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Blood Helper/Suppressor Lymphocyte Ratio in Sarcoidosis

Carlos Alfaro, Servando Rodriquez Pereda and Om P. Sharma

SUMMARY **The blood helper/suppressor ratio was measured in 38 patients with biopsy-proved sarcoidosis. There was no relationship between this peripheral helper/suppressor ratio and the activity of the granulomatous process. This test needs further evaluation before its routine use in assessing activity in sarcoidosis.**

Sarcoidosis is a disease characterized by hyperactivity of cellular as well as circulating immune systems.[1] In active sarcoidosis there is an increase in lymphocyte subtypes, particularly the T-helper cells in the bronchoalveolar lavage of these patients. At the same time, the T-helper cells in the peripheral blood decrease.[2,3] This study was designed to establish the value of the blood helper/suppressor ratio in assessing the activity of the sarcoid process.

MATERIALS AND METHODS

Thirty eight consecutive patients attending the Sarcoidosis Clinic at the Los Angeles County/ University of Southern California Medical Center were studied; 16 (42%) had had active disease; whereas 22 (58%) patients were diagnosed to have chronic stable disease. Tissue biopsy specimens were obtained from different sources including lung parenchyma, mediastinal nodes, skin and liver. All tissue specimens were stained and cultured to exclude tuberculosis and fungal infections. Active sarcoid was defined as the presence of progressive clinical symptoms, worsening chest x ray and an elevated serum angiotensin converting enzyme (SACE).

Serum angiotensin converting enzyme was measured by the method modified by Cushman and Cheung[4] using hippuryl-L-histidyl-L-leucine as substrate. Units are described as nanomoles of hippuric acid formed per minute at 37°C under standard assay conditions.[4,5] The angiotensin converting enzyme unit is expressed per milliliter of serum.

The percentage of T-helper OKT4 and T-suppressor OKT8 lymphocytes and their ratio were determined by a fluorescence activated sorter utilizing monoclonal antibodies.[6] The buffy coat from fresh heparinized venous blood was incubated with titrated monoclonal mouse antibody. After lysis of the red cells, the white cell pellet was washed and incubated with FITC goat $F(ab')_2$ anti-mouse IgG (Cappel Laboratory, Cochranville, PA) for 45 minutes at 4°C and washed again. Flow cytometry was performed on a cell sorter equipped with an argon laser, operated at 488 nm wave length and 400 nm for fluorescence excitation (FACS IV Becton-Dickinson, Sunnyvalle, CA or Cytofluorograph 50 H, Ortho Pharmaceuticals, Raritan, NJ). Samples were analyzed for forward light scatter and fluorescence. White blood cells were counted with a Coulter ZBI.

Leukocyte differentials were determined by counting 100 cells on a Giemsa-stained blood smear. Validation of lymphocyte analysis by flow cytometry was conducted by staining with OKM-1 monoclonal antibody which identified mono-

From the Sarcoidosis Clinic, LAC-USC Medical Center, USC School of Medicine, 1200 N. State Street, Los Angeles, CA 90033, USA.

cytes and polymorphonuclear leukocytes. The two-tailed *t*-test analysis was used in the statistical analysis.

RESULTS AND CORRELATIONS

The mean helper/suppressor ratio of the 16 patients with active sarcoidosis was 1.6 ± 0.9 SD; the normal value in our laboratory is 0.9 to 2.9. Only 3 (19%) of the 16 patients had a decreased helper/suppressor ratio (Fig. 1), with T-suppressor cells (OKT8) increased in 2 patients, and T-helper cells (OKT4) low and T-suppressor cells high in the third patient.

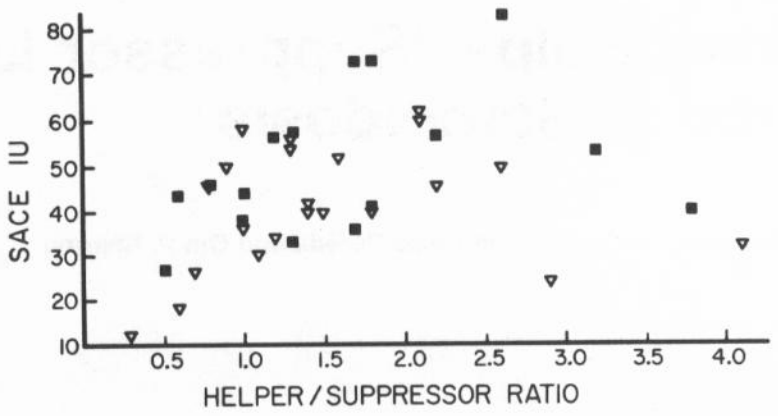

Fig. 1 Distribution of the Helper/Suppressor ratio in relation to SACE in active (■) and inactive (▽) sarcoidosis

We did not find a marked leukopenia in these patients. The mean white blood cell count was 6.3 ± 2.2 SD; only 2 (12%) of 16 patients had a WBC of less than 4.0/mm^3. The mean percentage of lymphocytes was 28% ± 12 SD; 8 (50%) patients had less than 30% lymphocytes in their blood.

The SACE was elevated in 13 (81%) patients with active disease, with a mean value of 50 IU ± 16 SD. The mean value of the serum gamma globulin was 4.0 gm/dl ± 0.5 SD, and was increased in 5 of 8 patients. There was no correlation between the high SACE or the hypergammaglobulinemia and the blood helper suppressor ratio (Fig. 1).

The chest x ray was classified following standard procedures as Stage I in 2 (17%) of 12 patients, Stage II in 7 (58%) and Stage III in 3 (35%) patients on films taken close to the H/S ratio measurement; this explains the difference in the numbers.

In the second group, twenty-two (58%) patients had inactive sarcoidosis. The mean helper/suppressor ratio in these patients was 1.5 ± 0.9 SD, and was low in 4 (18%) patients, a proportion similar to the one found among the active sarcoid patients (Table 1).

The mean white blood cell count in the inactive cases was 6.1 ± 2.3 SD, with a mean percentage lymphocytes of 35% ± 11 SD. Lymphopenia of less than 30% was present in only 7 (32%) patients.

The SACE was normal in all inactive sarcoid patients with a mean value of 26 IU ± 7 SD; this

Table 1 Comparison of the mean values between active and inactive sarcoidosis

	SACE IU	H/S Ratio	WBC cells/mm^3	Lymphocytes %	OKT4 %	OKT8 %	ESR mm	A-a DO2 mmHg	Globulins gm/dl
Normal	15-35	0.9-2.9	5.0-10.0	20-40	32-56	17-40	0-20	5-20	3.0-3.5
Active	50±16	1.6±0.9	6.3±2.2	28±12	40±9	35±12	26±20	49±19	4.0±0.5
Inactive	26±7	1.5±0.9	6.1±2.3	35±11	40±10	30±8	30±11	31±24	3.4±0.5
T-test	P≤ 0.05	NS	NS	NS	NS	NS	NS	NS	P≤ 0.05

NS, not significant

value was statistically different ($P \leq 0.05$) from the mean value of patients with active disease (Table 1).

The chest x ray in this group was read as Stage 0 in 3·(23%), Stage I in 1 (18%), Stage II in 2 (15%) and Stage III in 7 (54%) of 13 patients.

COMMENTS

In order to assess the value of the blood helper/suppressor ratio in determining the activity of the sarcoid process we measured this test in 38 patients with active and inactive disease. We correlated this ratio with the SACE (Fig. 1) and with the serum gamma globulin levels, and we did not find any relationship between this ratio and the two parameters, nor was this ratio able to indicate activity of the disease. However, we found a statistically significant difference ($P \leq 0.05$) in the mean values of SACE and serum gamma globulins between the active and inactive patients (Table 1).

Contrary to our expectation, the percentages of T-helper cells in most of the patients with active disease were within normal limits, and was low in only one patient. Similarly, the T-suppressor cells were in the normal range in 6 patients with an active disease, high in 3 and decreased in 2 patients.

The above findings suggest that the presence of the lymphocyte subtypes in the organs affected by the active sarcoid process may not be reflected by changes in their relative proportions in the peripheral blood cells. The blood helper/suppressor lymphocyte ratio has no role in assessing activity in sarcoidosis.

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REFERENCES

1. Sharma OP. Sarcoidosis Clinical Management. London : Butterworth, 1984.
2. Hunninghake GW, Crystal RG. Pulmonary Sarcoidosis: a disorder mediated by excess helper T-lymphocyte activity at sites of disease activity. N Eng J Med 1981; 305: 429-34.
3. Sharma OP. Lymphocyte subsets in tissue, blood and bronchoalveolar lavage fluid in sarcoidosis. Asian Pacific J Allergy Immunol 1985; 3: 60-2.
4. Cushman DW, Cheung HS. Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. Biochem Pharmacol 1971; 20: 1637-48.
5. Lieberman J. The specificity and nature of serum angiotensin converting enzyme (serum ACE) elevations in sarcoidosis. Ann NY Acad Sci 1976; 278: 488-97.
6. Modlin RL, Hofman FM, Meyer PR, Sharma OP, Taylor C, Rea T. In situ demonstration of T-lymphocyte subsets in granulomatous inflammation: leprosy, rhinoscleroma and sarcoidosis. Clin Exp Immunol 1983; 51: 430-8.