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Circulating immune complexes in sarcoidosis, a clinical role for the Raji assay?

D.P. Rooney, M.B. Finch, J.S. Elborn, C.F. Stanford

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ABSTRACT: Many immunological abnormalities, including circulating immune complexes (CICs), have been described in patients with sarcoidosis. The relationship of these abnormalities to clinical states is uncertain. In 31 proven cases of sarcoidosis we used the Raji assay in the investigation of the relationship between disease activity and the presence of CICs. Circulating immune complexes (>49 $\mu g \cdot ml^{-1}$) were found in 11 of 14 patients with evidence of recent significant disease deterioration, whereas only one of 17 patients with chronic stable sarcoidosis demonstrated CICs ($p<0.001$). At follow-up, it was found that eight of the 11 cases with active disease and detectable CICs had resolved clinically. Resolution was accompanied by normalization of CICs in seven and persistance in one. The remaining three patients did not improve clinically and continued to demonstrate CICs. Measurements of complement levels did not demonstrate any significant difference between the two groups of patients. It is concluded that elevated levels of CICs, as detected by the Raji assay, are commonly found in association with significantly deteriorating sarcoidosis, often normalize following disease improvement and, consequently, may have a useful clinical role as a marker of activity.

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Royal Victoria Hospital, Belfast BT12 6BA, Northern Ireland.

Correspondence: Dr D.P. Rooney, Ward 8, Royal Victoria Hospital, Belfast BT12 6BA, Northern Ireland.

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Since the report of HEDFORS and NORBERG [1] in 1974, a number of workers have demonstrated circulating immune complexes (CICs) in patients with sarcoidosis. Their findings, however, have been somewhat inconsistent. Differing methods between these studies, in both the clinical assessment of sarcoidosis activity and in the assay of CICs, have not helped to determine the significance of CIC detection in patients. Thus, CICs have been described with stages II and III chest X-ray appearance [2], disease duration >2 yrs [3], hilar adenopathy and erythema nodosum [4], recent onset disease [5], and extrathoracic sarcoidosis [6].

The Raji assay is a sensitive reproducible method of detecting the larger immune complexes which are thought to be the most efficient activators of the complement system [7]. Such activation probably accounts for many of the manifestations of immune complex mediated diseases. Relatively few previously published reports have used this assay to look for CICs in sarcoidosis [5, 8] and little is known as to whether or not such detected complexes persist following disease remission.

The purpose of this study was to compare the detection of CICs between patients separated into either active deteriorating or chronic stable sarcoidosis groups using the Raji assay and to follow up patients with elevated levels. It was hoped that by relying on investigations, such as high quality chest radiographs and standard lung function tests, which have been shown to have a high sensitivity in detecting disease deterioration [9], this would help to determine whether the Raji assay has a role in the clinical situation of assessing sarcoidosis activity.

Methods

A total of 31 patients with biopsy proven sarcoidosis, who were first referred for medical investigation on the basis of respiratory symptoms or the discovery of an abnormal chest X-ray, were prospectively studied. These patients represented the number with sarcoidosis attending the chest clinic at the Royal Victoria Hospital, Belfast, over a 12 mth period who fulfilled the entry criteria. Patients with complete resolution of symptoms, signs, chest X-ray and pulmonary function, as well as patients with other active disease processes which might confuse the assessment (*e.g.* cardiac failure) or who could independently demonstrate CICs (*e.g.* other inflammatory disease) were excluded.

Table 1. – Demographic data for the 14 patients with active sarcoidosis (Group 1) and 17 patients with stable disease (Group 2)

Patient	Disease duration yrs	Sex M/F	Age yrs	Chest X-ray stage	Clinical features	TLco % pred	SACE U·l^{-1}	CICs µg·ml^{-1}	Steroid therapy at study entry
Group 1									
1	2	F	50	III	Chronic pulmonary sarcoidosis	68.5	93	*	-
2	1	M	38	0	Severe iritis	120	47	130	10 mg prednisolone per day
3	4	F	59	II	Pulmonary sarcoid, acute deterioration	67	110	140	7.5 mg prednisolone per day
4	3	M	44	II	Chronic pulmonary sarcoidosis, iritis, skin plaques	80	147	100	-
5	9	M	36	III	Chronic pulmonary sarcoidosis	73	118	90	-
6	6	M	45	I	Lupus pernio, kerato-conjunctivitis	85	120	*	-
7	6	F	39	III	Acute on chronic pulmonary sarcoidosis	61	219	380	-
**8	0.5	M	66	III	Acute pulmonary sarcoidosis	62	142	375	-
**9	0.2	F	16	I	EN/BHL/ARTH	90	55	60	-
10	13	F	58	III	Acute on chronic pulmonary sarcoidosis	55	230	219	-
11	10	M	65	III	Acute on chronic pulmonary sarcoidosis	66	68	90	10 mg prednisolone per day
**12	0.3	F	68	III	Acute pulmonary sarcoidosis	55	320	190	-
**13	0.2	F	40	I	EN/BHL/ARTH	98	144	100	-
**14	0.2	F	50	I	EN/BHL/ARTH	113	77	*	-
Group 2									
1	6	F	42	III	Chronic pulmonary sarcoidosis, sarcoid skin plaques	70	41	*	20 mg prednisolone per day
2	2	M	45	III	Chronic sarcoid iritis	87	88	*	Predsol eye drops
3	5	F	39	0	Lupus pernio	100	80	*	-
4	10	F	78	0	Lupus pernio	60	58	*	-
5	3	F	59	III	Chronic pulmonary sarcoidosis	60	60	*	-
6	2	M	29	III	Chronic pulmonary sarcoidosis, widespread skin sarcoidosis	78	218	*	-
7	17	F	56	I	Hilar adenopathy, mild iritis	80	178	*	-
8	2	M	43	III	Iritis, chronic pulmonary changes	87	88	*	Predsol eye drops
9	5	F	50	0	Sarcoid skin plaques	90	42	*	-
10	10	M	37	II	Chronic pulmonary sarcoidosis, recently commenced on steroids (2 mths) for acute deterioration	60	51	*	20 mg prednisolone per day
11	6	F	61	I	Hilar adenopathy	87	60	*	-
12	7	F	37	I	Hilar adenopathy	69	49	*	5 mg prednisolone per day
13	2	M	46	0	3 mth steroid therapy for severe pulmonary sarcoidosis	85	67	*	20 mg prednisolone
14	4	M	50	0	Lupus pernio	92	152	*	-
15	6	M	39	0	Lymphadenopathy	95	60	*	-
16	15	M	52	0	Mild iritis	87	92	*	-
17	6	M	45	III	Skin plaques	85	152	80	-

*: value <49 µg·ml^{-1}; **: new patients; EN/BHL/ARTH: erythema nodosum, bilateral hilar adenopathy, arthropathy; TLco: transfer coefficient of the lungs for carbon monoxide; SACE: serum angiotension converting enzyme; CICs: circulating immune complexes.

Patients were questioned as to any symptomatic changes related to pulmonary and nonpulmonary sarcoid lesions since the last review. A full medical examination was performed and a chest X-ray and full respiratory function tests obtained. Transfer coefficient of the lungs for carbon monoxide (TLCO) was determined by the single breath method as modified by OGILVIE *et al.* [10]. In our pulmonary function laboratory the reproducibility in the same patients is ±4%. In the instance of nonpulmonary lesions (*e.g.* iritis), a specialist opinion was sought.

On comparison with similar measurements carried out 3–9 mths previously, the patients were subdivided into two groups (table 1). Group 1 consisted of 14 patients with clinically deteriorating disease. Clinical activity was defined as an increase in sarcoidosis related signs and symptoms (especially pulmonary, eye and skin lesions) associated with deterioration of chest X-ray appearances (as judged independently by a radiologist's assessment) and a fall in TLCO. Five new patients, three with erythema nodosum/ hilar adenopathy/arthropathy and two symptomatic patients with recent marked interstitial chest X-ray changes were considered to have acute deteriorating disease in the absence of previous respiratory clinic records, as was one case with recent severe sarcoid iritis. Group 2 consisted of 17 patients who did not fulfill the above criteria and were considered chronic stable. At study entry, all patients had blood taken for CIC, complement and serum angiotensin converting enzyme (SACE) assay. SACE levels were not used in the assessment of activity but were used instead to compare against CICs as predictors of activity.

CICs were measured by the Raji method with quantification using radiolabelled immunoglobulin as described by THEOFILOPOULOUS *et al.* [11].

Blood samples were allowed to clot at room temperature for three hours. Serum was separated by centrifugation and stored at -70°C in aliquots until assay. To minimize false positive results from antilymphocyte antibodies, assays were performed at 4°C.

Briefly, 25 µl of a fourfold dilution of duplicate serum samples was incubated at 37°C for 45 min with shaking, with 50 µl of serum-free medium containing 2×10^6 Raji cells. Control wells received 25 µl of a fourfold dilution of pooled normal serum. After three washes the cells were incubated at 4°C for 30 min with an optimum amount of ^{125}I-labelled rabbit anti-human immunoglobulin G (IgG) antiserum (Dakopatts Ltd, High Wycombe, Buckinghamshire, England). Cells were washed four times with serum-free medium, 1% bovine serum albumin, and radioactivity of the cell pellet was determined in a γ-counter.

Serum samples from 50 healthy adult blood donors of the regional population were used to determine a normal range for the immunoradiometric assay. The 50 CIC values were ranked and 95% confidence limits were obtained. In our population, the upper limit of normal has been found to be 49 µg of equivalent anti-haemophilic globulin per ml of serum. Values <49 $\mu g \cdot ml^{-1}$ were accorded the figure zero.

Total haemolytic complement (CH_{50}) was estimated by a method similar to that of MAYER [12] using sensitized sheep red cells and standardized antisera. C_3 and C_4 were assessed by a radial immunodiffusion technique [13]. The normal ranges in our laboratory are CH_{50}: 250–700 $U \cdot ml^{-1}$; C_3: 0.75–1.5 $g \cdot l^{-1}$ and C_4: 0.09–3.4 $g \cdot l^{-1}$. Serum angiotensin converting enzyme was assayed using a single reagent microcentrifugal procedure with 3-(2-Furylacryloyl)-L-phenylalanyl-glycyl-glycine (FAPGG) as the substrate [14]. Our normal adult range is 27–100 $U \cdot l^{-1}$.

All patients who had CICs detected at study entry were subsequently reinvestigated at least six months later.

Student's t-test was employed to compare the demographic features between groups 1 and 2. The comparison of CIC, SACE and complement values were made by the χ^2 test ($p<0.05$ was taken as significant).

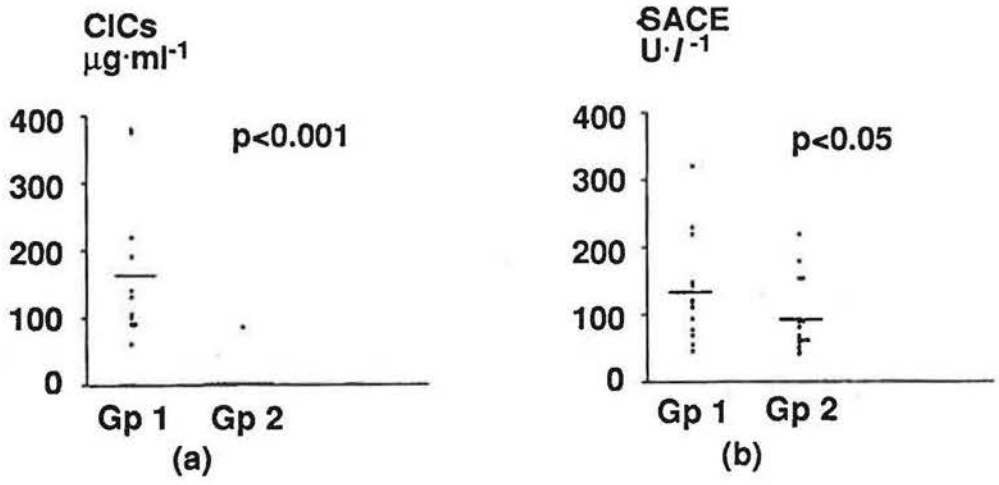

Fig. 1. – Comparison of (a) circulating immune complex (CIC) and (b) serum angiotensin converting enzyme (SACE) values between groups 1 and 2. Horizontal bars represent mean values; Gp: group.

Results

The two groups were well matched for age (48±14 yrs group 1 *vs* 48±12 yrs group 2), sex and disease duration (4±5.5 yrs group 1 *vs* 6.3±4.3 yrs group 2). Pulmonary function in group 1 showed a mean change of -10% from previous TLCO values at study entry, compared to a change of +2.7% in group 2 ($p<0.01$).

Elevated CIC levels were detected in the sera of 12 patients with concentrations ranging 60–380 $\mu g \cdot ml^{-1}$ (mean 164±105 $\mu g \cdot ml^{-1}$). CICs were, however, elevated in 11 out of the 14 patients from group 1 as opposed to only one patient from group 2 ($\chi^2=12.15$, $p<0.001$) (table 1). Employing the chest radiographic classification for sarcoidosis, CICs were detectable in one patient with stage 0 appearance, two with stage I, two with stage II and seven with stage III.

Elevated SACE levels (>100 $U \cdot l^{-1}$) were found at study entry in 13 patients, nine from group 1 and four from group 2 ($\chi^2=5.24$, $p<0.05$) (fig. 1). A significant positive correlation existed between SACE levels and elevated CICS ($r=0.54$, $p<0.002$). Values for complement (CH_{50}, C_3, C_4) were not statistically different between groups 1 and 2 and, in addition, no significant difference existed in the complement values between patients with detectable CICs and those without (table 2).

Clinical improvement subsequently occurred in eight of the 11 patients from group 1 who initially had elevated CICs, this was associated with CIC disappearance and normalization of SACE levels in seven (fig. 2). Five of these patients were started on corticosteroid therapy or had such therapy substantially increased following study entry.

The remaining three patients from group 1 demonstrated no improvement and continued to express both CICs and elevated SACE values. One case (patient 11, group 1, table 1) still had CICS detectable during clinical improvement despite steroid therapy.

Table 2. – Comparison of complement levels between the two groups with sarcoidosis and between those patients with and without elevated CICs result

	Group 1 (active disease) (n=14)	Group 2 (stable disease) (n=17)
C_3 $g \cdot l^{-1}$	1.16±0.18	1.10±0.26
C_4 $g \cdot l^{-1}$	0.37±0.10	0.42±0.15
CH_{50} $U \cdot ml^{-1}$	580±150	590±190
	Patients with no detectable CICs (n=19)	Patients with detectable CICs (n=12)
C_3 $g \cdot l^{-1}$	1.175±0.23	1.23±0.17
C_4 $g \cdot l^{-1}$	0.41±0.12	0.39±0.11
CH_{50} $U \cdot l^{-1}$	587.4±200	569.2±121

Complement levels expressed as mean±SD. CIC: circulating immune complex.

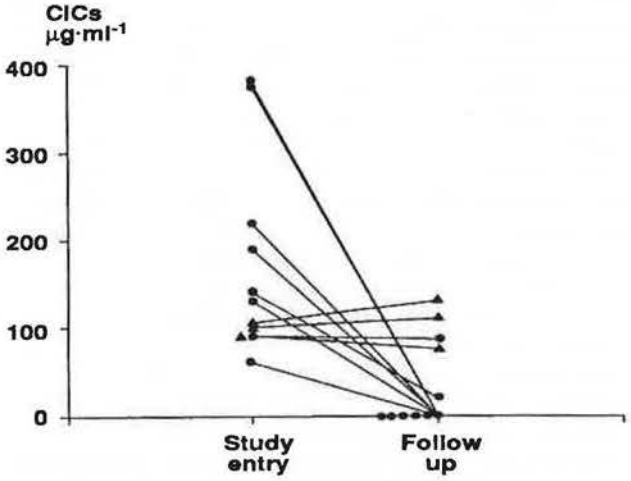

Fig. 2. – Circulating immune complex (CIC) values at study entry and follow-up in the 12 patients who had CICs detectable at study entry. ●: patients who demonstrated a significant resolution of disease activity at follow-up; ▲: patients who still had active disease at follow-up.

The only patient from group 2 positive for CICS (patient 17, group 2, table 1) continued to demonstrate immune complexes and did not alter clinically.

Discussion

The results demonstrate that patients with active deteriorating sarcoidosis, as determined by clinical criteria, often have circulating immune complexes present in their peripheral venous blood compared to infrequent detection in patients with chronic stable disease. Furthermore, clinical improvement, whether spontaneously or as a result of steroid therapy, may be mirrored by CIC disappearance.

This possible association to changes of disease activity has not been so clearly highlighted previously, although ROMER and SOLLING in a small longitudinal study [3] did detect a correlation between radiological disease regression and falling CIC levels in one case. DANIELLE *et al.* [5] also noted their absence in their patients with clinically resolved sarcoidosis.

The effects of corticosteroid therapy on CIC detection are difficult to define. It is possibile that institution of such therapy may result in suppression of immune complexes independently of clinical improvement. However, this would not explain their disappearance in two patients, whose disease resolved without therapy, or the detection of CICs in three patients already taking steroids at the time of study entry (table 1).

Complement fixation is a feature common to many immune complex mediated illnesses. We did not detect any change in complement levels in any of our patients with CICs, suggesting its fixation was not occurring (although this cannot be automatically assumed as the rate of synthesis may be sufficient to balance consumption). Moreover, immune complexes may be found with many diverse disease processes and although

they may have a role in the causation of some of the pathological changes, it is unlikely that they are often the direct mediators. It has been suggested that CICs may be generated by the process of inflammation [15] and it may be possible that in sarcoidosis their detection simply reflects a nonspecific reaction to a general inflammatory state. This could explain their much greater frequency in patients with acute disease deterioration, as presumably the inflammatory process, underlying the clinical features, has significantly increased in such cases.

What role might testing for CICs have in the assessment and management of sarcoidosis? As mentioned previously, the Raji assay is sensitive, reproducible and relatively simple to perform. Our results confirm the presence of CICs in sarcoidosis patients using this assay and also suggest a strong association with acute disease deterioration. The positive correlation we found between detectable CICs and elevated SACE suggests that the latter test would be a sufficient blood marker in many cases. However, CIC assay may be useful in those with active disease and normal SACE and could also prove a more sensitive test in reflecting changes of disease activity.

It is interesting to note, that although the difference was not statistically significant, a greater number of our group 1 patients had raised levels of CICS in their peripheral blood than SACE.

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Complexes immuns circulants dans la sarcoïdose. Un rôle clinique pour l'essai Raji. D.P. Rooney, M.B. Finch, J.S. Elborn, C.F. Stanford.

RÉSUMÉ: Beaucoup d'anomalies immunologiques, y compris la présence de complexes immuns circulants (CICs), ont été décrites chez les sarcoïdosiques. La relation de ces anomalies à l'état clinique est incertaine. Dans 31 cas prouvés de sarcoïdose, nous avons utilisé l'essai Raji dans l'investigation des relations entre l'activité de la maladie et la présence de CICs. Des complexes immuns circulants (>49 $\mu g \cdot ml^{-1}$) ont été décelés chez 11 des 14 patients ayant prouvé récemment une aggravation significative de leur maladie, mais chez un seulement des 17 patients dont la sarcoïdose était chronique et en état stable ($p<0.001$). Au cours du follow-up, l'on a trouvé que huit des onze cas atteints de maladie active et ayant des complexes immuns circulants décelables, avaient évolué cliniquement vers la résolution. La résolution s'accompagnait d'une normalisation des CICs chez sept patients, et d'une persistance de ceux-ci chez un. Les trois autres patients ne se sont par améliorés cliniquement et ont toujours des CICs démontrables. Les mesures du niveau du complément ne démontrent aucune différence significative entre les deux groupes de patients. L'on conclut que des taux élevés de CICs, comme ceux détectés par l'essai Raji, sont trouvés communément en association avec une sarcoïdose en cours de détérioration significative, se normalisent souvent après l'amélioration de la maladie, et pourraient donc avoir une utilité clinique évidente comme marqueur d'activité.

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