Secondary Amyloidosis and the Serum Amyloid Precursor in Leprosy: Geographical Variation and Association with Leukocytosis


Two distinct chemical types of amyloid fibrils have been found to correlate broadly with different clinical types of systemic amyloidosis. Amyloid of immunoglobulin origin is usually found in primary and myeloma-associated amyloidosis (7). Protein AA, a protein with no apparent relationship to immunoglobulin, is found in amyloid fibrils from patients with secondary amyloidosis (4, 12, 15). The detection of protein AA in fibrils from some patients who have amyloidosis associated with myeloma or macroglobulinemia (11) indicates that the correlation between clinical and chemical types of amyloid is not absolute. Additional evidence for this is the finding of light chain components, in addition to protein AA, in fibrils isolated from some patients with secondary amyloidosis (7, 33).

It is assumed that the immunoglobulin fragments that are deposited in amyloid fibrils are synthesized in plasma cells or lymphocytes, but the cellular origin of protein AA is unknown. It has been suggested that protein AA is derived from protein SAA, a serum protein detected with antisera to protein AA (16), and amino acid sequence studies show NH₂-terminal homology between the serum and fibril proteins (1, 24). Protein SAA is normally present in serum in trace amounts requiring radioimmunoassay for detection (23, 29). In amyloidosis and diseases which predispose to amyloidosis, the serum concentration of protein SAA is raised (10, 22).

Leprosy, particularly the lepromatous form of the disease, has long been known to cause amyloidosis. As the amyloid found in leprosy patients is of the AA-type, we have investigated the distribution of protein SAA in the different forms of leprosy and in patients with amyloidosis secondary to leprosy. Because protein SAA behaves as an acute phase reactant (18, 23) and population studies (2) have also established a correlation between elevated SAA levels and neutrophilia, the relationship between SAA concentration and leukocyte counts in leprosy patients has been examined.

The frequency with which amyloidosis complicates leprosy shows marked geographic variation (21, 25, 34). This has been attributed to dietary (34) and genetic factors (21), as well as to differences in the treatment of leprosy. In Papua New Guinea, where leprosy is a major cause of secondary amyloidosis, we have studied leprosy patients from two areas which differed in climate and disease pattern, as well as in diet and treatment of leprosy.

PATIENTS AND METHODS

These studies were carried out at two leprosy hospitals in Papua New Guinea, one at Togoba in the western highlands, and the other at Aitape on the north coast. The patients at Togoba hospital originated from throughout the highlands region, whereas Aitape hospital inpatients were from the Sepik river basin. These two regions differ in many respects. In the coastal area the climate is hot and humid, whereas the highlands are cool and temperate. The endemic diseases on the coast include leprosy, tuberculosis, filariasis, malaria, worm infections in addition to fungal and pyogenic skin infections. Of these diseases, only the worm infections and leprosy are common to the highlands. Diet in both areas is made up almost entirely of carbohydrates, with the
staple being sweet potato in the highlands and sago on the coast.

All patients were classified clinically, according to the Ridley-Jopling classification (\(\text{LL}\)), as polar lepromatous (LL), borderline lepromatous (BL), borderline (BB), borderline tuberculoid (BT), or polar tuberculoid (TT). The policy at the time of the study was to admit to the hospital infectious lepromatous patients for stabilization of therapy and only those noninfectious patients who required treatment for complications of neuropathies. The patients classified as BB and BT have been combined in a single group because of difficulty in separating the two on clinical grounds. Many of these patients were admitted with long-standing polynuropathy, and there was no record of the original skin lesions. Rectal biopsies were obtained from all patients, and Congo red-stained sections were examined for the presence of amyloid. Biopsies showing the characteristic amyloid birefringence with polarization microscopy were verified by electron microscopy.

Blood was taken for leukocyte counts for which standard hematological methods were employed. For the leprosy patients in Aitape differential leukocyte counts were also performed, and thick films were examined for the presence of malarial parasites. Protein SAA was detected by double immunodiffusion in sera, previously stored at \(-20^\circ\text{C}\), using an antiserum to protein AA prepared by immunizing rabbits with alkaline-degraded amyloid fibrils (\(\text{X}^2\)).

**RESULTS**

**Highlands study.** Protein SAA was detected in 41 of 199 patients with leprosy in Togoba hospital. SAA was more prevalent in LL patients (29%) than in BL (15.3%) or in BB + BT (12.1%) patients (Table I). In contrast to this trend, indicating an increased frequency of SAA towards the lepromatous pole of the leprosy disease spectrum, 4 of 11 (35.4%) of TT patients were SAA positive. A large proportion of the BB + BT and TT patients were in the hospital for treatment of neurotrophic ulcers. Ninety-two percent of the patients in this category, who had elevated levels of SAA, had trophic ulcers; whereas ulcers were present in 56% of the BB + BT and TT patients without detectable SAA (\(X^2=5.6, p<0.025\)). When the patients with ulcers (both SAA positive and negative) were excluded from the analysis, it was evident that the prevalence of SAA was highest at the lepromatous pole and progressively decreased towards the tuberculoid pole (\(X^2=7.9, p<0.025\)).

Amyloidosis was diagnosed by rectal biopsy in 17 of 190 patients. Twelve of these patients with amyloidosis were classified as LL, three as BL, one as BT, and one as TT. The five patients classified as other than LL all had severe neurotrophic ulcers to which their amyloidosis might be attributed. The lepromatous patients with amyloidosis were characterized by having had a stormy clinical course, punctuated by recurrent ENL reactions. The prevalence of SAA in patients with amyloidosis was 35%, which was not significantly higher than the prevalence of SAA in leprosy patients without amyloidosis (21%). In lepromatous patients there was no correlation between detectable levels of SAA and the number of documented ENL reactions in the past; nor was there a correlation between detectable SAA and age or sex.

**Coastal study.** The prevalence of elevated SAA levels was much higher in the coastal leprosy population (44%) than in the highlands (21%) (\(X^2=18.7, p<0.001\) (Table I)). As in the highlands, the prevalence of SAA varied in the different types of leprosy. SAA was detected in 53% of LL, 47% of BL, 29% of BB + BT, and 36% of TT patients. Trophic ulcers were common, particularly in the borderline and tuberculoid patients, but in contrast to the highlands study, there was not a significant correlation between SAA and ulcers in the BB + BT and TT patients. When patients with ulcers were excluded, in contrast to the highlands study again, there was still no significant variation in the distribution of SAA prevalence across the disease spectrum. As in the highlands, there was no variation with age or sex in the distribution of SAA.

Although the prevalence of SAA at Aitape was more than twice that in the highlands, only three patients with amyloidosis were diagnosed at Aitape. One was classified as TT, one as BT and the other as LL. This latter lepromatous patient was 1 of 67 LL and BL patients at Aitape who had had leprosy for more than two years. This contrasts sharply with Togoba, where 16% of patients in this category had amyloidosis.
TABLE I. Distribution of SAA across the leprosy spectrum at Togoba (highland) and Aitape (coastal) hospitals. Prevalence of trophic ulcers in SAA positive and negative patients within each classification group.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Togoba</th>
<th></th>
<th></th>
<th>Aitape</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SAA Positive</td>
<td>SAA Negative</td>
<td></td>
<td>SAA Positive</td>
<td>SAA Negative</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No. tested</td>
<td>Total</td>
<td>With</td>
<td>trophic ulcers</td>
<td>Total</td>
<td>With</td>
</tr>
<tr>
<td>LL</td>
<td>76</td>
<td>22 (29%)</td>
<td>5 (23%)</td>
<td>54 (71%)</td>
<td>10 (19%)</td>
<td></td>
</tr>
<tr>
<td>BL</td>
<td>46</td>
<td>7 (15%)</td>
<td>2 (29%)</td>
<td>39 (85%)</td>
<td>15 (39%)</td>
<td></td>
</tr>
<tr>
<td>BB + BT</td>
<td>66</td>
<td>8 (12%)</td>
<td>8 (100%)</td>
<td>58 (88%)</td>
<td>32 (55%)</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>11</td>
<td>4 (36%)</td>
<td>3 (75%)</td>
<td>7 (64%)</td>
<td>4 (57%)</td>
<td></td>
</tr>
<tr>
<td>Total patients</td>
<td>199</td>
<td>41 (21%)</td>
<td>18 (44%)</td>
<td>158 (79%)</td>
<td>61 (39%)</td>
<td></td>
</tr>
<tr>
<td>Amyloid cases</td>
<td>17</td>
<td>6 (35%)</td>
<td>1</td>
<td>11 (65%)</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

Amyloid cases 1 7 6 (35%) I II (65%) 4

Aitape 6

LL, polar lepromatous; BL, borderline lepromatous; BB, borderline; BT, borderline tuberculoid; TT, polar tuberculoid.

One patient absconded before examination.

The high prevalence of SAA in coastal leprosy patients and the lack of a correlation with the leprosy spectrum may be due to infections such as malaria, tuberculosis and filariasis, which are endemic on the coast but not in the highlands. Half of the patients at Aitape had enlarged, palpable spleens, but SAA was equally distributed in those with and without splenomegaly. The malaria parasite rate in thick blood films was under two percent but this does not reflect the true incidence of malaria, as chloroquine was freely available and taken routinely for fever. Blood was not examined at night for microfilaria but they were seen on four percent of thick films examined for malaria parasites. The true prevalence would be higher than this.

Leukocyte counts. The mean total leukocyte count in coastal leprosy patients (9,850/mm³) was significantly higher (0.01 < p < 0.02, Student t test) than in the highland patients (8,900/mm³). There was a significant correlation between the presence of SAA and elevated leukocyte count (Fig. 1). Patients with leukocyte counts greater than 11,000/mm³ were 2.9 times more likely to have a detectable level of SAA than patients with counts less than 11,000/mm³. Differential counts were not performed at Togoba but were done at Aitape. In the coastal patients there was no correlation between the presence of SAA and levels of lymphocytes, monocytes or eosinophils, although eosinophil levels were high. However, there was a significant correlation between SAA and neutrophil count (Fig. 1). Patients with neutrophil counts over 9,000/mm³ were 2.2 times more likely to have detectable SAA than those with counts less than 9,000/mm³. The mean neutrophil count in patients who were SAA positive was 33% higher (p < 0.01, Student t test) than in patients who were SAA negative.

DISCUSSION

The prevalence of SAA in the two populations of patients with leprosy studied here was very different. Paradoxically, the higher prevalence of SAA (44% compared with 21%) was found in the coastal population in which fewer cases of amyloidosis were diagnosed. Possible explanations for this paradox include differences in the incidence of other endemic infectious diseases and differences in the management of lepromatous leprosy patients in the two areas. It is known that SAA is elevated in a variety of acute
and chronic inflammatory states (11, 12, 21). Whereas leprosy is endemic in both highlands and coastal regions of Papua New Guinea, other infectious diseases such as tuberculosis, filariasis and malaria are endemic only on the coast. Evidence that intercurrent infections were more prevalent in the coastal population was the finding of a higher mean leucocyte count in the Aitape group. This could not be accounted for by differences in the severity of leprosy in the two populations. In studies of "normal" village populations in Papua New Guinea, the prevalence of SAA varied from 8% in a village with good medical care to 23% in a more isolated lowland area (11). This compares with the 22% reported for a small number of apparently healthy Ethiopians (14).

In the highlands group of leprosy patients, in which other infections were less common, elevated levels of SAA were more prevalent in patients toward the lepromatous pole of the leprosy spectrum as has been reported in Ethiopian leprosy patients (10) and in patients with neurotrophic ulcers. In the coastal population a similar trend in distribution of SAA across the leprosy spectrum was not statistically significant. A clearer trend was probably obscured by elevated levels of SAA secondary to other infections.

Why lepromatous leprosy patients have an increased prevalence of elevated SAA levels is not clear. Lepromatous leprosy is characterized by a specific cell-mediated immune defect to Mycobacterium leprae (reviewed in (1)), and elevated levels of SAA may be a direct reflection of this defect per se (16). Alternatively, the massive bacterial load carried by patients with lepromatous leprosy and the consequent hyperstimulation of the humoral immune response may be more important. We have previously reported that SAA levels are elevated in ENL reactions (17), which are a feature of some patients with I.L. and BL disease. The finding in this study that SAA levels correlated with a leukocytosis in both populations, suggests that the high prevalence of SAA in the I.L. and BL patients may, in part, be a result of ENL reactions. Indeed, the majority of leprosy patients who develop amyloidosis have a history of recurrent ENL reactions (17, 18). Levels of SAA in severe reactions may remain elevated for days or weeks before returning to lower levels (19). However, elevated levels of SAA were not detected more frequently in lepromatous patients with a past history of reaction than in other lepromatous patients. Therefore, it is probable that recurrent, rather than continuous elevation of SAA is a feature of leprosy patients who develop amyloidosis.

In this study only 35% of proven amyloid cases had elevated levels of SAA. This is similar to what we have found in other patients with amyloidosis in Papua New Guinea (17) but contrasts with studies elsewhere (10, 16), which have shown that majority of patients with secondary amyloidosis to have elevated levels of SAA. Renal disease cannot be the sole explanation for the failure to detect SAA in some of the patients studied here, as persistent proteinuria was found.

![Figure 1](image-url)
in only half of the patients in whom SAA was not detected. It appears that SAA levels may be undetectable in leprosy patients with amyloidosis at times when the ENL reactions, important for the development of amyloidosis, are quiescent. Thus, elevated levels of SAA reflect the activity of the predisposing disease process and intercurrent infections, and cannot be taken as a marker for the presence of amyloidosis.

The correlation between elevated levels of SAA and neutrophilia reported here in leprosy patients, concurs with observations in Papua New Guinean populations (2). Both neutrophilia and elevation of SAA concentration are associated with the acute inflammatory response and this correlation may, therefore, have no direct pathogenic significance. However, a number of conditions, including familial Mediterranean fever (15) and rheumatoid arthritis (4) in addition to leprosy, are associated with amyloidosis of the protein AA-type and are also characterized by episodes of neutrophilia and fever.

A marked difference between the two groups of patients studied here was the pattern of ENL reactions. At Aitape the classical crops of small, discrete, tender ENL skin lesions were seen regularly and iritis was associated with the reaction in several patients. In the highland group iritis was not seen although the skin lesions were generally more severe. The association between specific histocompatibility antigens and several diseases in which iritis is a prominent feature (reviewed in (20)) suggests that genetic factors may help to explain the different patterns of ENL reactions observed in this study. Most patients who had amyloidosis suffered from recurrent episodes of severe erythema nodosum leprosum, with extensive induration and panniculitis involving extensor surfaces. There was seldom any ulceration and the reactions usually settled with dense fibrosis and thickening of the skin. These patients had a stormy clinical course and were inclined to abscond from treatment, only to return with another reaction.

The different management of ENL reactions at Togoba and Aitape was probably also a contributing factor to the higher prevalence of amyloidosis in the highland patients. In 1967 a clinical trial of clofazimine (Lamprene or B63) was first started at Aitape. Since then, its value in the treatment of lepromatous leprosy and control of ENL reactions has become evident (30). Consequently, all patients who showed a tendency to recurrent ENL reactions at Aitape were changed from dapsone to clofazimine. On the other hand, clofazimine has only recently become available in other centers in Papua New Guinea, including Togoba, where few lepromatous patients had been treated with clofazimine at the time of this study.

The management of ENL reactions in the past has included the use of the anti-inflammatory drugs aspirin, chloroquine, antimony compounds, and steroids (reviewed in (31)). Recently, several new lines of therapy have been introduced. Thalidomide has a specific anti-ENL activity but it does not act against the leprosy bacillus (21). Clofazimine has, in addition to its bacteriostatic effect, a specific anti-ENL activity (43). Rifampicin is bactericidal (26) and might be expected to precipitate ENL reactions as antigens are released from rapidly degenerating bacteria. The fact that ENL reactions do not occur frequently may be due to an immunosuppressive action of rifampicin (20). The ability of these drugs to prevent the development of amyloidosis has not been examined but our results suggest that clofazimine prevents the development of amyloidosis by reducing the severity of ENL reactions. Colchicine has been shown to prevent the development of amyloidosis in experimental animals (30, 35). The place of colchicine in the management of leprosy patients who are at risk of developing amyloidosis has yet to be defined (20).

Recently, mouse SAA has been reported to inhibit antibody synthesis in vitro (4). If such an effect is confirmed in vivo, and in man, SAA will assume a significance in leprosy and in other diseases greater than that attributable to its presumed role as a precursor of amyloid fibril protein AA.

SUMMARY

The prevalence of the amyloid-related serum component, protein SAA, was investigated in two groups of leprosy patients from different areas of Papua New Guinea. Protein SAA was more prevalent in coastal leprosy patients (49% positive) than in highland patients (21% positive). Paradoxically, many more cases of amyloidosis were diagnosed in New Guinea, including Togoba, where few lepromatous patients had been treated with clofazimine at the time of this study.

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the highland group (17 of 199) than in the coastal group (3 of 112). The patient group, SAA was found to correlate with the leprosy disease spectrum, being more prevalent in patients toward the granulomatous pole. Borderline and tuberculoid patients who had detectable SAA usually had neutrophil ulcers. No such relationships were observed in the coastal patient group; probably because other infections, more common on the coast, were also responsible for causing increased concentrations of SAA which is known to behave as an acute phase reactant.

A correlation was observed between SAA positivity and neutrophil leukocytosis. This suggests that various inflammatory stimuli, such as erythema nodosum leprosum reactions, neutrophil ulcers and intercurrent infections, all contribute to the prevalence of SAA in leprosy patients.

RESUMEN

Se estudió la prevalencia de la proteína SSA, un componente sérico relacionado con el amiloidosis, en dos grupos de pacientes con lepra de diferentes regiones de Papua, Nueva Guinea. La proteína SSA se encontró con más frecuencia en los pacientes residentes en la costa (49% de positivos) que en aquellos residentes en las tierras altas (21% de positivos). Paradójicamente se diagnosticaron muchos más casos de amiloidosis en el grupo de las tierras altas (17 de 199) que en el grupo de la costa (3 de 112). En el grupo de pacientes de las tierras altas, la presencia de SSA se correlacionó bien con el espectro de la enfermedad y fue más frecuente en los pacientes del extremo lepromatoso. Los pacientes con lepra dimorfa y tuberculoid que tuvieron SSA detectable, generalmente, también tuvieron úlceras neurotróficas. Estas correlaciones no se observaron en el grupo de pacientes de la costa probablemente porque otras infecciones, más comunes en la costa, también contribuyeron a la alta frecuencia observada de SSA (la SSA se comporta como un reactivo de fase aguda).

 También se encontró cierta correlación entre la presencia de SSA y una leucocitosis de neutrófilos. Esto sugiere que toda una variedad de estímulos inflamatorios, tales como el eritema nodoso leproso, las úlceras neurotróficas y las infecciones intercurrentes, contribuyen a la prevalencia de SSA en los pacientes con lepra.

RESUMÉ

Dans deux groupes de malades de la lèpre, provenant de régions différentes de Papouasie-Nouvelle Guinée, on a étudié la prévalence de la Protéine SSA, constituant du sérum en rapport avec la substance amyloïde. La fréquence de la Protéine SSA était plus élevée chez les malades de la lèpre provenant de la région côtière (49 pour cent de positifs) que chez ceux provenant des collines (21 pour cent de positifs). Curieusement, un nombre plus grand de cas d'amyloïdose fut diagnostiqué dans le groupe originaire des collines (17 parmi 199), que dans le groupe venant de la région côtière (3 parmi 112).

Dans le groupe de malades originaire des collines, on a constaté que la Protéine SSA était en corrélation avec le spectre de la maladie lépreuse, étant plus fréquente chez les malades montrant un type de lèpre dans la gamme lepromatouse. Les malades atteints de lèpre dimorpha ou tuberculoid, qui présentaient un niveau décelable de Protéine SSA, souffraient généralement d'ulcère neurotróphique. Aucun relation semblable n'a été observée dans le groupe de malades de la région côtière, et ceci sans doute parce que d'autres infections, plus communes sur la côte, était également responsable pour une concentration plus élevée de Protéine SSA. On sait en effet que celle-ci réagit aux phases infectieuses aigües.

Une corrélation a été observée entre la positivité pour la Protéine SSA et une leucocytose neurotróphique. Ceci suggère que divers stimuli inflammatoires, telles que les réactions d'erythème noueux lepreux, les ulcères neurotróphiques, et les infections intercurrentes, peuvent tous contribuer à une prévalence élevée de Protéine SSA chez les malades souffrant de lèpre.

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REFERENCES


