CONVERSION OF THE C3 COMPONENT OF COMPLEMENT IN SERA OF HANSENIASIS PATIENTS

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ABSTRACT — The levels of total C3 (native C3 plus its degradation products) and the degree of conversion of native C3 into its breakdown products were studied in sera of Virchowian (V), tuberculoid (T), indeterminate (I), and Virchowian with erythema nodosum Hansenicum (ENH) patients. Sera from normal individuals (N) were also analysed. While the levels of total C3 were not significantly different among the groups, the percentage of conversion of C3 into its degradation products was significantly higher in V and ENH sera. The activation of the complement system and the involvement of immune complexes (IC) are discussed.

Key words: Hanseniasis. Complement 3.

1 INTRODUCTION

The reports on the complement system in hanseniasis, specially in hanseniasis complicated by ENH, are still conflicting 1, a, 11 A deficiency of the cell mediated immunity (CMI) in Virchowian patients is accompanied by an accumulation of bacilli in tissue lesions ; at same time humoral immunity is frequently increased. Immune complexes can contribute to the pathogenesis of some tissue lesions [13]. Many authors have observed the presence of IC reacting with C^1 q in agarose in sera of Virchowian and ENH patients [4, 6, 9, 10] as well as deposits of immuno - globulins and complement in lesions of ENH [13].

IC are activators of the complement system by the classical pathway mainly, leading to the cleavage, among other components, of C3. The purpose of this work is to look for a possible coincidence between the presence of circulating IC, observed in a previous work 6, and the complement activation.

2 MATERIALS AND METHODS

2.1 Sera

Table 1 shows the number of sera analysed.

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2.2 Antiserum

Antiserum to human C3 was raised in rabbits by the method of Mardiney & Müller-Eberhard [8].

2.3 Radial immunodiffusion (RID)

RID was performed by the technique of Mancini *et al.* [7] in plates having anti- -human C3 incorporated to the agarose. This antiserum reacted with human native C3 and with its breakdown products, so this method measured the total C3 (native C3 plus its breakdown products) of the sera. A pool of normal sera was used as standard in all plates.

2.4 Immunoelectrophoresis

The Laurell's crossed immunoelectrophoresis (CIE) was performed in a micro-technique as described by Weeke [12]. The measure of the area enclosed by the precipitates was performed by planimetry, after a 5.2 times enlargement. Since this area is related to the concentration of the antigen reacting with the antiserum incorporated to the agarose, the percentage of C3 conversion for each sample was calculated according to the formula:

$$\text{Percentage of conversion} = \frac{\text{C3 B.P. X 100}}{\text{C3 B .P + NC3}}$$

C3 B.P. = area enclosed by the precipitate formed by each C3 breakdown products and the antiserum incorporated to the agarose NC3 = area enclosed by the precipitate formed by native C3 and the antiserum incorporated to the agarose.

2.5 Statistical analysis

It was performed by the Kruskal Wallis testa at the level of significance of 0.001.

3 RESULTS

The mean value and the standard deviation (SD) of the quantitation of total C3 are in Table 2. As the mean C3 values were very close it was not applied the Kruskal-Wallis test. There were no differences, among the groups studied, for total C3.

Table 3 shows the statistical analysis for the percentage of C3 conversion.

The Kruskal-Wallis test for the difference among the groups studied for the C3 conversion was 223.52. It was observed that $H_{Tab} < H_{ob}$, so that the hypothesis that the groups were homogeneous was rejected. The test was significative at the level of 0.001, so that the variations could not be considered occasional.

The sera that presented a higher conversion of C3 (plus than 50%) were those in which it was observed the presence of IC previously.

It was observed that the serum of one patient with ENH and that presented IC had none conversion of C3.

T and I patients presented low C3 conversion.

4 DISCUSSION

The high conversion of the C3 component of the complement system and the presence of Clq binding IC, related in another paper [6], suggests that these IC are involved in the complement activation. These remarks observed in ENH patients agree with immune complexes diseases.

Bjovartn et al.[2] related that the presence of IC is not the only explanation for ENH as they are found in Virchowian patients with or without ENH as well. The only change in complement they observed was the breakdown products of C3.

The conversion of the C3 component could also be due to the presence of

circulating particulates IC undetected by the technique of Clq in agarose; to an activation of C3 by the classical as well as by the alternative pathway; to antibodies on the membrane of the bacillus ; to antigens or components of the bacillus. These components were scarcely studied in view of the difficulties in obtaining the Mycobacterium leprae.

Complement metabolic studies are important to explain the complicated and still obscure immunological processes of hanseniasis, specially in the pathogenesis of ENH.

TABLE 1 — Number of sera analysed

N	V	ENH	T	I
14	13	13	10	9

TABLE 2 — Mean and SD of total C3 measured by RID

	N	V	ENH	T	I
$\bar{X}$	1.8	1.4	1.8	1.8	1.9
SD	0.3	0.3	0.49	0.4	0.6

TABLE 3 — Mean, SD and Kruskal — Wallis test for the percentage of conversion of C3

	N	V	ENH	T	I
$\bar{X}$	5.6	23.1	25.5	18.8	14.8
SD	4.7	15.1	19.4	10.8	12.7

$H_{Obs} = 223.52 > H_{Tab}$

RESUMO — Os níveis de C3 total (C3 nativo mais seus produtos de degradação) e o grau de conversão do C3 nativo em seus produtos de degradação foram estudados em soros de pacientes V, T, I e de V com ENH. Soros de indivíduos normais foram também analisados, enquanto os níveis de C3 total não diferiram significativamente entre os grupos, a conversão de C3 em seus produtos de degradação foi significativamente maior nos soros de pacientes V e naqueles com ENH. A ativação do sistema complemento e o envolvimento dos IC neste processo são discutidos.

Palavras chave: Hanseníase. Complemento 3.

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