Serum Amyloid Protein SAA, C-Reactive Protein and Lysozyme in Leprosy[1]

M. A. Scheinberg, A. Masuda, M. D. Benson and N. F. Mendes[2]

Leprosy, like other chronic diseases such as rheumatoid arthritis, osteomyelitis and ankylosing spondylitis, can cause secondary amyloidois ([6]). A serum protein (SAA) antigenically related to AA, has been identified and appears to be a precursor of the amyloid fibril protein in patients with secondary amyloidosis ([2]).

The immunochemical and cellular aspects that are responsible for the pathogenesis of amyloid disease are presently unkown. One of the current working hypotheses is that activated macrophages occupy a central position in the cellular events responsible for the synthesis and laying down of amyloid protein in the tissues ([1]). Recently, attention was drawn by McAdam *et al*, to the relation between the circulating level of neutrophils, serum acute reactants and amyloidosis ([10]).

In this paper we have determined the serum levels of SAA in patients with the two polar forms of leprosy and compared these to the serum levels of C-reactive protein (an acute phase reactant) and lysozyme, a protein which is known to be elevated in disease states where macrophage activation is usually found ([4]).

MATERIALS AND METHODS

Serum samples from 36 leprosy patients were included in the study. The patients were all registered at the Diagnostic Service of the Health Institute of the State of Sao Paulo and were classified through histologic examination of biopsies in accord with the Ridley and Jopling system ([11]). Twenty-one patients were classified as polar lepromatous (LL) and 15 as polar tuberculoid (TT). None of the patients was previously treated, had reversal reaction or *erythema nodosum leprosum* at the time of sample collection. Serum samples from twenty normal healthy adults served as controls.

Serum amyloid A protein (SAA) determination. The level of SAA in serum samples was determined by radioimmunoassay as previously described, using antiserum to amyloid protein AA and 125 iodine-labeled AA ([12]). The antiserum was prepared in rabbits by immunization with purified AA ([12]).

Serum C-reactive protein and lysozyme determinations. Serum concentrations of C-reactive protein (CRP) were determined by the radial immunodiffusion technic according to Mancini *et al* ([7]). A monospecific antiserum was obtained from Behring Diagnostics (Frankfurt, West Germany).

Serum lysozyme levels were determined by the method described by Osserman and Lawlor without any modifications ([9]). In brief, *Micrococcus lysodeiticus* was suspended uniformly in a small volume of phosphate buffered saline (PBS), incubated with 1% agarose (60°-70° C) in M/15 PBS, pH 6.3 at a concentration of 50 mg organisms in 100 ml of agarose and placed in Petri dishes, 4 mm thick.

After the agarose solidified, 2 mm diameter holes were made, and the serum placed in the wells and run simultaneously with standard dilutions of purified egg-white lysozyme. After 12 to 18 hours, clearing zones developed, and their diameters were recorded. The diameters of the zones were proportional to the logarithmic concentrations of lysozyme present.

STATISTICAL ANALYSIS

The results of each study are expressed as the mean and standard error of the mean and the differences among the groups tested by the Student's t test. The Spearman rank correlation coefficient was used to make comparisons among the indices.

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[2]M. A. Scheinberg, M.D., Ph.D.; A. Masuda, Ph.D.; M. D. Benson, M.D.; N. F. Mendes, M.D., from the Division of Immunology, Escola Paulista de Medicina, Sao Paulo, Brasil, and the Division of Rheumatology, Indiana University, Indianapolis, Indiana, USA. Reprint requests to Dr. Scheinberg, Division of Immunology, Escola Paulista de Medicina, Caixa Postal 7144, Sao Paulo, Brasil.

RESULTS

SAA protein determination. SAA serum levels were markedly elevated in the LL patients when studied as a group although marked variations could be observed when cases were analyzed individually. SAA serum levels in patients with TT did not differ from those observed in the control group (Tables 1-3).

Serum CRP and lysozyme levels. As can be seen in Tables 1, 2 and 3, there was a trend for higher CRP and lysozyme levels in LL when compared to TT and the control group. On an individual basis, however, there was no correlation between the three parameters. A summary of the data and statistical analysis is shown in Table 3.

When lepromatous and tuberculoid leprosy patients were ranked by SAA, CRP and lysozyme levels, no significant correlation could be established among themselves or with disease duration (Table 4).

DISCUSSION

In the present investigation a high proportion of LL patients had elevated levels of amyloid-related serum component in their sera in spite of the absence of intercurrent infections in such patients. Our findings are in line with a previous report on SAA levels measured by a less sensitive technic ([5]). The presence of elevated serum lysozyme levels is in agreement with the observations made by others in leprosy and other granulomatous disorders ([10]).

C-reactive protein is a serum constituent that is elevated during the acute phase of most inflammatory reactions. Studies carried out in the past few years have associated CRP with phagocytosis and inhibition of antigen reac-

TABLE 1. *Disease duration, lysozyme, CRP and serum SAA levels in lepromatous leprosy patients.*

Patient	Disease duration (years)	Lysozyme (μg/ml)	CRP (mg/100ml)	SAA (μl/ml)
1	4	6.1	0.5	17
2	8	11.5	2.0	4000
3	4	7.5	5.9	2500
4	10	16.0	6.1	350
5	2	16.0	1.3	2200
6	5	13.0	0.5	32
7	6	13.0	1.8	150
8	8	11.5	1.4	425
9	2	18.0	0.5	18
10	4	20.0	0.5	45
11	5	6.0	0.5	12
12	5	12.0	1.2	750
13	3	7.4	10.8	650
14	10	14.0	0.5	160
15	8	28.0	0.5	90
16	10	41.0	1.5	240
17	4	7.3	1.0	335
18	5	28.0	0.5	53
19	8	41.0	1.0	2.8
20	10	28.0	0.5	75
21	5	18.0	0.5	115
Mean ± S.E.	6.0 ± 0.5	16.7 ± 2.3	1.85 ± 0.56	580 ± 47

TABLE 2. *Disease duration, lysozyme, CRP and serm SAA levels in tuberculoid leprosy patients.*

Patient	Disease Duration (years)	Lysozyme (μg/ml)	CRP (mg/100ml)	SAA (μl/ml)
1	2	11.5	0.6	115
2	3	9.4	0.5	7
3	4	13.0	0.5	-
4	3	18.0	0.5	115
5	3	5.5	1.0	65
6	1	7.4	0.5	16
7	5	8.6	0.5	19
8	6	8.6	1.0	48
9	3	10.0	0.5	25
10	5	5.5	-	5
11	5	12.0	1.0	23
12	2	18.0	0.5	10
13	4	6.0	0.5	30
14	5	18.0	0.5	26
15	3	7.4	0.5	17
Mean ± S.E.	3.6 ± 0.3	10.5 ± 1.1	0.6 ± 0.05	35.9 ± 9.4

TABLE 3. *Disease duration, serum lysozyme, CRP and SAA levels in leprosy (mean ± S.E.).*

	Lepromatous (21)	Tuberculoid (15)	p value
Disease duration	6.0 ± 0.5	3.60 ± 0.3	-
Lysozyme	16.7 ± 2.3	10.5 ± 1.1	< 0.05
CRP	1.85 ± 0.56	0.6 ± 0.05	> 0.05
SAA	580 ± 47	35.9 ± 9.4	< 0.02

Normal values: Lysozyme, 8 ± 1.2 μg/ml; CRP, 0.5 mg/100; SAA, 58 ± 5 μl/ml.

TABLE 4. *Ranked correlation in leprosy patients.*

	Lepromatous		Tuberculoid	
	t	p	t	p
SAA *vs* CRP	- 0.21	> 0.05	- 0.47	> 0.05
SAA *vs* lysozyme	0.08	> 0.05	0.11	> 0.05
SAA *vs* disease duration	- 0.24	> 0.05	0.02	> 0.05

tive lymphoid cells ([8]). Since suppression of immune functions by SAA has been reported in the murine model of secondary amyloidosis, we were interested in comparing the levels of these two proteins—CRP and SAA ([13, 14]). On the other hand, with the increasing evidence that macrophages may play a role in the pathogenesis of leprosy and amyloidosis, we have looked at serum lysozyme levels which are an index of monocyte turnover in these patients. However, no correlation could be established among these parameters, suggesting that their elevation in LL when compared to TT and controls may be due to independent factors that do not bear a strict relationship to the development of amyloidosis in such patients. Recently, CRP and SAA were sequentially evaluated after an inflammatory stimuli (etiocholanolone-induced fever). The CRP and SAA levels followed a similar time course, but the correlation between the concentration of both proteins was not significant ([11]).

None of the patients included in this study was known to have amyloidosis clinically, and by history these patients lacked clinical features of *erythema nodosum leprosum*, reversal reactions or chronic trophic ulcers, which have previously been shown to be associated with a higher frequency of amyloidosis. It is of considerable interest that in some of these LL patients extremely high levels of SAA were found which did not have a relationship to the disease. Whether these patients will be more prone to the development of amyloidosis and whether fluctuations of SAA may be a way to identify such patients is presently not known. Further studies are needed with longitudinal monitoring of SAA levels along the course of the disease to assess its value in the overall management of leprosy and the possible prevention of amyloidosis.

SUMMARY

Serum amyloid protein (SAA) appears to be the precursor of amyloid protein AA, the non-immunoglobulin fibril protein of secondary amyloidosis. Since amyloidosis is known to occur in high frequency associated with lepromatous leprosy (LL), we have examined the SAA levels in untreated LL patients and compared them to the levels observed in patients with tuberculoid leprosy (TT) and a large number observed in healthy controls. We found that SAA is markedly elevated in LL when compared to TT and controls. No clear correlation could be established with C-reactive protein, a well-documented acute phase reactant, or serum lysozyme levels that reflect the presence of monocyte activity. This study showed that SAA levels in leprosy do not appear to be a reflection of inflammatory activity or monocyte turnover. Whether amyloidosis will be more prevalent in patients who have higher SAA levels remains to be determined.

RESUMEN

La proteína amiloide sérica (SAA) parece ser la precursora de la proteína amiloide AA, la proteína fibrilar o inmunoglobulínica de la amiloidosis secundaria. Puesto que se sabe que le amiloidosis se presenta muy frecuentemente asociada con lepra lepromatosa (LL), hemos examinado los niveles de SAA en pacientes LL no tratados y los hemos comparado con los niveles observados en pacientes tuberculoides (TT) y en controles sanos. Hemos encontrado que la SAA esta notablemente elevada en el grupo LL. No se pudo establecer una clara correlación con la proteina C-reactiva, un "indicador de fase aguda" bien establecido, ni con los niveles de lisozima sérica, la cual refleja actividad monocítica. Este estudio mostró que los niveles de SAA en lepra no parecen ser un reflejo de la actividad inflamatoria o del intercambio monocítico. Queda por estudiarse si la amiloidosis es más prevalente en los pacientes que tienen los niveles más altos de SAA.

RÉSUMÉ

Il apparaît que la protéine amyloïde du sérum (SAA) est le précurseur de la protéine amyloïde AA, qui est la protéine fibrillaire non immunoglobulinique de l'amyloïdose secondaire. Puisque l'on sait que l'amyloïdose survient dans un nombre élevé de cas, en association avec la lèpre lépromateuse (LL) on a examiné les taux de SAA chez des malades LL non traités, et on a comparé ces taux à ceux observés chez les malades souffrant de lèpre tuberculoïde (TT), ainsi qu'à ceux relevés chez un nombre élevé de témoins en bonne santé. On a observé que la SAA est nettement plus élevée dans la lèpre lépromateuse LL, lorsque l'on compare les taux obtenus à ceux observés chez des malades TT et chez les témoins. Aucune corrélation nette n' a pu être établie avec la protéine C-réactive, un lysozyme du sérum, dont l'activité dans la phase aiguë est bien documentée, et qui réflète l'activité des monocytes. Cette étude a montré que les taux de SAA dans la lèpre ne semblent pas refléter l'activité inflammatoire ou la rotation des monocytes. Il reste à voir si l'amyloïdose sera plus fréquente chez les malades qui présentent des taux élevés de SAA.

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