

Leucocyte migration inhibition by heart extract and liver mitochondria in patients with myocardial infarction

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Leucocyte migration inhibition by heart extract and mitochondria can be shown in patients with myocardial infarction and angina. The active constituent of the heart extract in the migration test resides in the mitochondrial fraction. Migration inhibition by mitochondria is also seen in postoperative patients and subjects with a variety of autoimmune diseases, and seems to be a sensitive index of tissue damage. Possible uses of this test in differential diagnosis are discussed.

A variety of tissue damaging situations can be associated with cellular sensitization; cellular reactions to thyroid microsomes are seen in Hashimoto's disease (Wartenberg *et al.*, 1973a), to intrinsic factor in pernicious anaemia (Rose *et al.*, 1970), and glomerular basement membrane in glomerulonephritis (Macanovic, Evans, and Peters, 1972).

Earlier studies have directed attention to the serological reaction following tissue damage, as for example in carbon tetrachloride induced liver poisoning, where mitochondrial antibodies appear transiently (Weir, 1961). Similarly, autoantibodies to heart have been described in patients after myocardial infarction or cardiectomy (Van der Geld, 1964). One of the authors with a past history of viral myocarditis was particularly interested to see whether cellular sensitization also occurred in this situation. Previous attempts to induce lymphocyte transformation with heart extract in patients with Dressler's syndrome were unsuccessful (Lawrence and Wright, 1972), and we decided to employ another test thought to reflect delayed hypersensitivity, the leucocyte migration test. Preliminary experiments with this test using heart extracts suggested that positive results might be obtained with this system, and the present study was initiated, in which a group of patients with myocardial infarction was examined. Because of other studies in this laboratory indicating an unexpected reaction to liver mitochondria in patients with various chronic autoimmune

disorders (Brostoff, 1970; Wartenberg *et al.*, 1973b), the patients were also tested with mitochondrial preparations. This was done to test the possibility that the response to mitochondria represented a reaction to tissue damage alone.

Subjects and methods

Patients

A total of 23 patients (17 men and 6 women), mean age 62 years, range 32 to 82 years, attending the outpatient department or in the wards of The Middlesex Hospital, were selected for study. Fourteen patients were tested within 2 weeks of their infarct and two between 3 and 4 weeks. Seven subjects were tested at periods of time 3 months to 2 years after their last infarct. The diagnosis was confirmed by electrocardiography and enzyme studies.

One control group consisted of 30 apparently healthy laboratory personnel (17 men and 13 women), mean age 36 years, range 21 to 59 years. A further control group was composed of 12 older subjects (4 men and 8 women), mean age 68 years, range 46 to 95 years.

Tissue antigens

Fresh rat heart was minced with scissors and disrupted with three volumes of Eagle's minimal essential medium in a Potter-Elvehjem all glass homogenizer. It was centrifuged at 650 *g* for 15 minutes and the supernatant collected. For certain more detailed experiments this extract was further fractionated by centrifugation to provide mitochondrial, 'fluffy', and microsomal fractions as described by De Duve *et al.* (1955). Extracts were freshly prepared and concentrations expressed as

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dilutions of a standard weight of original tissue. Rat liver mitochondria were prepared by standard differential centrifugation techniques in sucrose (De Duve *et al.*, 1955) and freeze dried. Doses were calculated in this instance as dry weight of antigen per ml tissue culture medium. Preparations of human thyroid microsomes were obtained as described previously (Roitt *et al.*, 1964).

Leucocyte migration test

The technique of Bendixen and Soborg (1969) was followed. This depends on the fact that leucocytes from sensitized subjects do not migrate from a capillary tube in the presence of the relevant antigen as well as they do in its absence. Quadruplicate tests of migrations were made in the presence and absence of antigen, the area of migration being measured after 17 hours and expressed as a percentage of the value found in controls lacking antigen.

Results

Dose response of rat heart extract in the leucocyte migration test

As can be seen from Table I, doses of 1:100 and 1:250 of whole rat heart extract showed some inhibition of normal leucocyte migration, but at all dose ranges the patients inhibited to a greater extent than the controls.

Migration inhibition with heart extracts

None of the patients studied with infarction gave a normal leucocyte migration test with heart extracts at a dilution of 1:250. The mean migration of the patient group was 69 per cent ± 2.5 SEM, this being significantly different ($P < 0.0005$) from that of the control group; mean 94 per cent ± 1.5 SEM (Fig.). There was no correlation between the extent of the migration and the lapse of time between testing and infarction. Seven patients were tested up to 2 years after an infarct and at that time still showed significant inhibition. The effect of cellular sensitization is therefore very long lasting in comparison to the autoantibody response engendered by infarction. Two out of the three patients with angina pectoris who were tested also showed inhibition with this antigen (35%, 32%). One however, showed a normal response (104%).

TABLE I *Leucocyte migration inhibition with rat heart extract (dose:response)*

Patients	No.	Per cent leucocyte migration							
		Heart extract dilution			Liver mitochondria ($\mu\text{g/ml}$)				
		1:500	1:250	1:100	5	50	250	500	
Myocardial infarct	23	79 \pm 2.9*	69 \pm 2.5	57 \pm 2.7	85 \pm 4.4	82 \pm 3.9	66 \pm 3.8	57 \pm 4.1	
Controls	30	100 \pm 1.6	94 \pm 1.5	75 \pm 2.7	104 \pm 1.1	103 \pm 1.1	100 \pm 1.3	98 \pm 1.7	

* Figures represent mean per cent migration \pm standard error of mean (SEM).

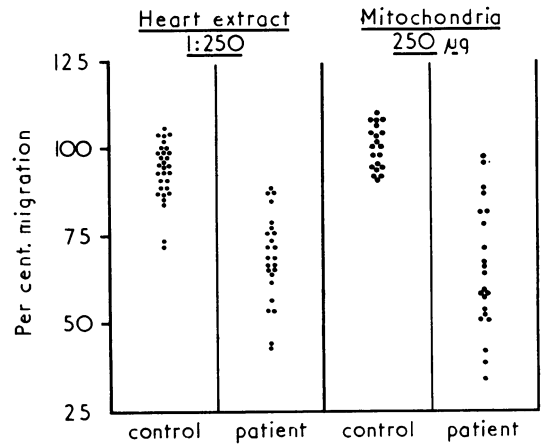


FIG. *Migration inhibition in patients with myocardial infarction.*

One of the control subjects giving significant inhibition with heart extract (67%) was the author with a previous history of myocarditis. In view of the subsequent findings it is interesting that this subject showed inhibition with heart extract but not with mitochondria.

Migration inhibition with rat liver mitochondria

At all doses of mitochondrial antigen the patients inhibited to a significantly greater extent than the control subjects. The results of individual tests with this antigen at a concentration of 250 $\mu\text{g/ml}$ are shown in the Fig. The mean migration for the two control groups was 102 per cent ± 2.1 SEM and the patient group 66 per cent ± 3.8 , the difference being highly significant ($P < 0.0005$). As with the heart extract, patients with angina pectoris also showed significant inhibition of migration with mitochondria (50%, 54%, 60%). It is interesting that with this antigen all three of the patients gave positive tests, even the one who showed no inhibition with heart extract. Again there was no significant correlation between the extent of the inhibition and the time

TABLE 2 *Leucocyte migration with fractions of rat heart extract in patients with myocardial infarction*

Patient No.	Per cent leucocyte migration				
	Whole heart extract 1:250	Whole liver mitochondria (250 µg/ml)	Heart extract fractions 1:250		
			Microsomes	'Fluffy'	Mitochondria
1	45	40	102	82	48
2	88	97	95	102	79
3	72	59	90	90	54
Control	104	92	99	114	88

lapse between infarct and testing. Some patients were tested up to two years after their infarct and still showed significant inhibition.

Leucocyte migration with fractionated rat heart extract

The fractions of rat heart extract were tested in patients with myocardial infarction in parallel with whole heart extract and liver mitochondria (Table 2). The whole heart extract gave migration indices comparable to those obtained with rat liver and rat heart mitochondria but the rat heart microsomes, which should manifest tissue specificity, showed little if any effect on the leucocyte migration pattern of patients with infarction. A further tissue specificity control was the use of human thyroid microsomes in these patients. As with rat heart microsomes no inhibition of migration was seen. Mean migration of controls with thyroid microsomes was 106 per cent \pm 3.9 and the mean migration of the patients was 96 per cent \pm 2.8, there being no significant difference between the groups.

Change in leucocyte migration after operation

Leucocyte migration was performed in two patients before and after laparotomy; one for hysterectomy and the other for hemicolectomy. Both mitochondrial and heart extracts were used to assess any change in the migration pattern. Normal responses to both heart extract and mitochondria were obtained before operation, but pronounced inhibition was seen with mitochondrial antigen 4 and 10 days after operation respectively (Table 3). No significant change at all was seen in the migration inhibition to rat heart extracts in either of the patients. The tissue damage both of infarction and operation have led to a pronounced inhibition of leucocyte migration to mitochondria and much less, if any, to heart extract.

TABLE 3 *Change in leucocyte migration after laparotomy*

Patient No.	Per cent leucocyte migration			
	Mitochondria (500 µg/ml)		Heart extract (1:250)	
	Before	After	Before	After
1	106	50	95	87
2	101	48	96	89

Discussion

All patients tested after myocardial infarction showed leucocyte migration inhibition with extracts of rat cardiac muscle, though none produced significant titres of antibodies against myocardium or showed evidence of organ specific autoantibodies. It was of interest that inhibition was found in the same patients using liver mitochondrial antigen. This *in vitro* response is long lasting, and significant inhibition may still be seen 2 years after infarction.

With fractionation of the heart extract by centrifugation, maximum activity in the migration system was found to reside in the mitochondrial fraction. The indication therefore is that the activity of the heart extract and mitochondria may well reflect a single antigen fraction, namely the mitochondria. However, until further studies of fractionation are carried out, the possibility remains that other components of cardiac muscle may be active in the migration system. There were two subjects who showed inhibition with heart extract and not with mitochondria, pointing therefore to this possibility.

In general the reactivity is seemingly related to tissue damage or injury since patients as early as 4 days after operation develop striking inhibition of migration with mitochondria and not with heart extract. Mitochondrial reactivity is not restricted to tissue damage of this sort: patients with a variety of autoimmune diseases also show significant inhibition of migration with similar preparations (Brostoff, 1970; Wartenberg *et al.*, 1973b).

Comparable findings to these have been described in guinea-pigs (Weir and Suckling, 1971) where peritoneal macrophage migration inhibition has been demonstrated with mitochondria, both in normal animals in which a peritoneal exudate had been induced and in those in which liver damage had been provoked with carbon tetrachloride.

Apart from these studies few others have investigated this cellular reactivity to tissue antigens, though autoantibodies have been examined. For example, in acute myocardial infarction in dogs,

autoantibodies detected by complement fixation have been demonstrated against crude heart extract. On fractionation, activity again resided solely in the mitochondrial fraction, microsomes and supernatant containing no activity (Pinckard *et al.*, 1971).

The question whether or not the leucocyte migration test is indeed a manifestation of cell-mediated immunity remains to be answered, but the phenomenon may be related to the localization of cells at sites of tissue damage and could be a component of the inflammatory process. The migration inhibition seen with mitochondria seems to be a sensitive assay of tissue damage and we are currently investigating the possibility that the test may have clinical applications such as in the differential diagnosis between cardiac neurosis and angina.

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