Skin Reactivity of Human Subjects to a Polysaccharide Component of BCG Culture Filtrates (35274)

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Unheated culture filtrates of *Mycobacterium bovis* (Bacillus Calmette Guérin [BCG]) have been found to contain several nondialyzable fractions with tuberculin activity in suitably sensitized guinea pigs. One of these fractions (GA) is acid soluble and is predominantly carbohydrate (90%). It produces delayed hypersensitivity reactions equivalent to an acid-insoluble protein fraction from BCG bacteria or purified protein derivative (PPD) in BCG-sensitized animals (1–3). Skin reactivity has been maintained unimpaired after trypsin proteolysis and reduction of the protein content in the carbohydrate fractions to 2.5%, (4, 5) and occurs in the absence of circulating antibody (6). Further evidence that the reactions observed were due to delayed hypersensitivity was shown by the ability of the polysaccharide component to inhibit migration of macrophages from sensitized animals in a degree similar to PPD (7). This surprising amount of reactivity of sensitized guinea pigs to this substance and its close agreement with PPD responses made it of interest to test this material in human subjects who have long been considered unreactive to polysaccharide antigens (8, 9). Accordingly, both nontuberculous individuals and patients with active tuberculosis were chosen for study in comparative testing with PPD. The results indicate that, unlike guinea pigs, human beings evince very little reactivity to BCG polysaccharide in the presence of well established PPD sensitivity.

Materials and Methods. Patients. The test group comprised a total of 49 individuals, including physicians, normal volunteers and a variety of patients hospitalized at the National Institutes of Health, and 20 patients with active pulmonary tuberculosis hospitalized at the Glenn Dale Hospital, Glenn Dale, Md. This latter group was kindly made available for study by Dr. M. Weiss. None of the patients tested were receiving steroids or other agents known to suppress delayed hypersensitivity responses.

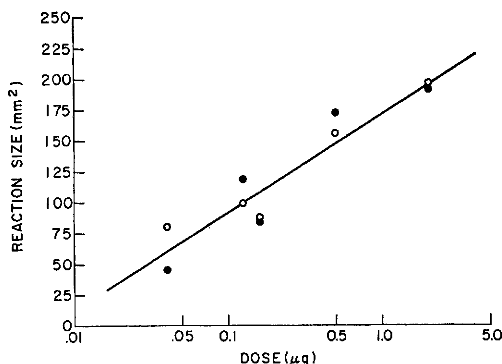

Fig. 1. Comparative skin reactivity of BCG-sensitized guinea pigs to intradermal testing with PPD (○); and BCG polysaccharide GA (●). Each point represents the mean of 5 animals.

Skin test antigens. BCG polysaccharide (GA) was obtained from unheated culture filtrates of *M. bovis* by previously described methods (1, 2). Dilutions in isotonic saline of 1.6 and 20 μg/ml (equivalent to 8 and 100 tuberculin test units (TU), respectively) were prepared in multiple dose vials and sterility and pyrogen tested prior to use.[1] Similar preparations were made of PPD (Parke Davis lots 15713 and 15714). Preliminary comparative testing of both GA and PPD in BCG sensitized guinea pigs established their equivalent potency (Fig. 1).

Test procedure. Initial testing in all individuals was performed by injecting 0.1-ml

[1] Kindly performed by Dr. Joseph Gallelli.

TABLE I. Skin Reactivity of PPD Negative[a] Subjects to PPD and a Polysaccharide (GA) Derived from BCG Bacteria.

	Test dose[b] 0.16 μg		2.0 μg	
Subject	PPD	GA	PPD	GA
K.A.	0	0	80[c]	0
R.C.	0	0	10	0
J.E.	0	0	90	42
W.G.	0	0	0	0
L.L.	0	0	0	0
G.L.	0	0	0	0
J.M.	0	0	0	0
F.M.	0	0	NT[d]	NT
P.R.	0	0	0	0
L.R.	0	0	9	0
A.S.	0	0	0	0
P.S.	0	0	NT	NT
S.S.	0	0	70	0
E.W.	0	0	0	0
R.J.	0	0	49	0
A.A.[e]	0	0	0	0
C.C.[e]	0	0	0	0
Total 17				
Mean[f]	0	0	7.1	0.2
Median	0	0	0	0

[a] Gave less than 100 mm² reactions with PPD, both test strengths.

[b] Dose of PPD or polysaccharide (GA) contained in 0.1 ml administered intradermally.

[c] Reaction size (induration) (mm²) within 48 hr obtained by multiplying longest by shortest diameters.

[d] Not tested.

[e] Had active tuberculosis (see text).

[f] Obtained by dividing the sum of the square roots of the individual observations by the number of observations and squaring the answer to reduce the effect of variation.

volumes containing either 1.6 μg of PPD or GA (8 TU) intradermally into the skin of opposite forearms. The sites were observed for immediate reactions and measured for induration at 24 and 48 hr after injection. All reactions were recorded as the product of the longest and shortest diameters (mm²) with "positive" reactions defined as induration of 100 mm² or more. All individuals with negative reactions (<100 mm²) to one or both antigens excepting those with active tuberculosis were retested within 1 week with the 2.0 μg (equivalent to 100 TU) dose of the nonreacting antigen.

Results. No immediate reactions were seen to BCG polysaccharide. PPD negative individuals including one with miliary tuberculosis (AA) found at autopsy and another with atypical pulmonary tuberculosis were uniformly negative to testing with GA (Table I). Subjects with positive reactions (>100 mm²) to either the 0.16 or 2.0 μg test strength of PPD and no evidence or history of active tuberculosis were similarly negative to testing with GA, although three persons displayed low degrees of reactivity to the polysaccharide (Table II). Nineteen patients with active pulmonary tuberculosis and well-developed skin reactions to the 0.16-μg dose of PPD likewise were uniformly negative to

TABLE II. Skin Reactivity of PPD Positive[a] Subjects Without Active Tuberculosis to PPD and a Polysaccharide (GA) Derived from BCG Bacteria.

	Test dose[b] 0.16 μg		2.0 μg	
Subject	PPD	GA	PPD	GA
B.B.	1050[c]	0	NT[d]	0
W.H.	0	0	100	0
M.H.	500	0	NT	49
L.H.	0	0	770	0
R.H.	0	4	770	0
L.L.	0	0	120	42
S.M.	0	0	132	72
B.R.	0	0	289	0
L.R.	0	0	180	0
P.R.	0	0	169	0
R.R.	1400	0	NT	0
G.W.	121	0	NT	9
T.W.	1050	0	NT	0
Total 13				
Mean[e]	108.8	0.02	271.0	3.7
Median	0	0	180	0

[a] Defined as 100 mm² or greater reaction with either test strength of PPD.

[b] Dose of PPD or polysaccharide (GA) in 0.1-ml volume given intradermally.

[c] Reaction size (induration) (mm²) observed within 48 hr after testing.

[d] Not tested.

[e] Obtained by dividing the sum of the square roots of individual observations by the number of observations and squaring the result.

TABLE III. Skin Reactivity of PPD Positive[a] Subjects with Active Tuberculosis to PPD and a Polysaccharide (GA) Derived from BCG Bacteria.

Subject		PPD[b]	GA[b]
R.C.		100[c]	0
T.C.		900	0
E.E.		400	4
G.F.		180	4
T.G.		550	0
C.L.		700	0
J.M.		360	6
H.S.		420	9
B.W.		225	0
V.A.		1140	0
O.B.		1050	0
H.C.		990	40
K.D.		2500	0
E.J.		1200	20
H.L.		100	0
J.M.		1280	130
M.M.		440	0
J.R.		440	0
E.S.		750	0
Total 19	Mean[d]	625	2.8
	Median	550	0
	Total pos.	19	1

[a] Reaction size to PPD >100 mm^2.

[b] Test dose 0.16 μg, given id, in 0.1-ml volume.

[c] Reaction size (mm^2).

[d] Obtained by dividing the sum of the square roots of individual observations by the number of observations and squaring the result.

equivalent doses of GA with the exception of one individual who developed 130 mm^2 of induration 48 hr after testing (Table III). As noted, her PPD response was 10-fold greater. Another patient with active tuberculosis and a low degree of reactivity of GA (40 mm^2) had a 25-fold greater PPD response (990 mm^2) (Table III).

Discussion. Three reasons seem possible to explain the negligible reactivity of the human subjects to BCG polysaccharide. First, it is known that sensitivity to the polysaccharide does not last as long as that to PPD in BCG-sensitized guinea pigs (7). After negative results were obtained in tuberculin positive patients who had no sign of active disease, it was considered possible that sensitization may have been so remote that whatever sensitivity to polysaccharide that might have once been present may have been lost. Accordingly, patients with active tuberculosis were studied and the finding of almost uniformly negative reactions to GA in this group would appear to rule out this possibility. Second, the use of a polysaccharide derived from BCG to test for dermal reactivity in patients sensitized with *M. tuberculosis*, a heterologous antigen, may have failed to detect sensitivity for reasons of antigenic specificity. Support for this is gained from studies on guinea pigs which indicated that while GA and PPD were equivalent in eliciting delayed hypersensitivity reactions in BCG-sensitized guinea pigs, the polysaccharide was significantly less active in animals sensitized with *M. tuberculosis* or *M. fortuitum* (10). Unfortunately, no BCG-sensitized subjects were available for study to further pursue this point.

Finally, the lack of reactivity to GA may reflect the general inability of polysaccharide antigens to act as immunogens for delayed hypersensitivity (8, 9). In the antigen used in the present investigations (GA) the possibility that the small amount of contaminating protein could be responsible for its reactivity, has been raised (9, 10). The persistence of sensitivity after extensive proteolysis would appear to rule this out. Other investigators, using a highly purified pneumococcal polysaccharide, have shown that guinea pigs can display well-developed delayed hypersensitivity to carbohydrate antigens (9). On the other hand, documentation of dermal reactivity other than Arthus or Jones-Mote reactions to nonhapten conjugated carbohydrate antigens in human subjects is lacking (9). In fact, an earlier study in which a different polysaccharide component of BCG culture filtrates was employed, failed to detect any skin reactivity in human subjects with positive reactions to PPD (11). In the present investigation one patient with active tuberculosis did develop 130 mm^2 of induration to GA, pointing out that some people can manifest delayed hypersensitivity to polysaccharide components of tubercle bacilli. However, such individuals clearly represent a minority and the reactions elicited are much smaller than those to corresponding doses of PPD. The use of the higher (100 TU) test

dose might have turned up more positive reactors to GA in the active tuberculosis group; however, the significance of this would only be questionable. Whatever the explanation, it is clear that polysaccharide derived from BCG bacilli is not clinically useful for the detection of either active or remotely acquired tuberculosis in human subjects.

Summary. A carbohydrate fraction of unheated BCG culture filtrates (GA) known to give equal tuberculin reactivity with PPD in BCG-sensitized guinea pigs was compared to PPD in clinical testing in human subjects. There were no immediate reactions to GA and no "false positives" in PPD negative individuals. PPD positive persons without active tuberculosis were generally unreactive to GA as were patients with active tuberculosis. The only significantly positive reaction was seen in a patient with active tuberculosis whose PPD response was 10-fold greater at the same dose level. Reasons for the unreactivity of humans to BCG polysaccharide despite well-developed PPD sensitivity are discussed.

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