A COMPARATIVE STUDY OF THREE ANTIGENS FOR THE LEPROMIN TEST

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Until about a decade ago lepromin was prepared only by grinding up cooked lepromatous nodules, suspending the material in 0.5% carbolized saline, and eliminating the larger particles of tissue by filtration through cotton gauze. The classical Hayashi-Mitsuda antigen thus produced is crude, contains a considerable amount of tissue matter, cannot be accurately standardized, and in case of strongly positive reactions often produces big, long-persistent ulcers.

Since then, however, two methods have been devised for separating bacilli from the tissue and making suspensions standardized by weight. Fernandez and Olmos Castro (2) separated them by centrifuging, first in strong saline to float them and then in alcohol to deposit them. A large proportion of the bacilli is lost during the process, and this method is little used. Dharmendra (1) separated the bacilli by grinding the tissue material in chloroform, evaporating off the chloroform, taking up the residue in ether, and centrifuging the other suspension. The lepromin prepared from the bacilli so separated contains very little tissue element; it produces well-marked early reactions, but weaker late reactions and no ulceration—this last point being considered a decided improvement.

A large degree of agreement was found between (*a*) the early reaction with this refined antigen and (*b*) the late reaction with the ordinary lepromin. Consequently, these different reactions with the two antigens were considered to be of the same significance.

It has been suggested, however, that the classical Mitsuda (late) reaction and the early (Fernandez) reaction may differ in significance. The study here reported was undertaken to find out the degree of agreement in results of the two reactions with the two antigens, and to see whether the discrepancy could be eliminated by using a variant of the Dharmendra antigen. If this were possible the results with the two tests could be said to have the same significance even if the two reactions are of different nature. Observations were made regarding the optimum interval after the injection—24 or 48 hours—for reading the early reaction.

MATERIALS AND METHODS

Antigens used.—Three antigens were used: the classical one, prepared by the improved method of Wade (Wade-Mitsuda, WM), the original Dharmendra antigen (DO), and the modification of the latter (DM).

The modification introduced was to reduce to a minimum the treatment with chloroform (about an hour) and the grinding of the bacillary powder (about 5 minutes). It was hoped that this change might result in stronger late reactions, in line with those caused by the classical antigen. The principal feature of Wade's improvement of the classical Mitsuda antigen [1] is that the suspension of lepromas (selected and pooled) is filtered through a single layer of very fine-meshed nylon (or silk) bolting cloth instead of multiple layers of highly absorbent cotton gauze, thus saving a great deal of antigen material that otherwise would be lost. This antigen was prepared according to instructions and with nylon fabric supplied by Wade (3).

For most of the work the three antigens were prepared from equal parts of pooled leproma material. From the same amounts of material the yield of Wade's lepromin (1 gm. of leproma to 20 cc. saline) was about one-half the volume of that of the other kinds (10 mgm. of bacillary powder per 100 cc.). In concentration of bacilli the first was about twice as strong as the others.

All patients were injected with 0.1 cc. of all three antigens. In addition, some were given a double dose of DM (DM_2), either as 0.2 cc. of the usual strength or 0.1 cc. of the double strength, for better comparison with the more concentrated WM antigen.

The Wade-Mitsuda antigen was a thicker suspension than the other two, and consequently it diffused from the point of injection and was absorbed more slowly than the others. The wheals were more prominent and sometimes persisted for more than 4 hours, whereas those raised by the other antigens usually disappeared within 1 to 2 hours. Smears showed that it contained large numbers of bacilli with some amorphous tissue matter; there were considerable numbers of acid-fast bacilli, though less than in WM, in the DM antigen; and only a few intact acid-fast bacilli in DO.

Readings.—In each case observations were made for: (1) the immediate flare,[2] watched for up to about 2 hours and sometimes longer; (2) the *early reaction,* readings made at both 24 and 48 hours after injection; and (3) the *late reaction,* with readings at weekly intervals up to 5 weeks.

The criteria of positivity employed were: for the early reaction—as usual—an infiltrated area at least 10 mm. in diameter, more or less erythematous, usually far exceeded in positive cases, occasionally slightly less; and, for the late reaction, a progressive induration going on to nodule formation usually 5 mm. or more in diameter, but sometimes less if of significant nature.

For grading the reactions we could not follow the recommendations of the WHO Committee (4). Although the positive reaction nodules were seldom below 5 mm. with the classical antigen, smaller nodules were very frequent with the Dharmendra antigens. For the purposes of the present observation the following uniform criteria were adopted:

1+, nodule at least 3 mm. in diameter, but less than 5 mm.

2+, nodule at least 5 mm. in diameter, but less than 7 mm.

3+, nodule at least 7 mm. in diameter, but less than 10 mm.

4+, nodule 10 mm. or more in diameter, or any with ulceration.

Cases tested.—A total of 110 cases was tested, 40 maculoanesthetic, 40 tuberculoid and 30 lepromatous. Two did not return for the early readings, and another 17 did not attend beyond the third week, as shown in Table 1.

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[1] Unpublished independently, but comprised in the WHO Committee report (reference 4).

[2] The immediate erythematous flare usually appears within one-half hour and lasts up to about 1 hour.

FINDINGS IN THE LEPROMATOUS CASES

The lepromatous cases were all active and bacteriologically positive. All but two had been previously lepromin tested (DO) on one or more occasions.

TABLE 1.—*Type distribution of the 110 cases tested, and the readings made.*

Type	Original number	Early reaction read in	Late reaction read in
Maculoanesthetic	40	39	29
Tuberculoid	40	40	36
Lepromatous	30	29	26
Total	110	108	91

Immediate flare.—This was seen in a small proportion of the cases with all the antigens—4 out of the 29 with WM, and in 3 with each DO and DM.

Early reaction.—All of these cases were negative with all the antigens by the criteria specified, although there were lesser degrees of this kind of response in some cases. A comparison of what responses there were showed that with WM and DO they were the same at the two readings, after 24 and after 48 hours, in one-half of the cases, while in the other half they were slightly the stronger at 24 hours. With DM and DM_2 only one-third were stronger, and only slightly so, at the 24-hour reading.

Late reaction.—Again the reactions were all negative. There was either no reaction at all or only a slight induration, most marked with WM. With that antigen tiny nodules about 2 mm. in diameter were seen in 9 cases, in fewer cases with the other antigens. No significance is attached to them.

FINDINGS IN THE MACULOANESTHETIC CASES

These cases were all of the form called "simple neural" (Cairo) or "indeterminate" (Havana), with bacteriologically negative smears. Most of them were lepromin tested for the first time.

Immediate flare.—Of the 40 cases, 17 showed flares with WM, 8 with DO, and 5 with DM; also 4 of the 20 tested with DM_2.

Early reaction.—The results of the early reactions are available in 39 of the 40 cases. These are shown in the first half of Table 2. It will be seen that positives were most frequent with DO, and least frequent with WM. There was no correlation with the immediate flare.

Comparison of readings at 24 and at 48 hours: (*a*) about equal in one-third of the cases with all antigens; (*b*) more marked at 24 hours in one-half the cases with WM and DO, and in one-third with DM and DM_2;

(*c*) more marked at 48 hours in one-sixth of the cases with WM and DO, and in one-third with DM and DM_2.

TABLE 2.—*Results of the early and late reactions in maculoanesthetic cases, obtained with the different antigens.*

Antigen	Early reaction				Late reaction			
	Cases	+	+	—	Cases	+	—[a]	2.5[b]
WM	39	21	1	17	29	28	1	—
DO		32	—	7		10	19	10
DM		25	1	13		13	16	8
DM_2	21	15	—	6	17	8	9	4

a Total negatives, including those in the 2.5 column.
b Negatives with nodules 2.5 mm. in diameter.

Among the cases in which the reaction was more marked at 24 hours, the difference was significant in 3, 7, and 5 cases with WM, DO, and DM respectively, the 24-hour readings being definitely positive while the 48-hour readings were doubtful. Among the cases in which the reaction was more marked at 48 hours, the difference was significant in only 2, with DO—doubtful at 24 hours, positive at 48 hours.

Strength of the early reactions with the different antigens: The positive reactions have been compared as regards strength as well as frequency. In the 21 cases in which the reactions to all these antigens were positive, they were slightly more marked with WM in two-thirds of the cases, and with DO in one-third.[3] When DM_2 was included, the reactions were most marked with this antigen.

Late reaction.—Only 29 of the cases presented for reading the late reaction. The results are shown in the second part of Table 2. This shows that positive reactions were most frequent with WM, and least frequent with DO—the reverse of the situation as regards early reaction. The question of whether, with the DO and DM antigens, a nodule of less than 3 mm. in diameter is significant will be discussed later.

Strength of the reactions: Besides giving the highest number of positive late reactions, WM caused stronger reactions as shown in Table 3. Only 1 case was negative with that antigen, and of the 28 positives no less than 10 had ulcerations. With the other antigens the stronger degrees of reaction were few, and ulceration did not occur with these cases (cf tuberculoids).

Correlation between early and late reactions.—A correlation between the two reactions with the various antigens is shown in Table 4. An important question is the degree of agreement—or disagreement—between

[3] As stated earlier, WM appeared to be about double the strength of DO.

the results of the early reaction with DO and of the late reaction with WM. There was agreement in positive reactions in 25 of the 29 cases; in 1 case there was a positive early with DO but negative late with WM; and in 3 cases there was a negative early with DO but a (weakly) positive

TABLE 3.—*Degrees of reactions in maculoanesthetic cases, obtained with the different antigens.*

Antigen	No. of cases	Negative[a]			Positive[b]			
		Nil	Nodule 2 mm.	Nodule 2.5 mm.	1+	2+	3+	4+
WM	29	1	—	—	10	6	1	11[c]
DO	29	5	4	10	7	3	—	—
DM	29	4	4	8	10	2	—	1
DM_2	17	3	2	4	6	1	—	1

a The cases with nodules 2.5 mm. in diameter, recorded as negative, should perhaps be regarded as positive in the case of the Dharmendra antigens.

b For the grading of the degrees of positivity, see text.

c Of the 11 cases 4+ with WM, 10 showed ulceration.

late with WM. These 3 last cases were also tested with DM_2; the early reactions in all were still negative, the late reaction was negative in 2, but in the third there developed a 2.5 mm. nodule.

TABLE 4.—*Correlation of the early and the late reactions in the maculoanesthetic cases.*

Antigen	No. of cases	Agreement		Disagreement	
		positive	negative	Early+ Late—	Early— Late+
WM	29	17	1	—	11
DO		10[a]	5	14	—
DM		13[a]	8	8	—
DM_2	17	8[a]	5	4	—

a If 2.5 mm. nodules with the Dharmendra antigens should be regarded as positive, these three figures would be increased by 10, 8 and 4 respectively, and those in the first column under Disagreements would be reduced correspondingly.

Previous and present results.—Among the maculoanesthetic cases there were 9 that had been previously tested with DO, with readings of only the early reactions, and that completed the readings in the present test. Previously, 7 were recorded as negative and 2 as doubtful; this time they were all negative for the early reaction. The results of the two tests have thus been found to be comparable.

FINDINGS IN THE TUBERCULOID CASES

Of the 40 cases in this group, 29 were minor and 11 major. Nine were in reaction, 1 being slightly positive bacteriologically. The others were torpid, and in 1 the disease had become inactive.

Immediate flare.—Flares were seen in 14, 11, and 7 cases with WM, DO, and DM, respectively; also in 7 of the 13 cases tested with DM_2.

Early reaction.—The results of the early reaction are available for all of the 40 cases tested. These are shown in the first part of Table 5. The frequency of positive reactions was high, and practically the same with all antigens. The fact that the 13 cases tested with DM_2 were all positive is a matter of coincidence, for they were all positive with the other antigens as well.

TABLE 5.—*Results of the early and late reactions in tuberculoid cases, with the different antigens.*

Antigen	Early reaction				Late reaction			
	Cases	+	±	—	Cases	+	—[a]	2.5[b]
WM	40	36	1	3	36	36	—	—
DO		37	1	2		17	19	4
DM		37	1	2		21	15	5
DM_2	13	13	—	—	12	8	4	3

a Total recorded as negative, including those in the 2.5 column.

b Negatives with nodules 2.5 in diameter which it is believed should be regarded as positive.

Comparison of the early reactions at 24 and at 48 hours: This comparison showed that: (*a*) the reactions in one-sixth of the cases were about equal at both readings with all the antigens; (*b*) they were more marked at 24 hours in one-half of the cases with WM and DM, and in two-thirds of the cases with DO; (*c*) they were more marked at 48 hours in one-third of the cases with WM and DM, and in one-sixth of the cases with DO.

Among the cases in which the 24-hour reaction was stronger the difference was significant in 5 cases with WM, and in 6 cases with DO and DM, since at 48 hours they had weakened to the doubtful stage. Among the cases in which the 48-hour reaction was stronger the difference was similarly significant in only 1 case, and with only 1 antigen (DM).

Strength of the early reaction with different antigens: In the 36 cases positive with all three antigens, the reactions were slightly more marked in about one-half of the cases with WM, in one-fourth with DO, and in the remaining one-fourth with DM. In the 3 cases in which WM was

negative, the reactions to the DM antigen were weakest and those to DO strongest.

Late reaction.—The late results are available for only 36 of the 40 cases, shown in the second part of Table 5. Regarding frequency, WM gave the highest number of positives, as before. In this instance no case was negative, whereas with DO slightly more than one-half were negative; somewhat more were positive with DM. The difference would be considerably less if the 2.5 mm. nodules were to be regarded as positive.

Strength of the reactions: Again the strongest late reactions were produced by WM, as shown in Table 6, and in 20 of the 36 cases ulcers were formed, several of them fairly deep and painful. DO produced no ulcers, DM did so in 3 of the 36 cases, and DM_2 in 1 of 12 cases. In all these 4 cases the nodules that ulcerated were only 4 or 5 mm. in diameter, and the ulcers were also small.

TABLE 6.—*Degrees of late reactions in the tuberculoid cases, obtained with the different antigens.*[a]

Antigen	No. of Cases	Negative			Positive			
		Nil	Nodule 2 mm.	Nodule 2.5 mm.	1+	2+	3+	4+[b]
WM	36	—	—	—	5	6	5	20
DO		11	4	4	15	2	—	—
DM		8	2	5	15	3	—	3
DM_2	12	—	1	3	5	2	—	1

a See the first two footnotes of Table 3.
b All of these 4+ reactions showed ulceration.

Correlation between early and late reactions.—The data on correlation between the two reactions with the various antigens show much the same trend as in the maculoanesthetic cases. The important question of agreement between the early reaction with DO and the late one with WM is relatively simple with these tuberculoid cases. Both were positive in 35 of the 36 cases. In the 1 case with disagreement DO gave a negative early reaction and WM a positive late one. This was in a minor tuberculoid case with a single lesion which, in the 5th week, showed a 4 mm. nodule at the WM site but only slight induration (negative) at the other sites.

Previous and present results.—The results of previous tests with DO, early reaction only, are available for 16 of the 36 cases. That reaction, previously, was positive in 13 and doubtful in 3; in the present tests it was positive in 15 and doubtful in 1 with all the three antigens. It is possible that the increase in reaction in the 2 cases may have been caused by repeated testing or by multiple injections in the same patient. The late reaction (present tests) was positive in 9 and doubtful in 7 with DO, and positive in 10 and doubtful in 6 with DM, against 16 positives with WM.

DISCUSSION

The immediate flare sometimes seen within ½ hour or so, regardless of case type or the kind of antigen (but most frequently with WM), cannot be correlated with either the early or the late positive reactions. It appears to be of nonspecific nature and of no significance.

Regarding the optimum time for reading the early reaction, in most cases this response is well developed within 24 hours and remains well-defined at 48 hours, so that it could be read at either time without making any difference in the results. There is, however, a small number of cases with well-developed reactions at 24 hours that become appreciably weaker by the end of 48 hours, and in such cases the earlier reading is better. On the other hand, there are a few cases which take longer than 24 hours for the reactions to develop fully; in such cases the reading has to be delayed until 48 hours. As a routine measure, therefore, the early reaction would best be read at 24 hours, and only those cases in which it is negative or doubtful at that time need be read again at 48 hours.

The use of the Dharmendra antigen gives rise to a question of the criterion for a positive late reaction with it. The usual specifications for grading the lepromin reaction nodules are based on results with the classical Mitsuda antigen. On the whole they are quite satisfactory for reading results with such antigens. Thus, in the present series the nodules of positive reactions with Wade's lepromin varied from 5 to 12 mm. in diameter, sometimes even bigger, except in 2 maculoanesthetic cases in which they were only 3 mm. When the Dharmendra antigens are used the nodules are much smaller, and when 3 mm. is adopted for the lower limit some cases which should actually be considered positive are excluded. It appears that with these antigens a distinctive 2.5 mm. nodule that persists to the 4th or 5th week is definitely significant. Such nodules with these antigens were not seen in any of the lepromatous cases, but they have been seen in considerable proportions of maculoanesthetic and tuberculoid cases with positive early reactions and also positive late reactions with the WM antigen. After all, it is not so much the size of the nodule as its nature that is characteristic of a positive reaction. It therefore appears that a characteristic nodule 2.5 mm. in diameter should be the criterion for positivity in the late reaction with the Dharmendra antigens.

Comparing the different antigens used in this study, they all gave entirely negative results in the lepromatous cases, but the frequency and strength of positive early and late reactions varied with the different antigens in the maculoanesthetic and the tuberculoid cases. Briefly, the Wade-Mitsuda antigen gave the most numerous late reactions in both of these case groups, and these reactions were stronger than those produced by the others. It produced positive early reactions in a high proportion—92 per cent—of the tuberculoid cases, and in 56 per cent of the maculoanesthetics. A great disadvantage is the frequent production of large

ulcers, often fairly deep and persistent. Ulcers were produced in 10 of the 29 maculoanesthetic cases, and 20 of the 36 tuberculoid cases.

Dharmendra's original antigen produced the highest number of early reactions—in 82 per cent of the maculoanesthetic and in 95 per cent of the tuberculoid cases. It produced the lowest number of late reactions, and no ulcer in any case. In general, there was agreement between the results of early reaction with this antigen, and those of the late reaction with the Wade-Mitsuda antigen; disagreement, however, was seen in 5 cases, and this is referred to later.

The modified Dharmendra antigen (DM), made with shorter exposure to chloroform and less grinding, slightly decreased the frequency of early reactions, making it of the same order as the WM antigen; it also increased slightly the late reactions, though not to the same extent as with WM. Doubling the strength (or dose) of this antigen made no change in the early reaction, but resulted in a further increase of late reactions. The positives in 29 cases increased from 11 with DO to 14 with DM and 16 with DM_2.[4] All of these cases were positive with WM.

Regarding agreement between early and late reactions, the DO antigen produced the most marked early reactions, and the WM antigen the most marked late ones. In general, the early reaction with DO and the late one with WM have been in agreement, both positive in 60 cases and both negative in 26 cases, totalling 86 of the 91 cases. There has, however, been disagreement in 5 cases, of which 4 were maculoanesthetic and 1 tuberculoid. In one maculoanesthetic the DO early was positive while the WM late was negative; the reverse occurred in 4 cases, 3 maculoanesthetic and 1 tuberculoid, the positive WM reaction being weak (5 mm.) in all instances.

The significance of this disagreement between the early and late reactions in a small proportion of cases remains a question, as does the problem of whether this discrepancy can be eliminated by any practicable variant of the Dharmendra antigen. The attempt to so modify the antigen that it would produce positive late reactions with about the same frequency as the classical Mitsuda antigen—i.e., the DM preparation used in this study—has been only partially successful. Another possible approach would be to modify the Dharmendra antigen so that it would produce positive early reactions with the same frequency as the positive late reactions of the classical Mitsuda antigen. It is proposed to investigate the whole matter further in maculoanesthetic cases, because the discrepancies have been seen mostly in such cases.

SUMMARY

A study is reported of the lepromin test done with three different antigens: (1) the classical Mitsuda antigen prepared by Wade's method,

[4] Six other cases would be added to these 16, making a total of 22, if 2.5 mm. were to be used as the lower limit of positivity.

(2) Dharmendra's refined bacillary antigen, and (3) a variant of the latter designed to increase the number of late reactions produced by it. Observations were made of: (*a*) the immediate erythematous flare, (*b*) the early reaction read after both 24 and 48 hours, and (*c*) the late reaction observed at weekly intervals up to 5 weeks. Of 110 patients tested, 108 returned for the reading of the early reaction, but only 91 attended regularly for the reading of the late reactions. These 91 were: 29 maculoanesthetic, 36 tuberculoid, and 26 lepromatous.

The immediate flare was seen in a number of cases of all types and with all the antigens used, but most frequently with the Wade-Mitsuda antigen. It appears to be of a nonspecific nature and of no significance in the present connection.

It is concluded that the early reaction can be read at 24 hours as routine. Only when it is negative or doubtful at that time need a 48-hour reading be made.

The characteristic nodule of the positive late reaction usually has a 5 mm. minimum with antigens of the classical Mitsuda type, but with the Dharmendra antigen the nodule is smaller. It is believed that 2.5 mm. should be the proper minimum, although 3 mm. was used in this study.

The Wade-Mitsuda antigen produced the highest number of positive late reactions and a fair number of early reactions. The Dharmendra original caused the highest number of positive early reactions, and the lowest number of late reactions. The Dharmendra variant gave results which were intermediate.

In the lepromatous cases both the early and the late reactions were negative with all the antigens. In the maculoanesthetic cases the early reaction was positive in 82 per cent and 56 per cent with the Dharmendra and the Wade antigens, respectively, and the late reaction was positive in 34 per cent and 96 per cent with these antigens. In the tuberculoid cases the corresponding figures were 95 per cent and 92 per cent for the early reaction, and 74 per cent and 100 per cent for the late one.

In general the early reactions with the Dharmendra antigen and the late reactions with the Wade-Mitsuda antigen were in agreement. There was, however, disagreement in 5 of the 91 cases—4 of the 29 maculoanesthetics and 1 of the 36 tuberculoids.

The Dharmendra variant gave stronger late reactions than the Dharmendra original, but this increase was far below the strength and frequency of the late positive reactions with the Wade-Mitsuda antigen. A better way of attempting to remove the disagreement between early reaction with Dharmendra's antigen and late reaction with Wade's antigen may perhaps be to try to increase the potency of the former.

RESÚMEN

Se presenta un estudio de la lepromina hecha con 3 antígenos diferentes.

1. Antígeno Mitsuda clásico preparado por el método de Wade.
2. El antígeno refinado bacilar de Dharmendra.

3. Una variación del número 2 designado a aumentar el número de reacciones tardías. Se hicieron las siguientes observaciones:
   (a) Reación eritematosa inmediata.
   (b) Reación precoz a las 24 y 48 horas.
   (c) Reación tardía observada semanalmente hasta 5 semanas.

De un total de 110 pacientes 108 regresaron pará la lectura de la reacción precoz pero solamente 91 atendieron regularmente para la lectura de las reacciones tardías. De éstos 91, 29 eran maculoanestésicos, 36 tuberculoides y 26 lepromatosos. La reacción inmediate se observó en algunos casos de todos los tipos y con todos los antígenos pero mas frecuentemente con el antigeno Wade—Mitsuda. Parece ser una reacción no específica y sin significado en ésta investigación.

Se concluye que la reacción precoz puede ser leída rutinariamente a las 24 horas. Cuando la reacción es negativa o dudosa a las 24 horas debe leerse de nuevo a las 48 horas.

El nódulo característico de la reacción tardía positiva es no menor de 5 mm. con el antígeno clásico de Mitsuda pero con el antígeno de Dharmendra el nódulo es mos pequeño. Aunque se cree que 2.5 mm. debe ser el verdadero mínimo en éste estudio se uso 3 mm.

El antígeno Wade—Mitsuda produjo el mayor número de reacciones tardías positivas y un número moderado de reacciones precoces. El antígeno original de Dharmendra produjo el mayor número de reacciones precoces positivas, y el menor número de reacciones tardías. El variante de Dharmendra produjo resultados intermedios.

En los casos lepromatosos las reacciones precoces y tardías fueron negativas con todos los antígenos. En los casos maculoanestésicos la reacción precóz fué positiva en 82% y 56% con los antígenos de Dharmendra y Wade respectivamente, y la reacción tardía fue positiva en el 34 y 96% con éstos antígenos. En los casos tuberculoides las figuras correspondientes fūren 95 y 92% en la reacción precoz, y 74 y 100% en la reacción tardía.

En general las reacciones precoces con el antígeno de Dharmendra y las reacciones tardías con el antígeno de Wade—Mitsuda estuvieron de acuerdo. Sinembargo hubo disparidad en 5 de los 91 casos, 4 de los 29 maculoanestésicos y 1 de los 26 tuberculoides.

El variante de Dharmendra produjo reacciones tardías más notables que el Dharmendra original, pero fueron mucho menor que las reacciones tardías con el antígeno de Wade—Mitsuda. La mejor manera de tratar de remover la disparidad entre los antígenos Wade y de Dharmendra debe ser tratando de aumentar la potencia del antígeno de Dharmendra.

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