbor, Me.	1:4–1:8 (3)[a]

[a] Pooled sera.

injection (titer, 1:10,240 to 1:20,480). The sheep received 7 mg in complete Freund adjuvant intramuscularly in each extremity on days 1, 7, 10, 14, and 17 with a booster 1 month later, and serum was collected 7 days after the last injection (titer, 1:20,480 to 1:40,960). These immune standard sera as well as the "normal" sera tested gave reproducible titers ± one twofold dilution on different days of assay and with successive preparations of *C. parvum* suspension.

Animal species assayed, the source of animal or serum, and the range of titers are given in Table 2. Normal rodents apparently have low levels of natural exposure and/or response. One rabbit serum had a titer of 1:128, but all others were 1:18 to 1:16. The explanation for the higher titers of the porcine sera is not apparent.

Human natural antibody immunity to *C. parvum* is not unexpected. *C. parvum* or *P. acnes* colonization is common in acne (13), in healthy skin (15), and probably in normal bone marrow (5). Although *Corynebacterium* diphtheroid disease in humans is rare (8), it is becoming more frequently recognized. It might be emphasized that a majority of diphtheroids are in the serogroup of the organism used for this assay. Response to other serogroup organisms has not been studied here.

Various biological roles for specific *C. parvum* immunity in immunotherapy have been suggested (12). Injections of *C. parvum* into hosts of widely differing immunities, either humoral or cellular, might be expected to result in variable therapeutic or adverse clinical results. Anti-*C. parvum* antibody, both total concentrations and specific class, is being studied in animal and human cancer therapy trials (7) for predictive value in type or severity of reactions. A possible role for delayed hypersensitivity to *C. parvum* in murine tumor models has been reported by Tuttle and North (14). Any final role for natural immunity to these organisms vis-à-vis acne or related host responses is a matter for speculation. In human tumor therapy trials involving *C. parvum*, it may be useful to include titrations of patients' sera for specific anti-*C. parvum* antibodies and studies of cellular hypersensitivity to these organisms.

LITERATURE CITED

1. **Cheng, V. S. T., H. D. Suit, C. C. Wang, and C. Cummings.** 1976. Nonspecific immunotherapy by *Corynebacterium parvum:* phase I toxicity study in 12 patients with advanced cancer. Cancer 37:1687–1695.

2. **Cummins, C. S., and J. L. Johnson.** 1974. *Corynebacterium parvum:* a synonym for *Propionibacterium acnes?* J. Gen. Microbiol. 80:433–442.
3. **Fisher, B., H. Rubin, G. Sartiano, L. Ennis, and N. Wolmark.** 1976. Observations following *Corynebacterium parvum* administration to patients with advanced malignancy. Cancer 38:119–130.
4. **Halpern, B. (ed).** 1975. *Corynebacterium parvum,* applications in experimental and clinical oncology. Plenum Press, New York.
5. **Hattori, T., and A. Mori.** 1973. Bacteriological survey of anaerobic Corynebacterium in human marrow and blood. Gann 64:7–14.
6. **Israel, L., and R. Edelstein.** 1975. Nonspecific immunostimulation with *Corynebacterium parvum* in human cancer, p. 485–504. *In* Immunological aspects of neoplasia, no. 26. The Williams & Wilkins Co., Baltimore.
7. **James, K., G. J. A. Clunie, M. F. A. Woodruff, W. H. McBride, W. H. Stimson, R. Drew, and D. Catty.** 1975. The effect of *Corynebacterium parvum* therapy on immunoglobulin class and IgG subclass levels in cancer patients. Br. J. Cancer 32:310–322.
8. **Kaplan, K., and L. Weinstein.** 1969. Diphtheroid infections of man. Ann. Intern. Med. 70:919–929.
9. **Oettgen, H. F., C. M. Pinsky, and L. Delmonte.** 1976. Treatment of cancer with immunomodulators *Corynebacterium parvum* and Levamisole. Med. Clin. North Am. 60:511–537.
10. **Robbins, J. B., J. C. Parke, R. Schneerson, and J. Whisnant.** 1973. Quantitative measurement of "natural" and immunization-induced *Haemophilus influenza* type B capsular polysaccharide antibodies. Pediatr. Res. 7:103–110.
11. **Scott, M. T.** 1974. *Corynebacterium parvum* as an immunotherapeutic anti-cancer agent. Semin. Oncol. 1:367–378.
12. **Shuster, S.** 1976. Biological purpose of acne. Lancet 1:1328–1329.
13. **Somerville, D. A., and C. T. Murphy.** 1973. Quantitation of *Corynebacterium acnes* on healthy human skin. J. Invest. Dermatol. 60:231–233.
14. **Tuttle, R. L., and R. J. North.** 1975. Mechanisms of antitumor action of *Corynebacterium parvum:* nonspecific tumor cell destruction at site of an immunologically mediated sensitivity reaction to *C. parvum*. J. Natl. Cancer Inst. 55:1403–1409.
15. **Whiteside, J. A., and J. G. Voss.** 1973. Incidence and lipolytic activity of *Propionibacterium acnes (Corynebacterium acnes* group I) and *P. granulosum (C. acnes* group II) in acne and in normal skin. J. Invest. Dermatol. 60:94–97.
16. **Woodruff, M. F. A., W. H. McBride, and N. Dunbar.** 1974. Tumour growth, phagocytic activity and antibody response in *Corynebacterium parvum*-treated mice. Clin. Exp. Immunol. 17:509–518.