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Antibodies to Phenolic Glycolipid-I During Long-term Therapy: Serial Measurements in Individual Patients[1]

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The characteristics of the humoral immune response to phenolic glycolipid-I (PGL-I), a species-specific antigen of *Mycobacterium leprae,* have been the subject of numerous studies since the description of this antigen by Hunter and Brennan in 1981 ([3]). The antibody response to PGL-I consists predominantly of IgM, although an IgG response is detectable in some patients ([4, 9]). The amount of circulating anti-PGL-I IgM as well as the percentage of seropositive patients have been found to correlate with both the disease classification and the bacillary index (BI) ([4, 8]) and, in the nine-banded armadillo, the anti-PGL-I level was directly related to the hepatic bacterial yield of *M. leprae* ([6]).

One of the proposed applications of anti-PGL-I serologic testing is to monitor the response to therapy in individual patients with the aim of preventing overt clinical relapses by early detection of treatment failure. The only currently available modality for monitoring therapeutic response, other than repeated clinical observation, is to perform serial skin biopsies or skin scrapings to determine the bacterial and morphological indices. These procedures are invasive, somewhat painful, require significant expertise for interpretation, and are susceptible to sampling error.

When cohorts of patients tested for varying lengths of time were studied, the mean level of anti-PGL-I IgM was found to decline slowly over several years ([1, 2]), but short-term studies in individual patients found a variable response of anti-PGL-I IgM during therapy ([8]). In addition, the wide range of levels in individual patients, even among those with the same disease class, makes it impossible to extrapolate from the behavior of large populations to that of specific patients. Data on the behavior of anti-PGL-I levels during several years of therapy in individual patients are therefore needed to accurately assess the potential for serologic monitoring. If individual patients' levels fluctuate erratically or fall exceedingly slowly, this test is unlikely to be clinically useful. To investigate this issue, we studied all of the patients followed at the Seattle Regional Hansen's Disease Clinic on whom serial sera specimens spanning at least the initial 5 years of treatment were available.

MATERIALS AND METHODS

Patients. Criteria for inclusion in this study were: a) availability of stored sera obtained over a period of approximately 5 years or more, b) first sera obtained prior to, or within 2 weeks of, the initiation of antileprosy treatment, and c) no prior treatment for leprosy. Approximately 85 unique Hansen's disease patients were seen at the Seattle clinic over the last 8 years, of whom 11 met eligibility criteria for this study. The most common reasons for exclusion were initiation of treatment prior to entering the clinic and diagnosis of Hansen's disease later than March 1982. All sera from each patient obtained at least 10 weeks after the last previous specimen were tested.

Patients were classified by standard clinical and histologic criteria as having polar tuberculoid (TT), borderline tuberculoid (BT), borderline (BB), borderline lepromatous (BL), or polar lepromatous (LL) disease, and all received both dapsone and rifampin as initial therapy. Erythema no-

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dosum leprosum (ENL) was treated with prednisone and clofazimine in most cases, and with thalidomide in most male patients. Reversal reactions were managed with prednisone.

PGL-I ELISA. Sera were obtained, aliquotted into small vials, and stored at −70°C until the day of the test. All sera on an individual patient were tested on the same day and on the same ELISA plate whenever possible. All sera were tested in duplicate at a dilution of 1:200 in both PGL-I-coated and blank wells as previously described ([5, 8]). This dilution provided the optimal signal: background ratio for the particular batch of PGL-I-coated plates used for this study. The positive control standard consisted of serial dilutions of pooled human leprosy sera, and the negative control consisted of pooled sera from healthy Seattle residents. The incubation time with the dye/substrate reagent was adjusted such that the lowest dilution of the positive control sera had A_{492} values of 1.2–1.4 (mean 1.3).

Data analysis. ELISA results were recorded as net A_{492} (the difference between the mean absorbance in the PGL-I-coated wells and the mean absorbance of the uncoated wells). To facilitate comparisons between patients, these absorbance values were then recalculated as percentages of each respective patient's initial absorbance values. Thus, the first specimen from each patient was arbitrarily given a value of 100%, and each subsequent serum specimen was assigned a percentage value proportional to its absorbance value.

RESULTS

Three paucibacillary patients (1 TT, 2 BT) had initial absorbance values in the low normal range (A_{492} = 0.00–0.07). Net absorbance values remained normal during the entire, uncomplicated treatment courses in all three of these patients.

Four patients with multibacillary disease (3 BB, 1 BL) had rapid steady declines in antibody level (Fig. 1). Two of these patients experienced reversal reactions during therapy and one had recurrent erythema nodosum leprosum (ENL). The mean initial net absorbance for this group was 0.68 ± 0.34. The mean levels in this group fell 53% in the first year, 66% by the end of the third year, and 86% by the end of the fifth year of treatment. At the completion of 5 years of therapy, two of these patients had net absorbance values within the normal range.

The last four patients (1 LL, 3 BL) had antibody levels which fell much more slowly, losing only 20% of their initial value in the first year (Fig. 2). The rate of decline in antibody level accelerated in subsequent years in three of these patients. At the end of 3 years of therapy, the mean levels had fallen by 56%, and by 67% at the end of the fifth year. All four of these patients experienced ENL reactions, but these reactions were unrelated to the transition from a slow to a rapid decline in antibody levels. The mean initial net absorbance for this group was 1.04 ± 0.13, and none had normal levels of antibody at the completion of 5 years of therapy.

Only one patient had a significant, sustained increase in antibody level following an initial fall. This patient had BB leprosy complicated by a reversal reaction. He was also noncompliant or poorly compliant with his basic regimen of dapsone and rifampin during the period shown in Figure 1. He did not experience a clinical relapse, and no biopsies or skin scrapings were taken during this period. No comparable periods of prolonged medical noncompliance were noted in the remaining 10 patients.

DISCUSSION

Antibodies of the IgM class are the first to appear during the immune response to a foreign antigen and rapidly decrease when the stimulating antigen is removed. The persistence of high levels of anti-PGL-I IgM in leprosy patients during the initial months and even years of therapy is most likely the result of ongoing antigenic stimulation. This hypothesis is supported by the observed correlation between the anti-PGL-I level and the bacterial index ([4]). Based on these findings, it has been proposed that anti-PGL-I IgM levels may serve as a useful monitor of therapeutic response.

The only published study which examined patients who had been extensively treated found that the mean absorbance in patients treated for 10 or more years was 14% of the mean absorbance in a different group of untreated patients, and that most

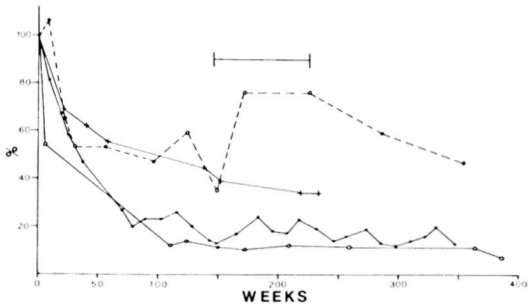

FIG. 1. Relative levels of IgM antibody to PGL-I in four multibacillary patients with rapid initial declines in antibody level. The bar marks the period of poor medical compliance in the patient whose antibody level rose sharply at week 171.

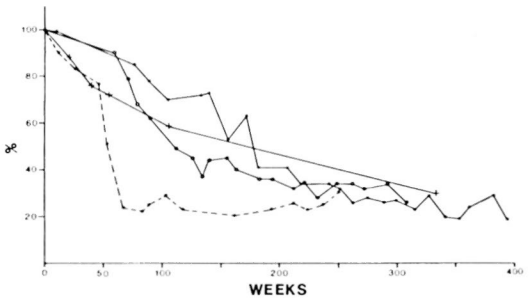

FIG. 2. Relative levels of IgM antibody to PGL-I in four multibacillary patients with slow initial declines in antibody level.

of this decline occurred in the initial 1–2 years of treatment ([1]). These results were encouraging but limited by the fact that since serial specimens from the same patients were not used, there may have been undetected differences between the various patient groups which affected the subsequent test results.

Antibody levels in our patients declined even more rapidly, falling to between 10% and 30% of the initial value after 5 years in most patients with initially detectable levels of anti-PGL-I IgM. More importantly, with one exception, each patient exhibited a pattern of diminishing amounts of anti-PGL-I IgM over time. We do not have frequent bacterial index determinations on these patients, so it is impossible for us to confirm the correlation between the IgM level and the bacterial index reported by others, but in the seven patients with consistently falling levels (and in the three patients with consistently negative levels), there was no evidence of clinical relapse or prolonged medical noncompliance.

Our sample population is biased toward patients with reactional states, primarily because these complicated patients were followed most closely by the clinic and therefore met the entry criteria for inclusion in this study. We could not detect any effect of ENL or reversal reactions on the pattern or rate of IgM decline in these patients, although there were few patients without reactional states included for comparison, and this study was not designed to screen for any such effect. However, in prior work, our laboratory and others have failed to find any correlation between ENL and anti-PGL-I IgM levels ([5]). Other investigators using slightly different assay systems have found that acute ENL is associated with a decrease in anti-PGL-I IgM levels ([4]), an effect that may be related to therapy with thalidomide rather than a primary effect of ENL ([7]). Three of our five ENL patients received thalidomide, and we cannot exclude an effect of thalidomide on the antibody levels in these patients.

In conclusion, we found that anti-PGL-I IgM antibody levels in multibacillary patients declined relatively rapidly during the initial 5–7 years of therapy, apparently irrespective of intercurrent reactional states. If these preliminary results are confirmed by larger studies which include frequent measurements of bacillary load, it may prove feasible to use serologic testing as a primary modality for assessing therapeutic response.

SUMMARY

Levels of IgM antibody to phenolic glycolipid-I (PGL-I) were measured in serum specimens collected over the initial 5 or more years of therapy from 11 leprosy patients. All three patients with paucibacillary disease had undetectable levels of antibody throughout their treatment. The eight patients with multibacillary disease had initially elevated levels which fell quite rapidly with treatment, reaching levels of 10% to 30% of their initial pre-treatment level after 5 years of therapy. The single patient with prolonged therapeutic noncompliance had an increase in antibody level, although clinical or bacteriologic relapse was not docu-

mented. These results in individual patients demonstrate that IgM antibody to PGL-I declines rapidly and consistently with treatment in multibacillary patients.

RESUMEN

Se midieron los niveles de anticuerpo IgM contra el glicolípido fenólico-I (GLF-I) en muestras de suero colectadas de 11 pacientes con lepra, durante los primeros 5 años ó más de tratamiento. Los 3 pacientes con lepra paucibacilar no tuvieron niveles detectables de anticuerpo a lo largo del tiempo del tratamiento. Los 8 pacientes con lepra multibacilar tuvieron títulos inicialmente elevados que disminuyeron rapidamente con el tratamiento alcanzando, después de 5 años de terapia, niveles del 10% al 30% en relación con los niveles de pre-tratamiento. El único paciente con un prolongado tratamiento irregular, tuvo un incremento en su nivel de anticuerpos pero, desafortunadamente, no se obtuvo la información sobre su recaída clínica o bacteriológica. Los resultados en los pacientes individuales demuestran que el anticuerpo IgM contra el GLF-I disminuye rapidamente y de manera consistente con el tratamiento en los pacientes multibacilares.

RÉSUMÉ

Chez 11 malades de la lèpre, on a mesuré les taux d'anticorps IgM à l'antigène phénoglycolipidique-I (PGL-I), dans des échantillons de sérum recueillis au cours des cinq premières années de traitement ou plus tard. Les trois malades atteints d'une affection paucibacillaire présentaient des taux non décelables d'anticorps tout au long du traitement. Les 8 malades qui souffraient d'une lèpre multibacillaire ont au début livrés des taux élevés d'anticorps, qui sont tombés assez rapidement au cours du traitement, pour atteindre après 5 années de traitement des taux qui se situaient entre 10% à 30% des taux initiaux relevés avant le traitement. Un seul malade, qui s'était signalé par un manque d'assiduité prolongé au traitement, témoignait d'une augmentation du taux d'anticorps, encore qu'aucune récidive clinique ou bactériologique n'ait pu être mise en évidence. Les résultats obtenus chez des malades individuels démontrent que les anticorps IgM au PGL-I déclinent rapidement et de manière cohérente au cours du traitement chez des malades multibacillaires.

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