

VACCINES CONTAINING *MYCOBACTERIUM VACCAE* AND THEIR USE IN THE CHILDREN OF LEPROSY PATIENTS IN IRAN

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ABSTRACT

The policy of vaccinating children who live with leprosy patients, and who have responses to leprosin A of 2mm or less, with BCG+killed *Mycobacterium vaccae* if they lack a BCG scar, or with killed *M. vaccae* alone if they have a BCG scar, has been followed over 3-4 years in two centers in Iran. Judged on the basis of skin test conversion to leprosin positivity, the policy has been highly successful. A way in which the vaccines may work is discussed, and supported by differences in apparent efficacy between the two study centers.

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INTRODUCTION

Despite ever-improving treatment of leprosy, there continues to be a need for effective vaccination against the disease, especially for those at particular risk such as the children of patients living in leprosy sanatoria or colonies. There are several publications about the use of vaccines containing killed *Mycobacterium vaccae* against leprosy,^{2-4,10} and in one of these it was recorded that a policy for their use had been adopted by Baba Baghi Leprosy Sanatorium in Iranian Azarbaijan.⁴ Subsequently this same policy has been applied in Behkadeh leprosy colony, Khorassan, Iran. The outcome of 3-4 years implementation of the policy is reported here.

MATERIALS AND METHODS

The Children Studied

These were children or grandchildren of leprosy patients living in Baba Baghi Leprosy Sanatorium or in Behkadeh leprosy community. All the children were skin tested and

examined for the scars of past BCG vaccination, and on the basis of the findings they were either vaccinated or considered to be optimally protected. In Baba Baghi, 139 healthy children (mean age 6.1 years; range 1-20 years) were examined in 1987 and 1988; sixty four (46%) of them required additional vaccination. In Behkadeh 490 young children (mean age 3.9 years; range 1-7 years), and 440 older children (mean age 10.9 years; range 1-17 years) were examined in 1988 and 1989; 313/490 (64%) and 338/440 (77%) required vaccination respectively (see Table I).

Skin Testing

The four reagents used were from the series of new tuberculin.⁷ These were tuberculin 2 µg/ml, leprosin A 10 µg/ml,⁹ scrofulin 2 µg/ml (prepared from *M. scrofulaceum*), and vaccin 20 µg/ml (prepared from *M. vaccae*). The dose of each reagent is 0.1ml given by intradermal injection on the volar aspects of the forearms, with at least 10cm between injections. In Behkadeh, children aged less than 3 years were tested with tuberculin on their left and with leprosin on their right, and those aged 3 years or more were tested with tuberculin and leprosin on their

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TABLE I. The children studied, and the vaccinations they received.

| | | |
|--------------------------------------|---------|---------------------|
| BABA BAGHI | | |
| No BCG scar, all needed vaccine B | 14/14 | mean age 4.6±4.8 Y |
| With BCG scar, not vaccinated | 75/125 | mean age 6.5±4.3 Y |
| With BCG scar, needing vaccine D | 50/125 | mean age 5.8±3.5 Y |
| BEHKADEH | | |
| Children under 7 years of age | | |
| No BCG scar, not vaccinated | 18/177 | mean age 3.4±1.5 Y |
| No BCG scar, needing vaccine B | 146/177 | mean age 4.3±1.4 Y |
| No BCG scar, needing vaccine D | 13/177 | mean age 4.1±1.3 Y |
| With BCG scar, not vaccinated | 159/313 | mean age 3.6±1.5 Y |
| With BCG scar, needing vaccine D | 154/313 | mean age 3.8±1.7 Y |
| Children aged 7 years or more | | |
| No BCG scar, not vaccinated | 7/157 | mean age 13.1±3.5 Y |
| No BCG scar, needing vaccine B | 143/157 | mean age 10.1±2.9 Y |
| No BCG scar, needing vaccine D | 7/157 | mean age 11.6±4.0 Y |
| With BCG scar, not vaccinated | 95/283 | mean age 11.5±2.0 Y |
| With BCG scar, needing vaccine D | 188/283 | mean age 11.0±2.5 Y |

Note: There was no significant difference between the numbers of children without a BCG scar requiring vaccination in the two centers. However, significantly more children with a BCG scar required further vaccination in Behkadeh than in Baba Baghi.

left, and scrofulin and vaccin on their right forearm. In Baba Baghi, all except one of the children were carefully examined for scars of previous BCG vaccination. All children were tested when first seen, and many were retested one to four

years after vaccination.

Vaccination Procedures

The two vaccines used were vaccine B which contained 10^6 live BCG (Glaxo) plus 10^7 killed *M. vaccae* per dose of 0.1 ml, and vaccine D which contained 108 killed *M. vaccae* alone per 0.1 ml dose.¹⁰ The *M. vaccae* (NCTC 11659), grown on Sauton's medium solidified with 1.5% agar, were harvested into M/15 borate buffered saline (pH 8.0) at concentrations of 1 mg and 0.1 mg wetweight of bacilli per ml. These suspensions, distributed in 5 ml multidose vials, were killed by autoclaving at 15 lbs/sq. inch for 15 minutes. The lighter suspension was then used to make up manufacturers' ampoules of freeze-dried BCG. Either vaccine was administered as an intradermal injection over the upper part of the left deltoid muscle.

Children without BCG scars and with responses to tuberculin of less than 5 mm, were vaccinated with vaccine B. Those without BCG scars with reactions to tuberculin of 5 mm or more, and to leprosin of 2 mm or less, were vaccinated with vaccine D, as were children with BCG scars but who had reactions to leprosin of 2 mm or less. Those with reactions to tuberculin of 5 mm or more, and to leprosin of 3 mm or more, were not vaccinated, whether they had a BCG scar or not.

Follow-ups

One, two, three, and four years after vaccination, many of the children were followed up by repeating the skin tests and examining the scars of our vaccinations.

TABLE II.
The initial skin test results obtained.

| | Tuberculin | Leprosin A | Scrofulin | Vaccin |
|-------------------|---------------------------|-------------------------|-------------------------|--------------------------|
| BABA BAGHI | | | | |
| aged 1-7 years | 2/14(14%) | 2/14(14%) | 2/14(14%) | 2/14(14%) |
| No BCG scar | | | | |
| With BCG scar | 14/55(25%) n.s. | 30/55(55%) P<0.007 | 18/54(35%) n.s. | 33/54(61%) P<0.002* |
| aged 7 and above | | | | |
| With BCG scar | 58/70(83%) P<0.00001 | 48/70(69%) P<0.0005 | 37/70(53%) P<0.01 | 50/70(71%) P=0.0001* |
| BEHKADEH | | | | |
| aged 1-7 years | | | | |
| No BCG scar | 34/146(23%) | 25/146(17%) | - | - |
| With BCG scar | 108/154(71%) P<0.00001 | 29/154(19%) n.s. | - | - |
| aged 7 and above | | | | |
| No BCG scar | 35/143(25%) | 29/143(20%) | 32/143(22%) | 33/143(23%) |
| With BCG scar | 121/188(64%) P<0.00001 | 71/188(38%) P<0.0005 | 75/188(40%) P<0.0006 | 98/188(52%) P<0.00001 |

* Significance of differences from those without a BCG scar shown in the top line.

TABLE III. Pooled data from both study centers of skin test results immediately before we vaccinated the children, and at follow-ups to four years.

| | Tuberculin | Leprosin | Scrofulin | Vaccin |
|--------------------------------|--------------|--------------|-------------|--------------|
| Recipients of Vaccine B | | | | |
| At start | 71/303(23%) | 56/303(18%) | 34/157(22%) | 35/157(22%) |
| After 1Y | 108/147(44%) | 60/147(41%) | 28/105(27%) | 32/105(30%) |
| After 2Y | 7/8(88%) | 5/8(63%) | 3/8(38%) | 6/8(75%) |
| After 3Y | 40/49(82%) | 43/49(88%) | 30/49(61%) | 37/48(76%) |
| After 4Y | 8/8(100%) | 8/8(100%) | 2/8(25%) | 5/8(65%) |
| Recipients of Vaccine D | | | | |
| At Start | 260/392(66%) | 103/392(26%) | 83/238(35%) | 116/238(49%) |
| After 1Y | 137/191(72%) | 62/191(32%) | 42/170(25%) | 68/170(40%) |
| After 2Y | 22/32(69%) | 22/32(69%) | 8/32(25%) | 19/32(59%) |
| After 3Y | 23/31(74%) | 24/31(77%) | 18/31(58%) | 24/31(77%) |
| After 4Y | 18/29(62%) | 23/28(79%) | 11/29(38%) | 16/29(55%) |

RESULTS

The initial skin test results are shown in Table II, with responses of 2mm or more being considered positive (the usual cut-off point with new tuberculin). Because of the low numbers of children in Baba Baghi without BCG scars (7 in each age group), only those with BCG scars are divided by age. Possession of a BCG scar was associated with increased responses to all skin tests in all groups, with the exception of leprosin A in the younger age group in Behkadeh. In general, BCG was more effective in enhancing skin test positivity in the older than in the younger children. The response to leprosin A in those with a BCG scar was significantly higher in Baba Baghi than in Behkadeh in both age groups ($P < 0.00001$ for both).

The changes in skin test positivity following vaccination with vaccine B (BCG+*M. vaccae*) are shown in the upper part of Table III, and the changes following vaccine D (*M. vaccae* alone) are shown in the lower part.

Vaccine B significantly increased ($P < 0.00001$) responses to tuberculin in the first year, and thereafter there was only a slight increase. In contrast, positivity to leprosin steadily mounted year by year from 18% when the vaccine was given to 88% in the third year of follow-up ($P < 0.00001$). Responsiveness to scrofulin and vaccin showed the same pattern of steady increases (too few were tested in year 4 to reach significance).

Vaccine D made no difference to the already high positivity to tuberculin, but steadily increased leprosin positivity from 26% at the time of vaccination to 79% after 4 years ($P < 0.00001$). Both scrofulin and vaccin positivity were also significantly increased by the third year.

The mean size of BCG scars present initially was 6.27 ± 4.23 mm; that following vaccine B, measured 1 to 3 years afterwards, was 4.00 ± 0.71 mm, and vaccine D rarely produced a scar.

DISCUSSION

The use of the two vaccines containing *Mycobacterium vaccae* significantly, perhaps maximally, increased skin test positivity to the soluble antigens of the leprosy bacillus in leprosin A to 87-89% amongst the children of leprosy patients. No problems or side effects were encountered with the use of the vaccines, and the scars formed were smaller than those produced by the BCG vaccine in routine use in Iran. None of the children in the study developed leprosy, and if positivity to leprosin is a measure of protective immunity from leprosy, then the vaccines were highly effective. Unpublished evidence from India supports this assumption.

The final levels of skin test positivity achieved three years after our vaccination procedures are shown in Table IV. The use of four skin test reagents in most of the children also allows an approximation to be made of the proportion of individuals responding to common, group I, mycobacterial antigen; the so-called category 1 responders.^{6,8} Since skin test recognition of these common antigens appears to be a measure of protective immunity, enhancement of the category also provides evidence of enhancement of protection.⁵ Table IV shows the effects of vaccination on category 1 responsiveness after correction for chance recognition of all four species by their species specific, group IV antigens.⁶ It can be seen that category 1 responsiveness in the group with an initial BCG scar has been increased from 39%, to 62%, and in those without evidence of previous BCG, it has been increased from 11% to 59%.

The annual increase in positivity to leprosin after vaccination is likely to be due to priming of the children for recognition of the antigens of *M. leprae* encountered in their surroundings,¹ rather than to a direct response to antigens present in the vaccines. Repeated skin testing is not likely to be the cause of increased skin test positivity, since the

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TABLE IV. The starting and final skin test results of the children studied in relation to which of our vaccines they received.

| | Tuberculin | Leprosin | Scrofulin | Vaccin | Category 1 |
|--|------------|-----------|-----------|--------|------------|
| All with a BCG scar (721) | 81% | 62%(23%)* | 55% | 68% | 38% |
| With BCG scar and no further vaccination needed 329/721(46%) | 98% | 100%(27%) | 75% | 91% | 73% |
| With BCG scar requiring Vaccine D 392/721(54%) | 66% | 26%(12%) | 35% | 49% | 14% |
| Positivity 3 years after Vaccine D | 74% | 77%(25%) | 58% | 77% | 52% |
| Final situation of group starting with a BCG scar | 85% | 87%(25%) | 68% | 82% | 62% |
| All without a BCG scar (348) | 33% | 27%(16%) | 26% | 27% | 11% |
| Without BCG scar and no vaccination needed 25/348(7%) | 100% | 100%(14%) | 86% | 100% | 86% |
| Without BCG scar requiring vaccine B 303/348(87%) | 23% | 18%(13%) | 22% | 22% | 5% |
| Positivity 3 years after vaccine B | 88% | 88%(31%) | 61% | 76% | 57% |
| Final situation of group starting without a BCG scar | 89% | 89%(30%) | 63% | 78% | 59% |

*Percent responding to species-specific, group IV antigens in leprosin A (i.e. total% positive minus % category 1 responders)

majority of children were only tested twice; initial test and 1 follow-up test. A small number of children who did not need either of our vaccines were retested one or more years after their initial tests, and there was little change in their responsiveness.

There were some differences in the efficacy of BCG given in the past to induce skin test positivity between the two study centers. Fewer children with BCG scars required vaccine D in Baba Baghi than in Behkadeh (50/125) compared with 342/598, $P < 0.0005$). Despite the low response to tuberculin of the younger children in Baba Baghi (14/55), their response to leprosin was significantly higher than the same age group in Behkadeh (30/55 compared with 25/146, $P < 0.0001$). The low response to tuberculin could have been due to several factors, such as the age when BCG was given, and the time elapsing between its administration and our tests. In the older age group, those in Baba Baghi with BCG scars were significantly more responsive to all skin tests than were those in Behkadeh, the difference being greatest for leprosin (48/70 compared with 71/188, $P < 0.00001$).

There were also differences between children in the two study centers in their conversion to leprosin after our two vaccines. Although the number receiving vaccine B in Baba Baghi was small, with 2/14 (14%) with responses of 2mm initially, 12 of the 13 (92%) followed up over three years produced responses of 2mm or greater whereas in Behkadeh, 41/227 (18%) started with responses of 2mm, and of the 181 followed up over three years, only 96 (50%) developed responses of 2mm or more ($P < 0.003$ for the difference between the two centers). Following vaccine D, the same trend was seen. At Baba Baghi, 3/50 (8%) children started with responses to leprosin of 2mm and 23 of them (46%) were positive over the three years of follow-up. At Behkadeh,

89/248 (38%) children started with responses of 2mm, and over the three years of follow-up 85 of 204 (42%) were positive.

BCG vaccine contains group I mycobacterial antigens which induce an expansion of T-cell clones recognizing them in a variable proportion of recipients, a number of whom will become category 1 responders to skin tests as a result. When a person with circulating T-cells responsive to group I antigens meets a mycobacterial challenge, this is rapidly recognized, and in most cases overcome. Secondary expansion of T-cell clones recognizing species-specific group IV antigens follows, with an increase in positivity to the skin test reagent prepared from that species. The addition of *M. vaccae* to BCG vaccine considerably increases the content of group I antigens and adjuvant.

Thus the increased responsiveness to leprosin after vaccination observed in Baba Baghi in comparison with Behkadeh is likely to be due to the greater contact with leprosy patients and bacilli experienced in the Sanatorium. The ratio of patients to children in Baba Baghi is approximately 1:1, whereas in Behkadeh it is about 1:5. New patients are continuously being admitted to Baba Baghi for a week or two to initiate treatment, whereas the population of patients in Behkadeh tends to be static, and the few entering the community have already received some weeks or months of treatment at other centers.

Of particular interest is the evidence that further vaccination with *M. vaccae* alone of those with a scar of past BCG brings up the level of skin test responses to that achieved with the BCG+*M. vaccae* mixture used in children without a BCG scar. This avoids the repeated use of a live vaccine, which is theroretically unsatisfactory and which is sometimes associated with more severe local reactions. It

may offer, also, a way of boosting immunity without risk in HIV infected persons and their dependents.

In conclusion, the vaccination policy adopted for children without leprosy living in Baba Baghi Leprosy Sanatorium has been highly effective.

The same policy applied in Behkadeh leprosy community appeared to be equally effective. If the efficacy of the vaccines is as good as the skin test data suggests, and similar data is obtained in other countries, then there is no need for the development of any other form of vaccination against the disease.

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