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Circulating immune complexes and monocyte Fc function in autoimmune diseases

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SUMMARY The phagocytosis of separated and adherent monocytes of patients with systemic lupus erythematosus is subnormal as compared to controls on the basis of latex and yeast uptake. The monocytes from the same patients react with antibody-coated sheep red blood cells in a significantly higher degree than normal monocytes. There is a correlation between the percentage of reactive monocytes and the serum immune complex content. After brief treatment of patients with levamisole the phagocytic function of monocytes is restored and at the same time the circulating immune complex content is decreased.

Patients with autoimmune connective tissue disorders display immunological hyperreactivity characterised by the formation of numerous autoantibodies and immune complexes (Johnson *et al.*, 1975; Mulli and Cruchaud, 1977; Winfield *et al.*, 1977). In addition to the alteration of humoral immunity there is an impairment of cell mediated immunity (CMI) (Utsinger, 1976). Recently Landry (1977) has shown that the impaired function of phagocytes rather than of lymphocytes may be responsible for alteration of CMI in systemic lupus erythematosus (SLE).

In this study we report impaired function of blood monocytes and the presence of circulating immune complexes (CIC) in the same patients with SLE. Alteration of monocyte function may be directly related to serum immune complexes (Rabinovitch *et al.*, 1975).

Patients and methods

PATIENTS

Twenty-nine patients with SLE were selected, 2 men and 27 women. Twenty of the patients were not receiving treatment at the time of the test. The patients fulfilled the preliminary ARA criteria for SLE (Cohen *et al.*, 1971). Nine patients had received levamisole 150 mg daily, every other day for a week. Sixteen controls were selected from among volunteers.

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CIRCULATING IMMUNE COMPLEXES

Immune complex assays were performed by a modification of the Onyewotu *et al.* (1974) technique. The ingestion of labelled aggregated IgG by guinea-pig peritoneal macrophages was inhibited in the presence of serum containing IC. 10^6 macrophages in 0·5 ml medium 199 (Flow) modified with Hanks's salts and HEPES buffer were incubated for 75 min at 37°C with 4 μg ^{125}I-aggregated human IgG and with 10 μl of a 1:5 dilution of SLE or control serum (Kávai *et al.*, 1977). All tests were performed in triplicate in siliconised glass tubes. After incubation the cells were washed 3 times, and the radioactivity of pellets was counted in a Packard automatic counter. The IC content of the sera was expressed in inhibition %.

SEPARATION OF MONOCYTES

Human monocytes were obtained by the method of Weston *et al.* (1975). 15 ml of heparinised venous blood from patients and controls was sedimented in Ficoll-Uromiro gradients. After washing, the cells were suspended in 1 ml of medium 199 and counted with a stardard haemocytometer. Trypan blue viability and neutral red phagocytosis were performed on the cells to determine the percentage of phagocytic cells and their viability.

MONOCYTE PHAGOCYTOSIS

A monolayer technique was used with the cells adherent to a glass slide in Boyden chamber. After washing, the monocytes (0·5–1·0 × 10^5 cells) were incubated with 25 μl of a 0·2% suspension of latex

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particles (Dow, 0·481 μ ϕ) or with 25 μl of a suspension of baker's yeast containing 5 × 10^6 particles, together with 0·5 ml medium 199. Incubation was at 37°C for 60 min in 5% CO_2 and 100% humidity with constant rocking. After washing, the monolayers were stained with Türk's or Wright's stain, and cells containing ingested particles were counted. The percentage of cells ingesting latex particles, and of cells ingesting or attaching yeast, and also the number of yeast particles ingested per cell (phagocytic index) was determined.

MONOCYTE OPSONISATION

Monolayers of adherent monocytes were incubated as above at 37°C for 60 min with 25 μl of 2 × 10^8 particles/ml yeast which had been treated with 0·1 ml of AB human serum as opsonin and washed. After washing and staining, the percentage of cells ingesting or attaching yeast and the number of yeast particles ingested per cell was determined.

In a separate experiment the yeast particles were incubated with AB serum in the same ratio for 60 min at 37°C. After washing, the opsonised yeast particles were incubated with fluorescein conjugated antihuman IgG, IgA, IgM, and complement C3 antisera (Hyland) and examined by fluorescent microscopy.

MONOCYTE EA ROSETTE

Monocyte Fc receptor activity was assayed by adherence of sheep red blood cells (SRBC) coated with a subagglutinating dilution (1:128) of an IgG fraction isolated on Sephadex G–200 from a rabbit anti-SRBC serum. The adherent monocytes were incubated with 0·2 ml of 2% sensitised SRBC in 0·3 ml medium 199 at 37°C for 30 min. After washing, the percentage of cells attaching or ingesting 3 or more SRBC was determined.

Results

CIRCULATING IMMUNE COMPLEXES

The patients' sera inhibited the ingestion of labelled aggregated IgG by macrophages to a significantly greater extent (48·1 ± 22·8% inhibition) than normal sera (7·8 ± 5·9% inhibition). The individual results are shown in Fig. 3.

PHAGOCYTOSIS OF MONOCYTES

Separated mononuclear leucocyte suspensions derived from peripheral blood by Ficoll-Uromiro density gradient centrifugation contained 25–30% monocytes. Monolayers of these mononuclear cells were prepared. The non-adherent cells were removed, then adherent cells, 85–99% monocytes, were analysed. In Fig. 1 it is shown that the phagocytosis of monocytes is subnormal in patients with SLE as compared with controls.

REACTIVE MONOCYTES

In this experiment the separated and adherent monocytes from the same patients and controls were incubated with sensitised SRBC or with opsonised yeast. Monocytes from the patients reacted with antibody-coated SRBC to a significantly greater extent than normal monocytes (Fig. 2).

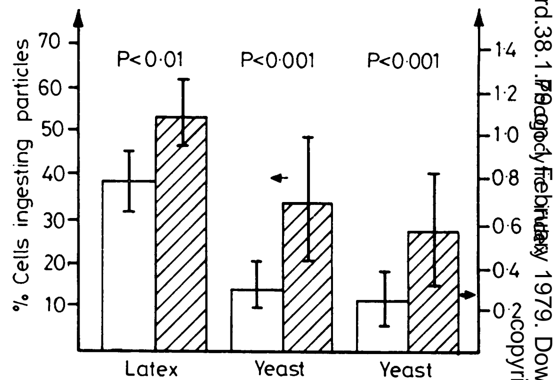

Fig. 1 *In vitro phagocytic activity of monocytes from patients* ☐ *and controls.* ▨

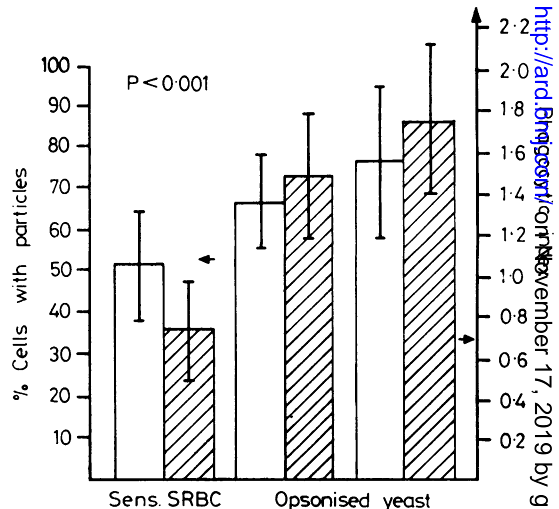

Fig. 2 *Percentages of monocytes from patients* ☐ *and controls* ▨ *reactive with sensitised SRBC or opsonised yeast particles.*

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No significant difference between the 2 groups was found in their ability to attach or phagocytose opsonised yeast. By immunofluorescence C3 could be demonstrated on the surface of each opsonised yeast particle but IgM only on 28% of the opsonised yeast particles.

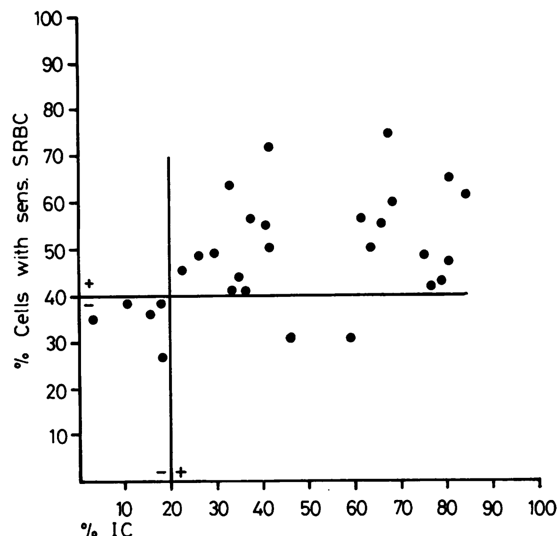

Fig. 3 *Correlation between the percentage of monocytes attaching or ingesting sensitised SRBC and the serum IC content of the same patient.*

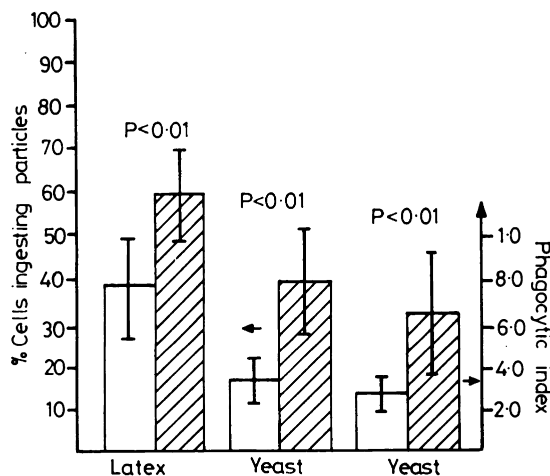

Fig. 4 *Effects of levamisole on phagocytic activity for latex and yeast of monocytes from patients.* ☐ = *before treatment,* ▨ = *after treatment.*

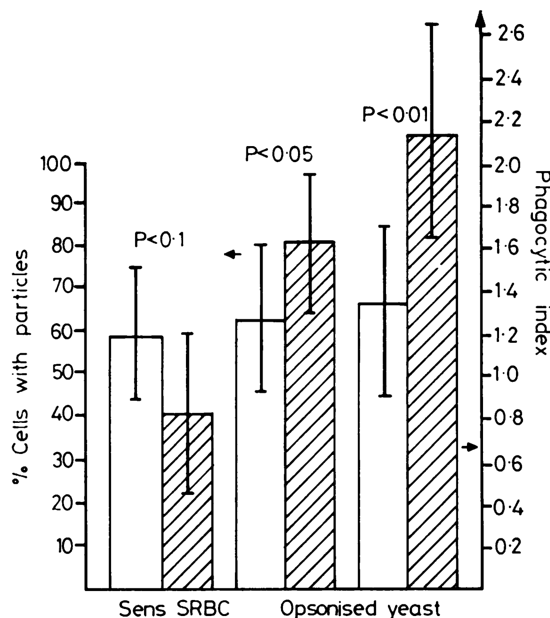

Fig. 5 *Effects of levamisole on patients' monocyte uptake of sensitised SRBC and opsonised yeast particles,* ☐ *before treatment,* ▨ *after treatment.*

Fig. 3 shows the correlation between the percentage of monocytes ingesting or attaching sensitised SRBC and the IC content of the same patients' sera. Patients having high circulating IC in most cases had monocytes with increased Fc activity.

LEVAMISOLE TREATMENT OF PATIENTS

Following levamisole treatment for 1 week phagocytosis of latex and yeast increased to normal levels (Fig. 4). The reactivity of patients' monocytes with opsonised yeast particles increased as well, but on the basis of reactivity with sensitised SRBC monocyte Fc activity was unchanged (Fig. 5).

In parallel with increased phagocytosis we observed decreased serum IC content except in 1 case (Table 1). The Table shows that the monocyte FC receptor activity was altered together with CIC of patients in 7 cases out of 9 after levamisole treatment.

Discussion

Adequate phagocytic capacity is essential for development of delayed hypersensitivity reactions. The reduced cutaneous response of patients with SLE may result from the reduced phagocytic ability of the

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Table 1 *Effects of levamisole on monocyte IgG receptor activity and on circulating immune complexes*

Patients	M-EA %		CIC %	
	Before treatment	*After treatment*	*Before treatment*	*After treatment*
1	58·9	71·0	0	0
2	35·2	65·2	0	72
3	43·3	42·0	23	16
4	50·6	51·0	63	40
5	75·0	32·3	67	22
6	56·3	24·1	65	51
7	55·9	32·7	40	25
8	82·5	13·4	33	12
9	72·3	41·3	38	24

M-EA %=% monocytes with erythrocyte-antibody.
CIC %=circulating immune complex %

polymorphonuclear leucocytes and monocyte-macrophages. Impaired phagocytic ability of polymorphonuclear leucocytes in SLE has been reported (Besana *et al.*, 1975; Zurier, 1976), and the phagocytic function of monocytes in SLE has also been questioned (Wenger and Bole, 1973; Landry, 1977). The present studies demonstrate impaired phagocytosis of latex particles and baker's yeast by monocytes in 29 patients with SLE. The CIC content of the same patients was significantly higher than that of controls. The data of Haakenstad and Mannik (1974) support our findings. *In vivo* administration of IC to the subject results in a subsequent decrease in the clearance rate of particulate matter from circulation.

We have also shown, however, that monocytes from patients with SLE react with antibody-coated SRBC to a significantly greater extent than normal monocytes, indicating increased monocyte IgG receptor activity. These data of normal monocytes are well supported by the same experiments of Reikvam (1977). The reason for this Fc receptor activation in SLE is unknown.

Increased activity of monocytes to bind antibody-coated SRBC was reported in patients with malignant lymphoma (LoBuglio, 1970; Saragome *et al.*, 1977) and in sarcoidosis (Douglas and Daughaday, 1976).

As the Fc receptor has a specially important role in the ingestion of IgG antibody-antigen complexes (Mannik *et al.*, 1974), the permanent presence of IC may elicit an increased net synthesis of membrane which contributes to activation of Fc receptor sites. This may account for differences in the immune and non-immune interaction of the monocyte-macrophage system with particles (Weiner and Bandieri, 1977; Walker, 1974).

In SLE Fc receptor activity towards opsonised yeast is not increased, as shown in Fig. 2. We could detect mainly C3 and IgM on the opsonised yeast particles. The IgG was only occasionally on a few particles. This was evidently too low a level of coating to reveal the increased reactivity demonstrated with sensitised SRBC.

Schmidt and Douglas (1976) have reported that levamisole increases *in vitro* phagocytosis of bacteria by mononuclear phagocytes. After brief treatment of the patients in the present study with levamisole the phagocytic function of their monocytes was restored, as reported by Symoeus (1976), and in parallel the CIC content decreased. After levamisole treatment the increased reactivity of the monocytes was demonstrable even with opsonised yeast particles.

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