

Leucocyte migration inhibition test in cases of ischaemic heart disease

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SUMMARY Cases of ischaemic heart disease have been studied for the cell-mediated immune response against human heart antigen by using the leucocyte migration inhibition test. In 30 cases of acute myocardial infarction, the leucocyte migration inhibition values started increasing from the first week reaching a peak in 3 to 4 weeks and then declining but still above control values 12 months after infarction. The leucocyte migration inhibition values were significantly higher than control values in another 10 patients with late complications of previous infarction and in those patients with acute myocardial infarction who were less than 40 years of age, who had extensive anterior infarction, or who had a past history of angina pectoris. The leucocyte migration inhibition values were negligible in all the 12 patients with stable angina pectoris, but were high in 2 of the 8 with unstable angina and in 3 of the 4 with the intermediate coronary syndrome. The leucocyte migration inhibition values were much higher in patients with complications, which may be the result of cardiac damage by a cell-mediated immune response.

Humoral autoantibodies have been widely studied in ischaemic heart disease (Gery *et al.*, 1960; Ehrenfeld *et al.*, 1961; Van der Geld, 1964; Heine *et al.*, 1966; Strausz and Dóbiás, 1967; Sharma *et al.*, 1970). These antibodies are cytotoxic to myocardial cells grown in tissue culture (Lotta, 1958; Thompson and Halbert, 1971) and to myocardium on passive transfer in experimental animals (Chaturvedi and Mehrotra, 1967a). Development of anticardiac antibodies in rats was shown as long ago as 1945 by Cavelti, but cell-mediated immune response against heart has only been demonstrated recently in experimental animals (Chaturvedi and Mehrotra, 1967a, b; Chaturvedi *et al.*, 1971b). Little work has been done on the cellular sensitisation which occurs in heart disease. Cell-mediated immune response against rat heart (Wartenberg and Brostoff, 1973) or human heart antigens (Chaturvedi *et al.*, 1977) has been shown in cases of myocardial infarction by using the leucocyte migration inhibition test. In the present study an attempt was made to study the relation of cell-mediated immune response to various clinical presentations in cases of ischaemic heart disease.

Subjects and methods

Sixty-four patients with ischaemic heart disease

were studied; these included 20 with angina pectoris, 4 with intermediate coronary syndrome, 30 with acute myocardial infarction seen within 1 week of onset, and 10 with old myocardial infarction (3 to 12 months after acute illness). There were 60 men and 4 women, aged 30 to 91 years (Table 1). Angina pectoris was diagnosed when a patient gave a history of ischaemic cardiac pain, usually lasting for 3 to 5 minutes and in some cases 10 to 20 minutes but never more than 30 minutes; the electrocardiograms at rest or after exercise showed evidence of myocardial ischaemia. The patients with intermediate coronary syndrome had had prolonged ischaemic cardiac pain lasting for more than 30 minutes but had no evidence of myocardial necrosis on serial 12-lead electrocardiograms or aspartate aminotransferase estimations performed during the following 3 days. Acute myocardial infarction was diagnosed in those with a history of prolonged ischaemic cardiac pain; the electrocardiogram showed pathological Q waves and characteristic ST segment and T wave changes. The old myocardial infarction cases were those who had suffered from proven acute myocardial infarction 3 to 12 months earlier. The patients with angina pectoris were classed as stable or unstable (Desai and Alexander, 1972). Angina was regarded as stable when symptoms in a given patient were predictably produced on exertion and relieved by rest or glyceryl trinitrate or other similar drugs. These patients had had angina for 6 months

Table 1 Age and sex distribution of the cases

	Total no. of cases	Sex		Age					Mean
		Males	Females	30-40	41-50	51-60	61-70	> 71	
<i>Study group</i>									
Angina pectoris	20	20	—	2	8	8	2	—	50.7
Intermediate coronary syndrome	4	4	—	1	2	1	—	—	46.0
Myocardial infarction	40	36	4	6	16	13	3	2	50.5
Total	64	60	4	9	26	22	5	2	50.4
<i>Control group</i>	20	18	2	5	8	5	1	1	48.9

or more, more or less constant in severity for at least 1 month. Angina was said to have become unstable when there had been a change in symptom pattern; pain was more easily provoked and/or started occurring spontaneously and lasted longer, and the previously successful methods of relieving pain were no longer effective. Associated risk factors, viz. diabetes mellitus, hypertension, hyperlipidaemia, positive family history, and smoking, were identified (Table 2). Cases of myocardial infarction were differentiated into anterior (including extensive anterior—Friedman, 1971) and inferior and/or posterior myocardial infarction; a note was also made of complications and past history of angina pectoris.

The control group consisted of 20 healthy, age-matched individuals who had no clinical, radiological, or electrocardiographic evidence of any heart disease.

LEUCOCYTE MIGRATION INHIBITION TEST

The heart of a 36-year-old man who died in a road accident was removed within 2 hours of death. The heart was cut in thin slices, washed with sterile phosphate buffered saline, and stored at -20°C . A 10 per cent heart extract was prepared in PBS and was used as antigen (Chaturvedi *et al.*, 1973b).

In the 30 acute myocardial infarct patients, the leucocyte migration inhibition test was done on heparinised blood collected at 1, 2, 3, and 4 weeks and 3, 6, and 12 months after infarction.

The leucocytes were separated from the plasma, washed thrice with Hank's basal salt solution, and introduced into glass capillary tubes, one end of which was sealed by heat; the tubes were centrifuged at 500 rpm for 20 minutes and then cut at the cell-fluid interphase, the portion containing cells being fixed in paraffin wax in migration chambers. The chambers were filled with medium minimum essential (Eagle's) containing 10 per cent newborn calf serum. The antigen (heart extract) was added to one set of chambers while another set remained without antigen for control. The optimum dose of antigen was determined in pilot experiments. The tests were done in triplicate both in controls and in the cases in the study group. The chambers were incubated at 37°C for 20 hours. The area of cell migration was drawn on Whatman No. 1 chromatographic paper with Camera Lucida using a magnification of $\times 15$. The area of migration on paper was cut and weighed on a precision balance. The results were expressed as per cent inhibition of leucocyte migration (LMI %) in the presence of antigen, according to the following formula:

$$\text{LMI (\%)} = \frac{\text{Migration without antigen} - \text{Migration with antigen}}{\text{Migration without antigen}} \times 100.$$

A migration inhibition of 20 per cent or more was considered significant (Ortiz-Ortiz *et al.*, 1974).

Table 2 Analysis of risk factors

Group	Total no. of cases	No. of cases without risk factors	Total no. of cases with risk factors	No. with various risk factors					
				+ve family history	Diabetes mellitus	Hypertension	Hyperlipidaemia	Obesity	Smoking
Angina pectoris	20	12	8	1	2	2	—	1	5
Intermediate coronary syndrome	4	2	2	—	1	—	1	—	2
Myocardial infarction	40	26	14	3	6	3	2	3	9
Total	64	40	24	4	9	5	3	4	16

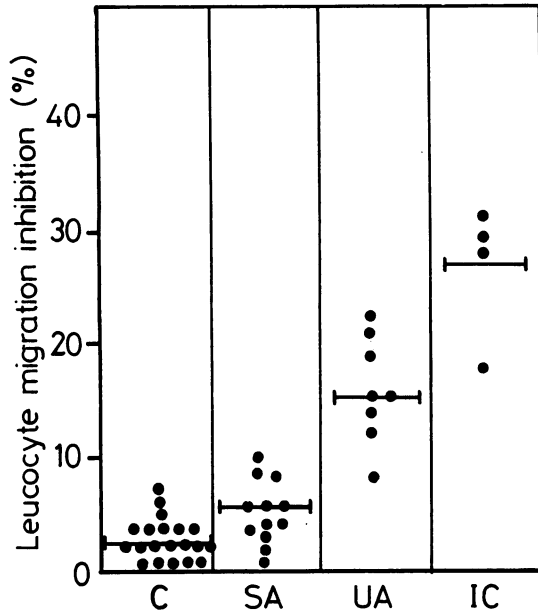


Fig. 1 Leucocyte migration inhibition in healthy controls (C), cases of stable angina pectoris (SA), unstable angina pectoris (UA), and intermediate coronary syndrome (IC): each point represents the mean of 3 estimations.

Results

In healthy controls the mean leucocyte migration inhibition was 2 ± 1 per cent. There was no significant relation between leucocyte migration inhibition and age or sex in the control cases. In cases of stable angina mean leucocyte migration inhibition was 5 ± 3 per cent and in unstable angina 16 ± 5 per cent (Fig. 1). Two of the 8 cases of unstable angina had leucocyte migration inhibition values above 20 per cent (21 and 22%) but the other 6 had lower values. Four cases of the intermediate coronary syndrome had leucocyte migration inhibition values of 32, 29, 27, and 17 per cent, with a mean value of 26 ± 6 (Fig. 1).

The cases of myocardial infarction were studied in greater detail and an attempt was made to correlate the leucocyte migration inhibition values with various possibly relevant factors.

TIME AFTER MYOCARDIAL INFARCTION

The results are presented in Fig. 2. The mean leucocyte migration inhibition value was 22 ± 8 per cent in the first week, 50 ± 15 per cent in the second week, 69 ± 8 per cent in the third week, 70 ± 9 per cent in the fourth week, 55 ± 8 per cent in the third month, 45 ± 14 per cent in the sixth month, and $39 \pm$

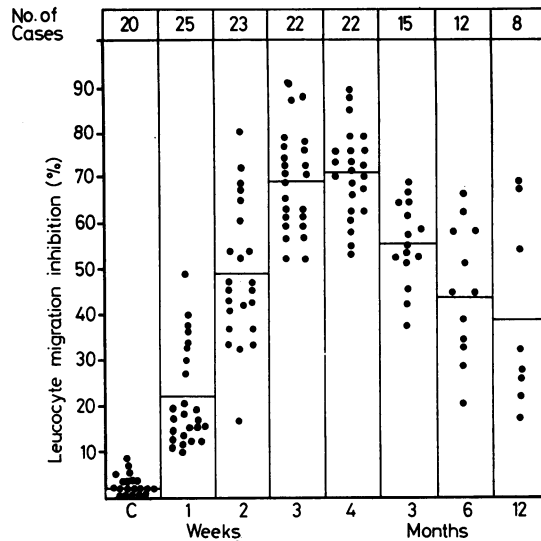


Fig. 2 Leucocyte migration inhibition in healthy controls (C) and at different times after acute myocardial infarction.

20 per cent in the twelfth month. It is evident from Fig. 2 that inhibition of leucocyte migration increased during the first 3 or 4 weeks reaching a peak value at that time, then gradually declined from the third to the twelfth month after myocardial infarction.

In the following paragraphs, the peak leucocyte migration inhibition value during the initial 4-week period has been related to the various factors.

AGE OF PATIENT

There were significantly higher leucocyte migration inhibition values in patients between 30 and 40 years of age than in those over 40 years ($P < 0.05$) (Table 3).

SITE OF MYOCARDIAL INFARCTION

The relation of the site of infarction and leucocyte migration inhibition is summarised in Fig. 3. The mean leucocyte migration inhibition value was

Table 3 Relation of mean leucocyte migration inhibition (LMI) to age of patients with acute myocardial infarction

Age (y)	No. of cases	Mean LMI \pm SD
30-40	5	77 \pm 5*
41-50	13	68 \pm 9
51-60	9	72 \pm 11
61-70	2	64
71-91	1	64

*Difference between mean value for patients aged 30 to 40 years significantly different from mean value for patients over 40 years ($P < 0.05$).

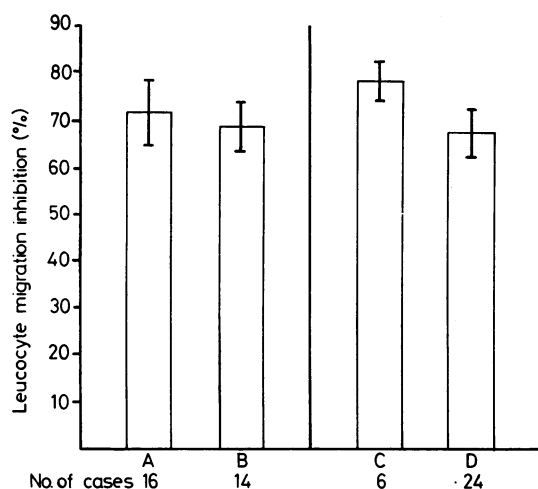


Fig. 3 Leucocyte migration inhibition in relation to site and extent of infarction. (A) Anterior infarction, (B) inferior/posterior infarction, (C) extensive anterior infarction, and (D) remaining anterior infarction/inferior/posterior infarction.

71 ± 10 and 70 ± 10 per cent in cases of anterior infarction and inferior/posterior infarction, respectively ($P > 0.05$). However, the cases of extensive anterior infarction showed 78 ± 7 per cent inhibition of leucocyte migration, compared with 68 ± 10 per cent inhibition of migration in all other infarction cases ($P < 0.01$).

RISK FACTORS

The risk factors taken into account were diabetes mellitus, hypertension, hyperlipidaemia, smoking, and positive family history. Mean leucocyte migration inhibition values in 11 cases of acute myocardial infarction with risk factors and in 19 cases without risk factors were 68 ± 9 and 71 ± 10 per cent, respectively ($P > 0.05$) (Fig. 4).

HISTORY OF ANGINA PECTORIS

Seven patients had had angina for 6 months or more. The mean leucocyte migration inhibition value in

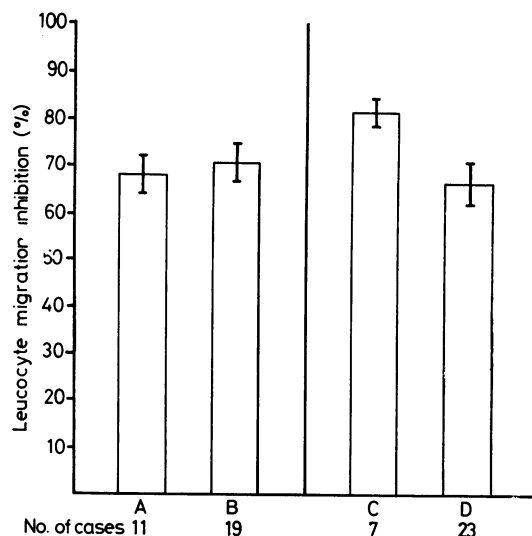


Fig. 4 Leucocyte migration inhibition in cases of acute myocardial infarction, (A) with risk factors, (B) without risk factors, (C) with past history of angina pectoris, and (D) without past history of angina pectoris.

such patients was 82 ± 8 per cent, while in another 23 patients who never had angina it was 67 ± 8 per cent (Fig. 4). The difference between these two groups is significant ($P < 0.001$).

COMPLICATIONS

Twelve patients with acute myocardial infarction had various complications (arrhythmias, left ventricular failure, congestive heart failure, or pericarditis). The mean leucocyte migration inhibition value in such patients was 67 ± 9 per cent (Table 4) and in the 18 cases with no complications the mean leucocyte migration inhibition value was 72 ± 10 per cent ($P > 0.05$). Myocardial infarct patients who were readmitted with angina, congestive heart failure, left ventricular failure, the shoulder hand syndrome, or Dressler's syndrome, 3 or 12 months after the initial illness, had mean leucocyte migration inhibition values significantly higher than those in uncomplicated cases (Table 4).

Table 4 Relation of leucocyte migration inhibition (LMI) to complications of myocardial infarction

	Acute infarction		Old myocardial infarction*					
	No. of cases	LMI ± SD	3 months		6 months		12 months	
			No. of cases	LMI ± SD	No. of cases	LMI ± SD	No. of cases	LMI ± SD
Uncomplicated	18	72 ± 10	11	52 ± 7	8	35 ± 14	5	25 ± 6
Complicated	12	67 ± 9	4	64 ± 4	4	61 ± 3	3	64 ± 6
P value		> 0.05		< 0.05		< 0.001		< 0.001

*This group includes patients originally seen as cases of acute myocardial infarction who had late complications, as well as those seen for the first time 3 to 12 months after their acute myocardial infarct.

Discussion

In the present study, myocardial infarct patients showed significant leucocyte migration inhibition which increased to a peak value 3 or 4 weeks after infarction and then declined, but which could still be shown after 12 months. Similarly, titres of humoral anticardiac antibodies are greatest 3 weeks after myocardial infarction (Sharma *et al.*, 1970). Also, in serial experimental studies peak values of humoral antibodies and cell mediated immune response were found 3 to 4 weeks after pericardiotomy (Chaturvedi *et al.*, 1973a). Wartenberg and Brostoff (1973) found significant leucocyte migration inhibition in all the 14 patients studied within 2 weeks and in 2 cases studied 3 to 4 weeks after myocardial infarction; their failure to show a correlation between leucocyte migration inhibition and time after infarction may be the result of the smaller number of patients and fewer observations on each patient.

The patients with stable angina pectoris showed insignificant leucocyte migration inhibition ($5 \pm 3\%$). Two of the 8 patients with unstable angina pectoris had more than 20 per cent leucocyte migration inhibition (21 and 22%). Wartenberg and Brostoff (1973) studied 2 cases of angina pectoris and found significant inhibition of leucocyte migration. Of 4 patients with the intermediate coronary syndrome, 3 showed significant leucocyte migration inhibition (mean $26 \pm 6\%$). Humoral antibodies have been found in 42 to 63 per cent of cases with angina pectoris (Davies and Gery, 1960; Heine *et al.*, 1966; Strausz and Dóbiás, 1967; Das *et al.*, 1974). These observations indicate release of cardiac antigens in such patients, which may be the result of necrosis of myocardial fibres (Hurst *et al.*, 1974).

Significantly higher leucocyte migration inhibition values were found in patients below the age of 40 years, with extensive anterior infarction, past history of angina pectoris, or late complications of infarction. The presence of coronary risk factors or complications of acute myocardial infarction did not affect the leucocyte migration inhibition values.

It was interesting to find that there were significantly higher leucocyte migration inhibition values in 11 myocardial infarct patients with late complications, including 3 with postinfarction angina, and 1 each of Dressler's syndrome and the shoulder hand syndrome. These higher leucocyte migration inhibition values may be the result of increased and/or recurrent release of cardiac antigens and consequent great sensitisation of T-cells. On the other hand, the sensitised T-cells may be damaging the heart thus causing these manifestations. The higher

leucocyte migration inhibition values may reflect greater T-cell activity. There is evidence that antibodies are produced as a result of cardiac injury (Chaturvedi, 1974). Antibodies may thus be produced as a result of myocardial infarction. Since anticardiac antibodies (both humoral and cellular) have been shown to produce cardiac damage on passive transfer to normal animals (Chaturvedi and Mehrotra, 1967a), it is likely that the above-mentioned complications may be caused by these antibodies.

It is interesting to speculate on why complications occur in some cases and not in others. This may depend upon certain factors which may potentiate cardiotoxic effects of antibodies: two of these have been reported in experimental studies, isoprenaline (Chaturvedi and Mehrotra, 1969), and Cocksackie B4 infection (Chaturvedi *et al.*, 1971a), but it is not known how far these experimental observations can be extrapolated to human patients.

Bauer *et al.* (1972) found no difference in anticardiac antibody titres in complicated compared with uncomplicated cases of myocardial infarction. There was no relation between antibody titre and the occurrence of the post-cardiotomy syndrome (to be published). It may, therefore, be that the cell-mediated immune response is more important as a cause of cardiac damage; this view is supported by observations in experimental animals (Natu and Chaturvedi, 1977).

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