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Fifteen year follow up of trial of BCG vaccines in south India for tuberculosis prevention

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A large scale community-based double blind randomized controlled trial was carried out in Chingleput district of south India to evaluate the protective effect of BCG against bacillary forms of pulmonary tuberculosis. From among 366,625 individuals registered, 281,161 persons were vaccinated with BCG or placebo by random allocation. Two strains of BCG were used, the French and Danish, with a high dose (0.1mg/0.1ml) and a low dose (0.01 mg/0.1 ml) in each strain. The entire population was followed up for 15 years by means of resurveys every 30 months, and selective follow up every 10 months and continuous passive case finding. There were 560 cases (189,191 and 180 from the high dose, low dose and placebo groups respectively) arising over 15 years, among 109,873 persons who were tuberculin negative and had a normal chest X-ray at intake. This represents a small fraction of the total incidence of 2.6 per 1000 person-years most of which came from those who were initially tuberculin positive. The incidence rates in the three "vaccination" groups were similar confirming the complete lack of protective efficacy, seen at the end of 7% years. BCG offered no overall protection in adults and a low level of overall protection (27%; 95% C.I. -8 to 50%) in children. This lack of protection could not be explained by methodological flaws, or the influence of prior sensitisation by non specific sensitivity, or because most of the cases arose as a result of exogenous re-infection. The findings at 15 years show that in this population with high infection rates and high nonspecific sensitivity, BCG did not offer any protection against adult forms of bacillary pulmonary tuberculosis.

Key words BCG trials - community based trials - tuberculosis prevention - vaccine efficacy

Tuberculosis remains one of the major health problems in India accounting for one million new cases every year. It is also the largest killer from a single major pathogen in adult life. Among women, tuberculosis accounts for as many deaths as maternal intrapartum and postpartum complications. While adequate and appropriate case management is an effective method of controlling tuberculosis, the results of even a good programme on reducing transmission rates will not be felt for several decades since most of the transmission would have occurred before detection of the case. Thus a more effective method of interrupting transmission is urgently required[1]. Vaccination with BCG is one possible method of stopping or at least slowing down transmission, since BCG is expected to prevent multiplication of bacilli in the body and prevent development of new cases of tuberculosis even though it cannot prevent primary infection.

The vaccine prepared from the Bacillus Calmette-Guerin has been in use for over 5 decades in the prevention of tuberculosis and indeed has been routinely

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used for tuberculosis control in several countries. It is not only an extensively used but also the most extensively studied vaccine and has been the subject of prolonged and bitter controversy over its efficacy. Eight major community-based prospective, controlled trials have been carried out to establish its efficacy. The observed levels of protection have ranged from 0-80 per cent[2]. Several factors, including the methodological issues[3,4] have been postulated to explain the variation in protective efficacy, but none of them has been wholly satisfactory.

A study in south India[5] had shown that BCG could protect only to the level of 31 per cent. However, the sample size in that study was small. It was felt, therefore, that a reliable estimate of the protective effect of BCG in this country was required, in order that its usefulness as a public health measure may be determined. It was against this background that the trial of tuberculosis prevention in south India[6] was designed with the objectives of obtaining *(i)* a precise estimate of the protective effect of BCG vaccination against tuberculosis in the non-infected; *(ii)* the protective effect of BCG vaccination in persons already infected; *(iii)* the protective effect of two different strains of BCG; and *(iv)* the influence of dosage of BCG on the protective effect. This study was expected to provide a conclusive answer with regard to the protective efficacy offered by BCG. For this reason, there were two expert committees that went into the possible issues that may obfuscate the interpretation of the results of the study. One dealt with vaccine related issues[7], and the other with the design of the study and the field operations (unpublished report: Indian' Council of Medical Research, Expert Committee on the Tuberculosis Prevention Trial, Madras, 1977). Both committees concluded that there were no reasons related either to the vaccine or to the conduct of the study, that could throw any doubt on the results of the study when they became known. The preliminary results of the study were published at the end of 7½ years. This showed that "BCG does not protect against bacillary pulmonary tuberculosis"[8,9]. This unexpected finding evoked a great deal of scientific interest, and several reasons were postulated for the lack of protection[10]. These issues were such that they could not be discussed even in the detailed report of the study published at 7½ years[6] since decoding of denominators had been deferred till the end of the trial. It was considered important to test some of these hypotheses, and to look for protection offered by BCG in the later years. Moreover, the number of cases occurring among those initially tuberculin negative, particularly in the younger age groups was small. Therefore, it was decided to continue the follow up for a longer period. The present report deals. with the findings of the trial after 15 years of follow up in those who were initially negative to tuberculin. The efficacy of BCG in the tuberculin positives has been briefly reported in the earlier report[8] and are not dealt with here.

Material & Methods

The trial of BCG vaccines in south India was carried out in a largely rural community with some semiurban areas. It was a large community-based double blind randomized controlled study. Two vaccine strains, one Danish and the other French, were evaluated against a placebo control, and within each strain, two doses were evaluated; 0.1 mg/0.1 ml (high dose) and 0.01 mg/0.1 ml (low dose). All individuals aged one month or more in the study area, which covered 209 *panchayats* and 9 town blocks, irrespective of the tuberculin status were allocated randomly to receive vaccine or placebo, such that one third of subjects received placebo, one third the Danish and one third the French strain. Half the individuals (randomly selected) in each of the vaccine groups received a high dose and the other half a low dose. Randomization was at the individual level and not by cluster. The total population in the study area who received the "vaccine" was 281,161 among whom there were 117,718 who were classified as "uninfected", *i.e.*, those showing a reaction size of 7mm or less to 3 IU of purified protein derivative (PPD-S) of mammalian tuberculosis. Analysis of protective efficacy was restricted to this latter group.

The details of the methodology, the basis for selection of vaccine strain and the basis for definitions of infection and disease have been presented in detail earlier[6,8,9]. A brief summary of the field work and analysis methods is presented here.

At intake, all individuals were registered on individual cards and those aged 1 yr and above were tested with both 3 IU of PPD-S and 10 units of mycobactin prepared from Battery strain (PPD-B),

randomly allocated to either forearm. At the time of testing, all individuals aged one month or more were given, by random allocation, either high dose or low dose of the vaccine or a placebo. Vaccine strains Danish 1331 and French 1173 P2 were used. Vaccine and placebo were supplied to the project staff in coded ampoules. The codes were retained at the headquarters of the Indian Council of Medical Research and were not available to any of the project staff. Rigorous steps were taken to ensure that the cold chain was not broken. To facilitate identification of each individual over the entire trial period, finger prints were taken on the back of the card on which the vaccine code was entered.

Case finding was almost continuous. All individuals aged 5 yr and above were screened by mass miniature radiography once every 30 months (regular surveys). All "suspects" (those with abnormal X-ray), symptomatics and absentees in the regular surveys were X-rayed once in 10 months (selective follow up). To identify cases occurring between these surveys, a network of passive case finding centres were established in the Government run peripheral health institutions *i.e.*, primary health centres (PHC) in the area where individuals with symptoms who self reported could be examined for tuberculosis. The medical officers of these institutions referred the symptomatics to the trial staff. These persons were X-rayed using an MMR (GENERAY, Italy) machine which was taken to the PHC at least once a week. These methods were expected to maintain a continuous surveillance and pick up almost all the cases occurring in the study area. Each round of examination (resurvey or selective followup) was concluded only after obtaining at least 90 per cent coverage.

All X-rays were read independently by two readers, who were unaware of the identity of the individual or the vaccine group to which he/she belonged. In case of disagreement in X-ray reading between the readers, X-rays were submitted to a third ("umpire") reader. One spot and one overnight specimens of sputum were collected from each individual whose X-ray was read as abnormal by either reader and subjected to smear for acid fast bacilli (AFB) and culture for *Mycobacterium tuberculosis*. Finger prints were again taken at the time of sputum collection. The identification of the individual from whom the specimen was collected was not available to the bacteriologist.

Case-status was objective and very rigourously defined. Only those who were culture positive on at least one specimen of sputum were regarded as cases of tuberculosis for estimating the protective efficacy of BCG vaccination. The scar status, tuberculin status or the vaccination status were not considered in defining a "case".

The study was started in 1968 and intake phase was completed by 1971. Fifteen years follow up was completed in September 1987.

Statistical methods: Once a "case" was defined, the finger prints taken at the time of sputum collection were compared with those at intake to ensure identity. Since only 52 per cent of the total population remained at the end of the study period, denominators were defined in terms of person-years. If a person was seen at least once during one round of examination (resurvey or selective follow up or passive case finding) he or she was considered to have contributed 2½ person-years to the study. The incidence was calculated from the total number of cases occurring in each round divided by the number of person-years of observation in the round. Protective efficacy was calculated from the formula 100 (1-RR), where RR is the risk ratio, obtained from the ratio of incidence among the vaccination and the placebo groups. Confidence intervals were calculated using appropriate formulae[11].

The "vaccinated" cohort considered for analysis is those with 0-7 mm reaction size to PPD-S and normal chest X-ray at intake. Persons less than 10 yr of age at intake were ineligible for X-ray. It was assumed that these persons were normal on chest X-ray.

Results

Epidemiological characteristics of the study area: The study area selected included 209 contiguous *panchayats* and one town, from Thiruvellore and Thiruttani *taluks* in Chingleput district, Tamil Nadu, south India. A detailed description of the study area along with the baseline characteristics has been reported previously[6]. The overall prevalence of tuberculosis infection was 54 per cent in males and 46 per cent in females with an annual risk of infection of 4.1 per cent. The prevalence and incidence (over the first 2½ years) of bacillary disease was 1055 per 100,000 and 250 per 100,000 per annum

respectively[6]. These characteristics were examined over 15 years, to document the trend of the disease over the duration of the study period.

Prevalence of culture positive cases, at each of the 7 rounds, is shown in Table I. The prevalence of bacillary disease has come down from 1055 per 100,000 to 775 per 100,000 over 15 years. In males, this decrease has been from 1655 to 1307, and in females from 454 to 250 per 100,000. The age distribution was uniform over the years.

The incidence of culture positive cases over 15 years (Table II) has also shown a decline from 383 to 232 per 100,000 per year; from 576 to 361 and from 187 to 102 per 100,000 per year, in males and females respectively. During the first round of follow up, 94 and 92 per cent of new cases in males and females respectively came from those initially infected, *i.e.*, those showing a reaction size of 12 mm or more to PPD-S. This proportion showed a decline over the years; but even so, during the sixth round of follow up, 80 per cent of new cases in males and 78 per cent of new cases in females came from those initially infected (Table III).

Base-line characteristics of the study population: Since the allocation of individuals to the three groups, 0.1 mg of BCG, 0.01 mg of BCG and Placebo, was at the individual level, it was expected that the distribution of persons allocated to the three groups would be similar with respect to various known and unknown factors. The distribution of persons in the three groups according to some important factors is shown in Table IV. The number of persons allocated to each of the three groups was similar with over 93,000 persons in each group. The age distribution of the individuals and the proportion of males and females was similar in each group. This similarity was also maintained in the three groups with respect to initial tuberculin status. Equal number of individuals had been allotted to the "Danish" and the "French" strains. Thus the three groups were similar with respect to age, sex, initial tuberculin status and strain of vaccine used.

Coverages: The entire population was surveyed seven times using the same techniques. At each survey, the coverage of the available population was over 90 per cent. However, the numbers in the original cohort of individuals included for "vaccination" declined. These

Table I. Prevalence of culture positive cases at rounds I-VII

Age group, yr	Prevalence (rate per 100,000) at round						
	I	II	III	IV	V	VI	VII
				Males			
10-24	226	179	146	113	180	151	79
25-44	1934	1787	1908	1629	1588	1606	1369
45+	3613	3818	3869	3719	3490	3389	3248
10+	1655	1634	1675	1528	1484	1454	1307
	(37519)	(42788)	(44449)	(49479)	(51303)	(53032)	(52729)
				Females			
10-24	124	120	80	95	85	89	59
25-44	546	449	485	421	310	256	331
45+	867	642	792	790	430	574	399
10+	454	364	396	377	249	262	250
	(37386)	(41710)	(44347)	(49225)	(5 1359)	(54138)	(55283)
				Both sexes			
10-24	176	150	113	104	134	120	69
25-44	1206	1085	1162	996	918	898	825
45+	2306	2307	2405	2325	2034	2050	1893
10+	1055	1000	1037	953	868	859	775
	(74905)	(84498)	(88796)	(98704)	(102662)	(107170)	(108012)

Standardised rates calculated on the basis of the age-sex distribution of population at round I

Figures in parentheses give number X-rayed

Table II. Incidence of culture positive cases

Age group, yr	Incidence (rate per 100,000) at round					
	I-II	II-III	III-IV	IV-V	V-VI	VI-VII
			Males			
10-24	137	118	73	103	65	83
25-44	627	639	417	418	449	403
45+	1270	1012	736	747	767	787
10+	576	511	350	365	365	361
	(29463)	(3263 1)	(35196)	(38655)	(40033)	(40103)
			Females			
10-24	57	66	41	43	37	35
25-44	231	166	145	107	125	132
45+	317	326	263	145	215	156
10+	187	165	133	92	113	102
	(29072)	(32432)	(35792)	(38850)	(41243)	(42634)
			Both sexes			
10-24	100	94	58	75	52	61
25-44	413	383	270	250	274	256
45+	814	684	509	459	503	485
10+	383	339	242	230	240	232
	(58535)	(65063)	(70988)	(77505)	(81276)	(82737)

Standardised rates calculated on the basis of the age-sex distribution of population at round I
Figures in parentheses give number X-rayed

Table III. Incidence of culture positive cases, according to size of reaction to PPD-S at round I (Age group: 5+ yr)

Reaction to PPD-S (Round I)	Incidence (rate per 100,000) at round					
	I-II	II-III	III-IV	IV-V	V-VI	VI-VII
			Males			
0-11 mm	48	64	62	109	109	156
	(14199)	(14717)	(16139)	(15263)	(13975)	(12415)
12+ mm	775	647	481	538	582	617
	(19368)	(15641)	(14544)	(13863)	(12591)	(11351)
% of cases with 12+ mm	94	91	87	83	84	80
			Females			
0-11 mm	25	48	24	34	36	59
	(16618)	(16013)	(17007)	(15451)	(13562)	(1 1623)
12+ mm	290	212	213	145	202	208
	(16340)	(13550)	(12689)	(11937)	(10954)	(10067)
% of cases with 12+ mm	92	82	90	81	85	78
			Both sexes			
0-11 mm	36	55	43	72	73	109
	(30817)	(30730)	(33146)	(30714)	(27537)	(24038)
12+mm	554	445	356	356	405	425
	(35708)	(29191)	(27233)	(25800)	(23545)	(21418)
% of cases with 12+ mm	94	89	89	83	85	80

Figures in parentheses give number X-rayed

losses were similar in the three groups, as can be seen from Table V. On an average, 11 per cent had either died or migrated by 2% years, 17 per cent by 5 years, 22 per cent by 7½ years; 28 per cent by 10 years, 35 per cent by 12½ years and 42 per cent by 15 years. Thus, at the 7th round, 52 per cent of the cohort was examined, 7 per cent had died, 35 per cent had migrated and 6 per cent absented themselves for the X-ray examination.

Case finding: Most of the cases were diagnosed at the time of resurveys where the whole population was X-rayed. However, a proportion of the population (about 10%) was re-X-rayed during selective follow up, in order to diagnose cases arising out of those who were "suspects" in the resurvey rounds. Similarly, symptomatic individuals seeking treatment between the rounds (about 2%) were investigated at the passive case finding centres. Similar numbers of individuals were examined at each round by each of these methods in the three groups. There was no difference in diagnostic methods employed between the three groups as can be

Table IV. Base-line characteristics of the study population

Factor	0.1 mg of BCG	0.01 mg of BCG	Placebo
Age, yr :			
1m-4	11350 (12.16)	11413 (12.22)	11497 (12.18)
5-9	13138 (14.08)	13187 (14.11)	13165 (13.94)
10-14	12615 (13.52)	12724 (13.62)	12911 (13.67)
15-24	15508 (16.62)	15564 (16.66)	15593 (16.51)
25-44	25181 (26.99)	25110 (26.88)	25496 (27.00)
45+	15520 (16.63)	15433 (16.52)	15757 (16.69)
Sex :			
Males	47247 (50.63)	47514 (50.85)	48039 (50.88)
Females	46065 (49.37)	45917 (49.15)	46379 (49.12)
Strain of BCG :			
Danish	47241 (50.63)	47039 (50.39)	47625 (50.44)
French	46071 (49.37)	46392 (49.65)	46793 (49.56)
Tuberculin status (mm) :			
0-7	39681 (42.53)	39012 (41.75)	39025 (41.33)
8-15	14212 (15.23)	14297 (15.30)	14459 (15.31)
16+	35723 (38.28)	36415 (38.98)	37220 (39.42)
Absent	3696 (3.96)	3707 (3.97)	3714 (3.93)
Total	93312 (100.00)	93431 (100.00)	94418 (100.00)

Figures in parentheses give percentages to total

Table V. X-ray coverages over 15 years

Period of		0.1 mg of BCG		0.01 mg of BCG		Placebo	
0	Cohort	37005	100.00	36463	100.00	36405	100.00
I	X-rayed	30670	82.88	30119	82.60	30139	82.79
(0-2.5)	Dead	582	1.57	673	1.85	604	1.66
	Left	3445	9.31	3438	9.43	3422	9.40
	Absent	2308	6.24	2233	6.12	2240	6.15
II	X-rayed	28382	76.72	28050	76.96	27997	76.92
(2.5-5)	Dead	1026	2.77	1086	2.98	1022	2.81
	Left	5272	14.25	5073	13.92	5223	14.35
	Absent	2314	6.26	2240	6.15	2157	5.93
III	X-rayed	27084	73.26	26591	73.02	26648	73.23
(5-7.5)	Dead	1411	3.82	1476	4.05	1427	3.92
	Left	6585	17.81	6390	17.55	6471	17.78
	Absent	1891	5.11	1958	5.38	1841	5.06
IV	X-rayed	24719	66.91	24281	66.71	24241	66.67
(7.5-10)	Dead	1788	4.84	1830	5.03	1794	4.93
	Left	8442	22.85	8317	22.85	8300	22.83
	Absent	1997	5.41	1970	5.41	2023	5.56
V	X-rayed	22095	59.86	21681	59.62	21709	59.78
(10-12.5)	Dead	2200	5.96	2241	6.16	2193	6.04
	Left	10550	28.58	10355	28.48	10421	28.70
	Absent	2067	5.60	2086	5.74	1989	5.48
VI	X-rayed	19228	52.14	18987	52.28	18857	52.00
(12.5-15)	Dead	2531	6.86	2572	7.08	2537	7.00
	Left	13046	35.38	12757	35.12	12865	35.47
	Absent	2072	5.62	2003	5.52	2007	5.53

31, 69, 71, 115 and 125 culture positive cases diagnosed during periods I to V respectively were excluded for subsequent periods

seen from Table VI. Thus the three groups were similar with respect to susceptibility (base-line characteristics), surveillance and diagnostic testing (case-finding).

Protective efficacy of BCG: Protective efficacy was calculated from among those who were considered tuberculin negative *(i.e.,* reaction size of 0-7 mm to PPD-S) at intake and infants- who were assumed to be non-reactors. Those who had normal chest X-ray, or were ineligible for X-ray were considered as non-cases at intake. A new (incidence) case is defined as an individual with an abnormal X-ray, who has produced at least one positive culture on sputum examination. The incidence of new cases by vaccination status from among these persons in each time period is shown in Table VII.

It was seen that in the first 5 years after vaccination, the average annual incidence of tuberculosis was higher in the vaccinated groups as compared to that in the placebo group. In the next 7½ years, there was some evidence of protection with a higher incidence of disease in the placebo group as compared to that in the group receiving the high or the low dose of BCG. Between 12½ to 15 years, however, there was again an increase in the annual rates among the vaccinated as compared to that among those receiving the placebo. Considering the entire period of follow up, however, there was no difference in the attack rate of tuberculosis in the three groups, the average annual rates being 50, 51 and 48 per 100,000 in the high dose, low dose and placebo groups respectively. The differences within each period, in favour of, or against the vaccine were not statistically significant, except for low dose BCG in the second

Table VI. Percentage of total examined in the three "vaccine" groups for each of three case finding methods over 15 years

Period of follow up, yr	"Vaccine" group	% of total examined in each of		
		Resurvey follow up	Selective follow up	Passive follow up
0 - 2.5	0.1 mg BCG	33.8	35.6	34.4
	0.01 mg BCG	33.1	32.4	34.6
	Placebo	33.1	32.0	31.0
		(82152)	(3399)	(1161)
2.5 - 5	0.1 mg BCG	33.6	33.4	36.3
	0.01 mg BCG	33.2	33.0	32.7
	Placebo	33.2	33.5	31.2
		(82009)	(11039)	(1489)
5 - 7.5	0.1 mg BCG	33.7	34.4	34.9
	0.01 mg BCG	33.1	32.8	31.9
	Placebo	33.2	32.8	33.2
		(77322)	(11205)	(2137)
7.5 - 10	0.1 mg BCG	33.7	34.1	34.7
	0.01 mg BCG	33.2	33.5	32.2
	Placebo	33.1	32.4	33.1
		(69882)	(9333)	(1884)
10 - 12.5	0.1 mg BCG	33.7	33.8	34.1
	0.01 mg BCG	33.1	33.4	33.2
	Placebo	33.2	32.7	32.7
		(63616)	(5172)	(1457)
12.5 - 15	0.1 mg BCG	33.7	34.0	33.9
	0.01 mg BCG	33.2	34.2	33.7
	Placebo	33.2	31.8	32.4
		(549 14)	(6397)	(1201)

Figures in parentheses give total examined

Table VII. Annual incidence by vaccination status for all ages

Period of follow up, yr	0.1 mg of BCG		0.01 mg of BCG		Placebo	
	Person-years	No. of cases	Person-years	No. of cases	Person-years	No. of cases
0 - 2.5	76675	11 (14)	75298	14 (19)	75348	6 (8)
2.5 - 5	70955	23 (32)	70125	34 (48)	69993	12 (17)
5 - 7.5	67710	25 (37)	66478	17 (26)	66620	29 (44)
7.5 - 10	61797	34 (55)	60702	35 (58)	60603	46 (76)
10 - 12.5	55238	35 (63)	54202	44 (81)	54272	46 (85)
12.5 - 15	48070	61 (127)	47468	47 (99)	47142	41 (87)
0- 15	380445	189 (50)	374273	191 (51)	373978	180 (48)

Figures in parentheses give average annual rate per 100,000

period. Thus, the overall protection, for all ages, must be taken to be nil.

Although the estimated protective efficacy for all ages over 15 years was nil, the possibility that BCG may offer some protection in children was examined (Table VIII). It is seen that in children aged 1 month to 4 yr, the protective efficacy is of the order of 32 and 18 per cent respectively for the high and low doses of BCG, and in children aged 5 to 9 yr, it is 24 and 23 per cent; *i.e.*, in children aged 1 month to 9 yr, a moderate protective efficacy of 27 and 21 per cent is seen with the high and low doses of BCG respectively. However, these levels of protection were not statistically significant with the limits of the confidence intervals lying on either side of zero. In age groups 10- 14 yr and above, the attack rates in those who received either dose of vaccine is slightly higher as compared to that in those who received the placebo, except for low dose of BCG in age group 10-14 yr. None of these differences were statistically significant.

The protective efficacy of BCG in children, aged 1 month to 9 yr, was examined by period of follow up, by comparing the incidence rates of tuberculosis in the vaccinated and placebo groups (Table IX). The pattern of results in these children is similar to that seen among persons of all ages. The incidence rate among those vaccinated with either dose of BCG in the first five years of follow up was higher than that in those receiving placebo; these differences were not statistically significant. Subsequently upto 12½ years, the incidence rates in the placebo group was much higher ($P<0.01$) as compared to that in the vaccinated groups, suggesting a protection of 69 per cent with the high dose and 59 per cent with the low dose. Between 12½ to 15 years, the incidence rates in the vaccinated group were again higher than that in the placebo group; these differences were not statistically significant. Nevertheless. over 15 years, the average annual rates were 20, 22 and 27 per 100,000 in those receiving the high dose, low dose. or placebo respectively resulting in a marginal protection of 27 per cent with the high dose and 21 per cent with the low dose BCG respectively. However, as the confidence intervals are wide, and lie on either side of zero, the 'protection' seen in this age group must be interpreted with caution.

Table VIII. Protective efficacy of BCG vaccine by age

Age group, yr	0.1 mg of BCG		0.01 mg of BCG		Placebo		Prot. effect (%)	
	Person-years	No. of cases	Person-years	No. of cases	Person-years	No. of cases	0.1 mg of BCG (95% CI)	0.01 mg of BCG (95% CI)
1m-4	110150	15 (14)[a]	110118	18 (16)	110280	22 (20)	32 (-32, 65)[b]	18 (-53, 56)
5-9	109425	29 (27)	108212	29 (27)	108973	38 (35)	24 (-23, 53)	23 (-25, 53)
1m-9	219575	44 (20)	218330	47 (22)	219253	60 (27)	27 (-8, 50)	21 (-15, 46)
10-14	62342	45 (72)	61478	35 (57)	61560	42 (68)	-	17 (-31, 47)
15s-24	41378	49 (118)	40420	45 (111)	38960	34 (87)		
25-34	28365	23 (81)	25775	29 (113)	26115	20 (77)		
35+	28785	28 (97)	28270	35 (124)	28090	24 (85)		
Total	380445	189 (50)	374273	191 (51)	373978	180 (48)		

[a] Figures in parentheses give average annual rate per 100,000

[b] 95% confidence interval

Protective efficacy according to strain of BCG: The overall protection by BCG, for all ages, was seen to be nil (Table VII). The very remote possibility of one strain being associated with a protective and the other with a harmful effect was examined. As no such difference was observed the results of both strains have been combined. It was also examined whether the moderate protective effect seen among children, aged 1 month to 9 yr, was similar with the two strains (Table X, upper half). With the high dose of BCG, the protective efficacy was 27 per cent for either strain (P>0.9), and with the low dose of BCG, it was 25 per cent for Danish strain and 17 per cent for French strain (P>0.7), the levels of protection in all four instances being statistically not significant. Thus, the two strains behaved similarly in the children studied.

Influence of non-specific sensitivity: Influence of prior exposure to environmental mycobacteria on the protective effect of BCG was examined (Table X, lower half). A reaction size of 10 mm or more (reactors) to PPD-B is taken to indicate prior exposure to environmental mycobacteria[6]. Only children are included in this analysis, as the proportion of adults who are non-reactors to

Table IX. Annual incidence by vaccination status in children aged 1 month to 9 yr

Period of follow up, yr	0.1 mg of BCG		0.01 mg of BCG		Placebo	
	Person-years	No. of cases	Person-years	No. of cases	Person-years	No. of cases
0 - 2.5	43790	5 (11)	43305	6 (14)	43555	3 (7)
2.5 - 5	41390	7 (17)	41112	9(22)	41388	3(7)
5 - 7.5	39465	3 (8)	39150	3 (8)	39528	12 (30)
7.5 - 10	36153	6 (17)	36000	6 (17)	36078	8 (22)
10 - 12.5	31995	3 (9)	31833	7 (22)	31862	19 (60)
12.5 - 15	26782	20 (75)	26930	16 (59)	26842	15 (56)
0 - 15	219575	44 (20)	218330	47 (22)	219253	60 (27)

Figures in parentheses give average annual rate per 100,000

Table X. Influence of strain of BCG and non-specific sensitivity on protective efficacy

	0.1 mg of BCG	0.01 mg of BCG	Placebo	Prot. effect (%)	
				0.1 mg of BCG	0.01 mg of BCG
(a) Strain of BCG :					
Danish strain					
Person-years	110620	108725	109315	27	25
No. of cases	23	23	31	(-26,57)	(-28, 57)
French strain					
Person-years	108955	109605	109938	27	17
No. of cases	21	24	29	(-28, 58)	(-43, 52)
(b) Non-specific sensitivity :					
0-9 mm to PPD-B					
Person-years	125985	122268	122313	39	12
No. of cases	20	28	32	(-6, 65)	(-45.47)
10+mm to PPD-B					
Person-years	93590	96062	96940	11	32
No. of cases	24	19	28	(-53.49)	(-23, 62)

Figures in parentheses give 95% confidence limits

PPD-B is very small. With the high dose of BCG, the protective efficacy was 39 per cent among the non-reactors (0-9 mm to PPD-B) and 11 per cent among the reactors (P>0.2), and with the low dose of BCG, it was 12 per cent among the non-reactors and 32 per cent among the reactors (P>0.2). In all four instances the confidence intervals were wide, and included zero, the levels of protection being statistically not significant. Thus the protective efficacy is not influenced by prior exposure to environmental mycobacteria as measured by size of reaction to PPD-B.

Also, if prior exposure to environmental mycobacteria was protective, the attack rates in the reactors should be much less than that in the non-reactors, at least in the placebo group, where there has been no interference with BCG. It is seen, however, that the attack rates among the reactors is 28.9 per 100,000 as compared to 26.2 per 100,000 in the non-reactors (P >0.7). This is contrary to what is expected if prior non-specific sensitization offered protection.

Discussion

Chingleput was chosen as the study area for the BCG trial since the area had a high incidence of tuberculosis as well as a high prevalence of sensitivity to non-tuberculous mycobacteria; in addition BCG was not a current Public Health measure. This report presents the findings of the trial after 15 years of follow up. BCG has offered no overall protection in adults and a low level of overall protection (27%; 95% C.I.: -8% to 50%) in children.

The tendency for excess cases among the vaccinated seen in the first five years reappears at 12½ to 15 years in contrast to the finding of fewer cases in the vaccinated between 5 and 12½ years. The same trends are seen even in those who were less than 10 yr old at intake. The varying pattern of results by period of follow up seen in this trial was also observed in the BCG trial at Madanapalle, south India[5]. More cases were seen among the vaccinated during the first three years, thereafter up to the 9th year fewer cases were seen among the vaccinated indicating protection by BCG, and during the last two to three periods the incidence among the vaccinated was higher than that among the controls. The authors explain that the vaccinated cases occur later than the unvaccinated and that the group of vaccinated persons continue to produce cases when the corresponding controls have ceased to do so. In the British BCG trial[12], it was observed that in the sixth period of observation, *i.e.*, from 12½ to 15 years, and beyond, the incidence of cases among the vaccinated was higher than that among the unvaccinated controls contrary to what was seen in the earlier periods. A gradual increase in the level of resistance in the unvaccinated group and a gradual decrease in the level of resistance in the vaccinated groups are given as possible reasons.

The estimates of protective efficacy are based on rates per 100,000 person-years obtained from complete decoding of the trial population. They need to be evaluated taking into account several criticisms and hypotheses that have been put forward after the publication of the earlier report at 7½ years.

Clemens *et al*[3] have suggested that methodological flaws could account for the variations in the observed efficacy levels of protection by BCG observed in the various trials. They cited four main sources of bias, namely, susceptibility bias, surveillance bias, diagnostic testing bias and diagnostic interpretation bias, and suggested that the Chingleput trial might have suffered from the first three. Such issues could not be addressed earlier as the trial was ongoing and the key to the vaccine codes was not available with the project staff. With complete decoding, however, it has become obvious (Tables IV-VI) that the study population was equally distributed between the three groups with respect to base-line characteristics that could have influenced the development of tuberculosis. The coverages of the population available at each round were similar for the three groups, as also the proportion of the original cohort available for follow up at each round. The proportion of dead and those who had migrated were also very similar for the three groups. Thus biases in allocation (susceptibility) and surveillance (differential follow up of the groups) could not have operated in the trial. Three methods were used for case detection which ensured that almost all cases arising in the area would have been registered. The number of persons examined at each round by each of these methods was equally distributed between the three groups. The trial covers a large population, in fact the largest among the reported

community trials of BCG efficacy, which has been systematically and meticulously followed up for 15 years with bias free management (the trial was kept double blind) and objective definitions of case (bacteriological confirmation) in order to avoid diagnostic testing bias. The trial was designed to detect a difference of 50 per cent with 80 per cent power, based on the assumption that the prevalence of bacillary disease in the area would be about 4 per 1000. However, it was seen that the prevalence was actually 11 per 1000, about 1½ times the expected value. Thus, a re-examination of the trial size showed that the sample size of the trial was large enough to detect a vaccine efficacy of 50 per cent (even among the subgroups).

It is thus unlikely that methodological flaws or lack of power could account for the lack of protection seen in the trial. This has been endorsed by a committee of experts convened by the WHO and the ICMR in Madras in 1977 before the analysis of results was begun. This committee reviewed carefully both the design and the field procedures and concluded that there were no obvious deficiencies that could influence the resultsof the trial (unpublished report: Indian Council of Medical Research, Expert Committee on the Tuberculosis Prevention Trial, Madras, 1977).

The vaccine strains have been evaluated carefully, both prior to selection and during the vaccination phase, and were found to produce good post vaccination allergy. The evidence regarding lack of influence by strain variation has been discussed at length in previous report[6,8,9]. The consensus appears to be that strain differences or variations, or mutations in the strains used, could not have contributed to the lack of protective efficacy. *In vitro* tests and animal experiments (VC) have shown that there were no differences in the strains used.

The high prevalence of non-specific sensitivity is another factor that is invoked as a reason for the lack of protection by BCG[10]. It is argued that environmental mycobacteria have sensitizing properties similar to those of BCG and that BCG does nothing to add to this prior protection. This hypothesis is suggested by the findings in mice[13] and guineapigs[14] in which challenge with a variety of non-tuberculous mycobacteria (NTM) induced protection and late immunization with BCG could not improve on this protection. These results were confirmed more recently by Narayanan *et al*[15]. This hypothesis would appear to be plausible given the fact that NTM were isolated from 8.6 per cent of sputum specimens collected from the study area[16]. If this were true in humans and BCG does indeed have some protective efficacy, this should be seen in those who were initially non-reactors to PPD-B, in whom the possible protection offered by BCG would not be "masked" by prior exposure to environmental mycobacteria. The data, however, show that even in children below 10 yr of age who were initially not reacting to PPD-B, the protective efficacy, with both doses of BCG, was only marginal and not statistically significant. Also, the protective efficacies seen among non-reactors to PPD-B did not differ significantly from those seen among reactors to PPD-B. Further, if exposure to environmental mycobacteria could afford protection, then there should be substantially less cases among those who were initially infected with such mycobacteria. Such protection is also not seen (Table X); in the placebo group, in children reacting with 0-7 mm to PPD-S, the attack rate (28.9 per 100,000) among "reactors" to PPD-B was similar to that (26.2 per 100,000) seen among "non-reactors" to PPD-B. Similar findings are seen in the study in Puerto Rico[17,18] where the same low protection (31%) was seen in those positive and those negative to the high dose test. Thus, it seems unlikely that non-specific mycobacteria could have masked the effect of BCG if the later had indeed been protective in human beings.

BCG is not expected to offer protection against exogenous reinfection. Since Chingleput is known to be an area with a high prevalence of tuberculosis, it has been hypothesized that most of the disease that occurred in the study population, was a result of exogenous reinfection and that this could be a reason why BCG had failed to show protection in the study area. It has been suggested[19] that if most of the disease was from endogenous reactivation, then a great proportion of new cases arising in the study area would come from those initially infected. The data (Table III) show that even after 15 years, 80 per cent of cases have come from among those who were found infected (12 mm or more to PPD-S) at intake. It has also been suggested[20] that if the disease was due to endogenous reactivation, the proportion of new cases with INH resistance would probably remain constant over the years and would be substantially less than that among all prevalence cases.

In this area, the prevalence of INH resistance has remained steady over the years even in fresh cases (Table XI). There is no significant difference in the trend. When this is seen in conjunction with the fact that the proportion of new cases occurring from the initially infected has declined only very slightly from 94 to 80 per cent over 15 years, it is possible that most of the cases occur due to endogenous reactivation. Though this is indirect epidemiological evidence, it would appear that exogenous reinfection could not have contributed to a significant proportion of disease and masks a protective efficacy due to BCG, if such an effect had indeed been present. However, whether a given case has occurred as a result of endogenous reactivation or exogenous reinfection is very difficult to decide.

Genetic differences in human ethnic groups leading to different mechanisms of action of BCG in different races was postulated as yet another reason why BCG failed to show any protection in this population. Investigations carried out by the Tuberculosis Research Centre show that no significant differences in the patterns of macrophage induced killing of *M. tuberculosis* was found between Indian and British subjects either before or after BCG vaccination[21,22].

This trial was designed to test the value of BCG as a public health measure, *i.e.*, in cutting down transmission of tuberculosis. This potential benefit of BCG needs to be reviewed in the light of the epidemiology of tuberculosis in the study area and in other parts of India. The study area is characterised by high infection rates in children and high disease rates in middle aged and older people. Unlike in Britain[12] most of the disease (94 to 80%) came from those who were infected rather than uninfected initially. The pattern of incidence is similar in Madanapalle[23] and Bangalore[24] where 75 and 65 per cent of the burden of disease, respectively, was contributed by those initially infected. Thus even if BCG offered protection in those initially uninfected (which it does not) the public health value of BCG can be only in preventing childhood mortality caused by disease resulting from haematogenous spread. The impact on infectious cases can at best be only minimal. Styblo and Meijer[25] examining the impact of BCG vaccination programmes in children and young adults on the tuberculosis problem, have shown that the impact of such vaccination on the incidence in persons aged over 30 yr, is "not readily discernible". Rodrigues and Smith[26] arrived at similar conclusions after reviewing the efficacy of BCG and the epidemiology of tuberculosis in several geographical regions. Sutherland[1] has argued that a policy of BCG vaccination among infants or adolescents cannot prevent disease in older individuals who would have already been infected and hence can have no impact on transmission.

Table XI. Resistance to INH of culture positive cases over 15 yr

Round of examination	Prevalence cases		Incidence cases	
	no.	% resistant	no.	% resistant
I	707	12.7	-	
II	729	18.4	519	5.8
III	792	21.5	481	10.8
IV	884	15.4	409	5.6
V	824	20.1	423	10.9
VI	873	21.2	457	10.7
VII	776	21.4	443	10.8

Resistant to INH : MIC >1µg/ml

The impact of BCG vaccination on childhood forms of tuberculosis was not investigated in the Chingleput trial. This was probably because, BCG was expected to reduce the risk of transmission by reducing the sputum positive cases and this was rare in children. Since the publication of the 7½ year report, this question has been extensively investigated by the World Health Organisation[27,28]. The protection has ranged from 0-95 per cent with case-control studies and less than 50 per cent from contact studies. Observational studies generally suffer from biases which may even be undetectable. Not withstanding this, the efficacies reported have repeated the pattern seen in the community-based studies.

In conclusion, the Chingleput trial, which was designed to evaluate BCG as a public health measure, has shown that BCG offers no protection against adult type bacillary tuberculosis. Consequently, BCG cannot be expected to reduce the transmission due to tuberculosis. This observation of failure to protect, could not be attributed to defects in methodology, inadequate

sample size, prior exposure to environmental mycobacteria or to most of the disease being a result of exogenous reinfection. These unexpected results have led to several studies which would eventually increase our understanding of the host responses and immune mechanisms in tuberculosis.

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