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RECOGNITION OF A CATEGORY OF RESPONDERS TO GROUP ii, SLOW-GROWER ASSOCIATED, ANTIGENS AMONGST KUWAITI SENIOR SCHOOL CHILDREN, USING A STATISTICAL MODEL

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Summary

A mathematical model previously developed to test the validity of categorisation of skin test responders has been applied to data obtained from 3 age groups of Kuwaiti school children. Two specially designed sets of 4 new tuberculins were tested on senior school children to determine whether extra categories of responders might exist amongst them.

Strong statistical evidence has been obtained that a proportion of the children respond to group ii, slow-grower associated antigen, creating a fourth responder category, but no evidence was found for responses to group iii, fast-grower associated antigen. The significance of group ii antigens in immune protection from tuberculosis has never been considered specifically. It is of especial interest to note that responders to these antigens have been readily found in Kuwait, a country where BCG is thought to be effective, whereas no such category could be found in India or Sri Lanka, where the efficacy of the vaccine is less certain.

Résumé

Un modèle mathématique mis au point antérieurement pour tester la validité de la catégorisation des sujets réagissant aux tests dermiques a été appliqué aux données obtenues à partir d'écoliers du Koweit de 3 groupes d'âge différents. Deux batteries spécialement développées de tests comportant l'utilisation de 4 nouvelles tuberculines ont été testées chez les enfants des classes les plus âgées pour déterminer si de nouvelles catégories de sujets réagissant pouvaient exister parmi eux.

Il a été démontré, avec un degré élevé de signification statistique, qu'une proportion des enfants répondait au groupe ii, antigène associé aux mycobactéries à croissance lente, créant ainsi une quatrième catégorie de sujets réagissant; mais aucune preuve de réponse au groupe iii, antigène associé aux mycobactérie de croissance rapide, n'a été mise en évidence. L'importance des antigénes du groupe ii dans la protection immunitaire contre la tuberculose n'a jamais été considérée de façon spécifique. Il est tout particulièrement intéressant d'observer que l'existence de sujets réagissant à ces antigènes a été facilement mise en évidence au Koweit, un pays où l'on pense que le BCG est efficace, tandis que cette catégorie de sujets n'a pas été retrouvée en Inde ou au Sri Lanka, où l'efficacité du vaccin est moins certaine.

Resumen

Un modelo matemático desarrollado anteriormente para probar la validez de la categorización de los sujetos que reaccionan a los tests cutáneos fue aplicado a los datos obtenidos en escolares de Koweit de 3 grupos de edad diferentes. Se probaron dos baterías de tests de 4 nuevas tuberculinas, especialmente preparadas, en niños de los cursos superiores, para determinar si entre allos podían existir otras categorías de sujetos reactivos.

Se demostró, con alto grado de significación estadística, que una proporción de los niños respondía al grupo ii, antígeno asociado a micobacterias de crecimiento lento, creando una cuarta categoría de sujetos reactivos; pero no hubo ninguna evidencia de respuesta al grupo iii, antígeno asociado a micobacterias de crecimiento rápido. La importancia de los antígenos del grupo ii en la protección inmunitaria contra la tuberculosis nunca ha sido considerada de manera específica. Es especialmente interesante observar que la existencia de sujetos que reaccionan a estos antígenos fue puesta en evidencia en Koweit, país donde se piensa que el BCG es eficaz, mientras que esta categoría de sujetos no se ha encontrado en India o Sri Lanka, donde la eficacia de la vacuna es menos segura.

Introduction

In a previously reported study of skin testing of Kuwaiti school children [1], a number of the findings were unexpected and a number of questions were left open. In the present study we have reinvestigated the reported data by applying a mathematical model [2] to test some of the claims made and have carried out a further skin test survey with two sets of reagents designed to answer specific questions.

In the model, individuals are assumed to be in one of the three categories of responders already established [3].

Category 1=those positive to all 4 reagents due to responses to group i, common mycobacterial antigens [4].
Category 2=those negative to all 4 reagents due to homoeostatic regulation of responses to small doses of antigens.
Category 3=those positive to some reagents and negative to others due to differential recognition of group iv species specific antigens.

Individuals who happen to have responses to group iv, species specific antigens of all 4 species with which they were tested will also fall into category 1, and individuals who have not met any of the 4 species with which they were tested will fall into category 2, although

the almost universal application of BCG in our study group makes this last possibility extremely unlikely. Thus it is not possible to determine the precise reasons for an individual belonging to either of the first 2 categories. The statistical model can be tested as a reasonable description of the data, since there should be a significant impairment in the goodness of fit if either the category 1 or category 2 responder types are dropped from the model, and this has been shown to be the case [2].

The model as described so far does not take into account possible responses to group ii, slow grower associated, or group iii, fast grower associated antigens, which would manifest as an excess of individuals who respond to all the reagents containing group ii or group iii antigens respectively. Such responsiveness had been sought in India and Sri Lanka but no evidence for it was found [2]. Among the unexpected findings in Kuwaiti school children [1] was an apparent responsiveness to group ii antigens, and to investigate this an additional component has been added to the statistical model.

An unusual feature of the Kuwaiti data, as compared with those previously studied from India and Sri Lanka, was the very high response rate to Tuberculin (85–90 %). This means, since Tuberculin was one of the two slow-growing species tested, that any responses to group ii antigens would be empirically confused with responses to the group iv antigens of other slow-growing species. The data already obtained cannot therefore, provide a strong test for the hypothesis of a response to slow-grower associated antigens.

Materials and methods

The materials for this study were the data obtained in our previous work [1] using reagent sets 1–7, and that obtained from a new series of tests carried out along the same lines as the earlier ones, but using two new sets of reagents on Kuwaiti school children aged 17–18 years. As before, each reagent set included Tuberculin.

Set 8 was designed to circumvent the empirical problems complicating the search for a group ii antigen response in the initial study. To demonstrate such responses it is necessary to have reagents prepared from 2 slow growing species, as well as Tuberculin (to which almost everyone responds) and a reagent prepared from a fast growing species in the same set. The chances of successfully demonstrating responsiveness to group ii antigen are increased if the one fast grower reagent included in the set has a low species-specific positivity rate in the community, so that responders to it are likely to belong to category 1. Similarly one at least of the slow growers should have a relatively low positivity rate, so that responses to it are more likely to be due to its group ii antigens than its species-specific antigens. Set 8 therefore, contained Tuberculin, Xenopin and Gordonin, prepared from slow growers, and Gilvin, prepared from a fast grower.

Set 9 was designed to test whether responsiveness to group iii, fast grower associated antigens was expressed in skin tests. Leprosin A lacks antigens of both groups ii and iii, whereas both Chitin and Flavescin contain group iii antigens. Chitin was chosen as a reagent to which Kuwaitis frequently respond and Flavescin was included as a representative of fast growing mycobacteria found to be an uncommon cause of sensitisation in the Kuwaiti environment [1].

When the tests were read 72 hours after injection, BCG scars, and subsequently vaccination record cards were checked; 413 children received set 8, amongst whom 342 had received BCG once and 71 had been revaccinated; 382 children received set 9, amongst whom 272 had received BCG once and 110 had been revaccinated.

The methods of analysis were those previously described [2] and as modified to detect responses to groups ii and iii antigens, as described below.

Results

Analysis of the earlier data [1]

There were 14 individual data sets comprised of the 7 sets of new tuberculins in each of the 2 age groups (shown in Table 1). The model permitted a good fit in 8 of the 14 cases with $p>0.05$, and was less good in the other cases, although the maximum chi-square for goodness of fit was only 38.04, with 9 degrees of freedom (df). The effect of dropping category 1 or category 2 individuals from the model resulted in a significant decrement in fit in all 14 cases (change in chi-squared, 1 df: minimum=17.97, or 15.86, $p<0.001$; maximum=100.9 or 129.8, $p<0.001$ respectively). Taken overall, the model as previously proposed [2] can be regarded as a reasonable description of the data. Table II shows the estimates of the proportions of individuals in each of the 3 categories.

Only 12 of the 14 data sets were suitable for analysis of the importance of responses to group ii, slow grower associated antigens in categorisation of responders. Reagent set 3 was unsuitable for this analysis as explained below. In the 12 data sets examined the addition of a term for responsiveness to group ii antigens produced significant evidence of an improved fit in only 2 cases (with chi-squared values, 1df, of 4.06: $p<0.05$ and 7.69: $p<0.01$). Combining all 12 sets gave a chi-square (12 df) of 21.34, which is just significant with $p<0.05$.

In general, and within the limits of sampling variation, the proportions of category 1 responders and category 2 non-responders (Table II) are broadly similar in all reagent sets except sets 3 and 5 which in both age groups show far higher incidences of category 1 responders. In set 3 all 4 new tuberculins were prepared from slow growing species, and

Table I. The percentages of reactions of 2 mm or more in diameter divided according to age. Data taken from earlier study [1].

Reagent	*Elementary school (mean age 9.2±0.9 y) Per cent*	*Intermediate school (mean age 13.1±1.1 y) Per cent*
Tuberculin	86.8	89.6
Set 1 Nonchromogenicin	45.4	43.5
Xenopin	39.8	43.5
Rhodesin	27.8	30.1
Set 2 Gilvin	14.6	37.3
Marinin	70.5	80.7
Neoaurumin	45.9	65.1
Set 3 Aviumin A	82.6	90.2
Aviumin B	70.4	73.5
Aviumin C	80.9	88.5
Set 4 Chitin	54.1	71.3
Burulin	51.9	64.0
Diernhoferin	41.4	60.3
Set 5 Leprosin	82.8	76.3
Scrofulin	82.8	74.6
Vaccin	73.3	74.6
Set 6 Ranin 1	27.3	46.2
Gordonin	42.8	55.8
Ranin 2	31.1	50.0
Set 7 Duvalin	38.5	49.6
Kansasin	78.2	83.3
Flavescin	23.1	26.0

hence the excess of individuals in category 1 might well be due to individuals responding to group ii, slow grower associated antigens. Set 5 is unusual in that it contains both Leprosin A and Vaccin, for which a special association has also been claimed [1, 5], and such an association would increase the numbers of individuals apparently in category 1.

Table II. Proportions of subjects in each of the three categories of responders, for each of the seven reagent sets used, amongst elementary and intermediate school children.*

Elementary school children (mean age 9.2 years)						
No.	*Set*	*Category 2*	←———	*Category 3*	———→	*Category 1*
		− − − −	− − − −	−/+	++++	++++
107	1	9.3	0.0	74.0	1.6	15.1
281	2	11.0	0.1	75.1	0.3	13.5
115	3	2.5	0.1	33.1	2.6	61.7
133	4	3.3	0.4	64.6	1.3	30.3
220	5	4.5	0.5	29.4	3.7	61.9
264	6	15.9	5.3	60.2	0.9	17.6
78	7	5.1	0.0	71.8	1.6	21.5
Total and weighted mean values						
1198	—	8.7	1.3	57.9	1.6	30.5

Intermediate school children (mean age 13.1 years)						
N	*Set*	*Category 2*	←———	*Category 3*	———→	*Category 1*
		− − − −	− − − −	−/+	++++	++++
246	1	15.7	0.6	59.3	0.7	23.7
82	2	3.6	0.0	60.2	0.7	35.5
347	3	3.9	0.1	29.5	10.5	55.9
136	4	3.4	0.3	44.9	1.3	50.1
117	5	5.6	2.1	27.9	1.3	63.1
50	6	19.7	2.0	46.3	2.5	29.5
247	7	4.0	0.1	74.8	2.4	18.7
Total and weighted mean values						
1225	—	7.0	0.5	48.9	4.0	39.6

*Category 3 also contains the calculated proportion of individuals who, by chance, would react with none (− − − −) or all (++++) of the four reagents used.

Table III. The results obtained with reagent sets 8 and 9 in senior school children, some of whom had been revaccinated with BCG some 5 years earlier.

Reagent	*+ve/N*	*%+ve*	*Mean response size*
Tuberculin	783/795	98 %	11.6 mm
Gilvin	263/413	64 %	5.1 mm
Gordonin	362/413	88 %	6.5 mm
Xenopin	345/413	83 %	6.0 mm
Flavescin	260/382	68 %	5.2 mm
Chitin	365/382	96 %	7.8 mm
Leprosin A	359/382	94 %	7.7 mm

The new data

The results obtained with reagent sets 8 and 9 are shown in Table III: 413 children received set 8, and 382 children received set 9. Both groups had a mean age of 18 years.

Analysis of the new data

Analysis of the set 8 data by the standard model with category 1, 2 and 3 responders showed highly significant effects for the presence of categories 1 and 2 (category 1: chi-squared, 1 df=114.77 $p<0.001$; category 2: chi-squared, 1 df=30.63 $p<0.001$). The model represented a fairly good fit to the data (chi-squared=29.23, 9 df, $p<0.001$). However, addition of an extra term specific to the slow-grower associated antigens present in 3 of the 4 new tuberculins produced a highly significant improvement (change in chi-square, 1 df=27.61, $p<0.001$), with the model now showing an excellent fit (chi-squared=1.63, 8 df, NS). Calculations based on the parameter estimates for the fitted model suggest that 23 % of the population recognise group ii antigen, that 53 % recognise group i, common mycobacterial antigen (category 1), 22 % respond only to group iv, species specific antigens (category 3), and only 1.5 % are category 2, non-responders. Of course some individuals in category 1 might also recognise groups ii and iv antigens, but they cannot be identified using the present method. The proportions in each category were very similar whether or not those tested had received a second BCG vaccination.

Analysis of the set 9 data with the standard model of category 1, 2 and 3 responders produced a moderately good fit (chi-squared=20.38, 9 df, $p<0.05$), with significant evidence for both category 1 and 2 responders, 66.2 % of subjects being in category 1 and 1.3 % in category 2. The possible existence of responses to group iii, fast grower associated antigen was sought by the addition of an extra parameter specific to sensitivity to both Flavescin and Chitin. This parameter produced only a marginal improvement in goodness of fit (chi-squared, 1 df=0.118, NS). We may therefore, conclude that there is no evidence that subjects can respond to group iii antigens.

Discussion

Re-analysis of the data obtained previously [1] from primary and intermediate school children who had been vaccinated with Japanese glutamate BCG just before starting school confirmed that the basic model of three categories of responders to tuberculins can be applied to them. It was not possible to obtain definitive evidence for an extra category of responders to group ii, slow grower associated antigen from these data alone, although, as previously suggested there was presumptive evidence for it. The cause of the difficulty was an empirical confusion between categories because of the very high (86 %+) positivity to Tuberculin which was 1 of the 2 slow grower derived reagents in each of the sets suitable for analysis. The new data obtained from senior school children allowed resolution of the problem, and there is now no reasonable statistical doubt that a group ii antigen responsive fourth category exists in this population which was not found in the previously studied data from India and Sri Lanka [2]. Because of the lower incidence of BCG vaccination and lower positivity to Tuberculin in those countries, we can be confident that responses to group ii antigens did not occur there. Although this is by no means the first time that response to group ii antigen has been suggested in the literature, it is the first time that it has been formally proved to be distinct from responsiveness to group i antigen. The so-called cross-reactivity between PPD reagents prepared from *Mycobacterium tuberculosis* and *M. intracellulare,* which is less between these reagents and a PPD made from *M. fortuitum*

[6, 7, 8], was presumptive evidence for reaction to group ii antigen. Indeed the lack of specificity in PPD preparations is likely to be due to such responses [9]. That this responsiveness is present in some countries but not in others is a new observation of our study.

Further analysis of all the Kuwaiti data confirms that there is not a category of individuals responding to group iii, fast grower associated antigen, in agreement with the Indian and Sri Lankan data.

In conclusion our study has resulted in a potentially important observation about the ability to make cellular responses to mycobacterial antigens upon which little attention has previously focussed. That group ii antigen should be recognised in Kuwait but not in South India or Sri Lanka indicates that the part it might play in post-BCG protection from tuberculosis needs to be investigated.

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