In vivo assessment of granulocyte migration to diseased bowel in Crohn's disease

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SUMMARY It has been suggested, on the basis of impaired granulocyte migration to skin windows, that there is a fundamental granulocyte defect in Crohn's disease. *In vitro* tests of granulocyte function have, however, failed to confirm this. We have studied granulocyte migration to inflamed bowel in Crohn's disease using a new approach which utilises dynamic gamma camera imaging after injection of ^{111}In labelled autologous granulocytes. In 20 of 22 studies there was rapid migration to diseased bowel, compatible with no migration delay. Only two patients showed delays in migration of 12 and 15 minutes respectively, but neither had any clinical characteristics to distinguish them from the other 20 patients. This study shows that the majority of patients with Crohn's disease in relapse have rapid granulocyte migration to diseased bowel and provides evidence against a significant migration defect in this condition.

The similarity between the intestinal lesions in chronic granulomatous disease and Crohn's disease has led to the suggestion that defective granulocyte function may be involved in the pathogenesis of Crohn's disease.¹ Support for this hypothesis is a striking impairment of *in vivo* granulocyte migration into inflammation induced in skin windows.² Further studies have shown a delay of between two to four hours before leucocyte accumulation begins.³ The possibility that identification of a granulocyte defect might provide the basis for specific therapy for Crohn's disease has led to a detailed assessment of granulocyte function *in vitro*. Because the results of such studies have been conflicting for both chemotaxis and phagocytosis,⁴ however, they have been of little value.

To determine whether there is a granulocyte migration defect in Crohn's disease there is a need for a direct *in vivo* assessment of granulocyte migration into diseased bowel. Recent work with ^{111}In labelled leucocytes has provided a possible approach. Thus, we⁵ and Segal *et al.*,⁶ utilising a mixed ^{111}In labelled leucocyte preparation, have shown intense accumulation of

labelled cells in Crohn's disease within two to four hours of injection. In a single patient in whom the kinetics of ^{111}In labelled leucocytes were studied, a significant delay of 20-30 minutes before the start of granulocyte accumulation was shown in ileocaecal Crohn's disease. We have recently improved this technique by separating and labelling a pure granulocyte fraction without isolation from plasma. By using dynamic gamma camera imaging immediately after injection of labelled granulocytes we have monitored granulocyte migration into sites of inflammation. In the present study we have used this technique to measure the migration delay, if any, in Crohn's disease.

Methods

PATIENTS

Twenty two patients with clinically active Crohn's disease were studied. None had received any medication in the previous four months. The diagnosis was based on typical clinical and radiological features and, in 15 patients, confirmed by histology. In 12 patients, the disease involved the ileum alone in five, the ileum and colon, and five, the colon alone. Apart from ankylosing spondylitis in one patient, there were no extra intestinal complications. The Table summarises patient details.

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Received for publication 15 June 1984

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Table Clinical details and results in patients studied

Patient	Disease distribution	CDAI	ESR (mm fall in 1st h)	B-b/A (% per min)	A/a	D (min)
1	Ileal	224	66	3.8	1.15	10
2	Ileal	211	31	0.9	1.08	10
3	Ileocolonic	190	38	1.8	1.15	10
4	Ileal	156	16	0.9	1.04	10
5	Colonic	348	100	2.9	1.15	10
6	Ileocolonic	159	35	1.2	1.12	10
7	Colonic	162	33	2.8	1.08	10
8	Ileal	178	18	3.9	1.03	10
9	Ileal	331	15	2.6	1.02	5
10	Ileal	280	90	6.4	1.13	5
11	Ileal	193	31	2.6	1.12	5
12	Ileal	177	20	3.9	1.37	5
13	Ileal	188	53	2.8	1.40	5
14	Colonic	183	39	1.6	1.30	5
15	Colonic	216	28	1.6	1.1	5
16	Ileal	250	72	1.0	1.2	5
17	Ileal	202	35	4.0	1.14	5
18	Ileal	157	45	5.6	0.83	12
19	Colonic	262	56	3.0	0.94	15
20	Colonic	208	34	2.4	0.83	5
21	Colonic	158	73	1.0	1.10	5
22	Colonic	249	52	1.5	1.24	5

B-b/A, A/a and D – see Fig. 1a. CDAI – Crohn's disease activity index.

LABELLING TECHNIQUES

Granulocytes were isolated in plasma enriched density gradient media and subsequently labelled in plasma with ^{111}In tropolonate as previously described.⁷

IMAGING

The labelled granulocytes were injected with the patient supine, positioned beneath a gamma camera (IGE 400T) interfaced to a computer (MDS A²). Dynamic imaging using a frame time of 15 sec from 0–5 min and one minute subsequently was performed over a period of 40 min after injection. For the first 10 minutes (in the initial eight patients) or first five minutes (in the subsequent 14 patients) the camera was positioned over the chest, in order to record lung activity, and then moved to the abdomen to record activity from the site of diseased bowel. Time activity curves were constructed over the chest for the first five or 10 minutes and over the site of inflamed bowel and a control abdominal region for either the subsequent 35 or 30 minutes. In most cases the area of inflammation was not central, and the control region was taken symmetrically from the opposite side. In two cases with pancolitis (patients 21, 22) and one case (patient 20) with a diseased segment of transverse colon the control region was not symmetrical and selected from an uninvolved area of the abdomen with an attempt to

ensure the inclusion of similar amounts of bone marrow and major blood vessels. The control and test regions had areas of equal size.

DATA ANALYSIS

Based on dynamic imaging over the lungs it was established in each case that pulmonary leucostasis was absent in that by five minutes the count rate over the lungs had fallen to less than 40% of initial maximal value and by 10 min to 30%.⁷

The counts collected in each minute over the test and control regions of the abdomen were subjected to computerised least squares linear regression analysis using the MINITAB programme. The significance of the difference between the slopes of the test and control regions was calculated using the respective standard deviations of the slopes. An estimate of the rate of accumulation of activity in the inflammatory site was made by subtracting the slopes of the control and test regions and expressing the difference in terms of the zero time intercept of the test region. The corrected slope $(B-b)/A$, is the early granulocyte accumulation index (EGAI) and gives the rate of accumulation of granulocytes in inflamed bowel as a fraction of the zero time count per unit time (Fig. 1a). D, the maximum estimated migration delay, is the interval between injection and the time at which the count rate over the test region starts to rise. If there was no delay before granulocyte accumulation began the ratio of the extrapolated zero time intercepts of the test and control regions (A/a) would represent the relative blood pools of each region, and should be equal to or greater than unity. If there was a significant delay, the ratio of the extrapolated zero time intercepts would be expected to fall below unity (Fig. 1b).

Results

All 22 of the patients had positive scans within 40 minutes of injection of the labelled granulocytes (Fig. 2a). In several cases abnormal activity could be seen at 10 min (Fig. 2b). In 20 patients the count rate was rising over the test region at the start of the abdominal dynamic imaging (which in 12 cases was at five minutes and in the other eight cases at 10 min). An example of such a dynamic pattern is shown in Fig. 3. This study shows that the count rate was rising over the test region at five minutes and shows that this rise cannot be accounted for by background accumulation as the control region showed minimal change. In two patients (18 and 19) the count rate remained constant over the test region until 12 minutes and 15 minutes respectively after injection and then began to rise. An example

Fig. 1 Method of determining migration delay and corrected gradient of uptake of activity. (a) Maximum estimated migration delay (D) less than five minutes. y_1 and y_2 are the count rates recorded over the test and control regions respectively. A and a are the corresponding zero time intercepts and B and b the gradients. The corrected slope is equal to $B - b/A$. A/a is greater than unity. (b) Maximum estimated migration delay (D) equals 10 minutes. y_1 , y_2 , B , b and a as in Fig. 1a. A' (zero time intercept of test slope) is less than a because of the migration delay of 10 minutes and so A'/a is less than unity.

Fig. 2 (a) $^{111}\text{Indium}$ granulocyte scan in patient 12 at 40 minutes showing abnormal activity in the right iliac fossa corresponding to ileal Crohn's disease. (b) Scan taken between 5 and 9 minutes already showing faint activity in the right iliac fossa.

Fig. 3 Dynamic sequences showing a migration delay of less than five minutes in patient 12. Ordinate: counts collected in one minute frames. Abscissa: time from injection. $\blacktriangle$ = inflammatory focus; $\circ$ = control region.

of this dynamic pattern is shown in Fig. 4. The Table summarises the values of the EGAI $[(B-b)/A]$, A/a , and D , the maximum estimated granulocyte migration delay.

In the studies where localisation was in progress at the start of the dynamic imaging the maximum estimated delay in granulocyte migration was 10 minutes in patients 1–8 and five minutes in patients 8–17. The ratios of the extrapolated zero time intercepts of A and a were greater than unity in all 17 patients in whom symmetrical test and control

Fig. 4 Dynamic sequences showing a migration delay of 12 minutes in patient 18. Ordinate: counts collected in one minute frames. Abscissa: time from injection. $\blacktriangle$ = inflammatory focus; $\circ$ = control region.

regions were available (mean $1.15 \pm \text{SD } 0.11$). Only one patient (20, with the isolated segment of transverse colon involved, and an asymmetric control region, had an A/a ratio of less than unity. In the two patients where granulocyte accumulation was not in progress at the start of dynamic imaging there were significant delays of 12 and 15 minutes. In both cases symmetrical control regions were analysed and the ratios of the extrapolated zero time intercepts were less than unity. There were no clinical features to distinguish these two patients from the other 20 with regard to activity, distribution, or duration of disease.

The EGAI varied from 0.9 to 6.4% per minute. In all cases there was a significant difference ($p < 0.001$) between the slopes of the test and control areas.

Discussion

Crohn's disease is a chronic inflammatory condition which is characterised by acute relapses interspersed with periods of remission. It may be that a defective acute inflammatory reaction resulting from granulocyte dysfunction is the cause of this condition. Despite considerable study, however, both *in vivo* and *in vitro*, the available evidence is inconclusive.

Using a novel approach to assess granulocyte function *in vivo*, the present investigation showed rapid migration of labelled granulocytes to inflamed bowel in Crohn's disease, compatible, in the majority of patients, with no migration delay at all. The significance of the delayed granulocyte accumulation showed in two of the patients is not certain. The ratios of the extrapolated zero time intercepts of less than unity suggest that this was a true migration delay specially as all but one of the remaining patients had ratios greater than unity. In previous studies, based on mixed leucocytes labelled in saline media, the immediate pulmonary granulocyte sequestration that was apparent may have been the cause of delayed localisation. In the present study, however, dynamic imaging showed rapid pulmonary granulocyte transit.

The observation of rapid granulocyte accumulation in inflamed bowel is difficult to reconcile with the results of skin window studies. Although it is an elegant method to examine granulocyte function *in vivo* the skin window technique has several important drawbacks. Firstly it is intrinsically insensitive in defining the magnitude of a migration delay, because as granulocyte accumulation in skin windows is counted at intervals of an hour, the delay is approximated to the nearest hour. Secondly, factors apart from granulocyte migration, namely granulocyte adhesion and tissue generation of chemotactic factors, influence the results. Finally it

is not known whether it is valid to extrapolate from induced skin inflammation to naturally occurring inflammation in intestinal tissue.

In vitro studies are almost equally divided in support of the presence or absence of granulocyte dysfunction in Crohn's disease. Five groups have shown normal *in vitro* chemotaxis^{2 3 8-10} while six have found abnormalities, either reduction of cellular chemotaxis or presence of serum inhibitors.^{4 11-15} These discrepant findings do not appear to result from differences in disease activity, duration of treatment or site of disease. They may, however, be related to problems associated with *in vitro* tests. Thus, many workers have subjected granulocytes to extensive *in vitro* manipulation during the separation procedures which result in cell damage. This cell damage is often too subtle to be detected by *in vitro* function tests yet may profoundly influence *in vivo* kinetics.¹⁶ A further drawback with *in vitro* chemotaxis assays – for example, the Boyden Chamber, is that, by using a leading front method, a non-representative subpopulation might be assessed. It appears likely that the existence of a granulocyte defect in Crohn's disease will not be resolved by *in vitro* testing because of problems of individual test modifications and lack of standardisation.

An *in vivo* scanning technique offers several advantages over the standard skin window technique and *in vitro* chemotaxis assays. Thus, the ability of ¹¹¹indium granulocytes to monitor bowel inflammation renders the results directly relevant to Crohn's disease. As the skin window technique examines the total time for (a) the generation of chemotactic factors, (b) subsequent intravascular margination, (c) extravascular migration and, (d) accumulation in skin filters, an impairment at any of these stages could produce abnormal results. Because ¹¹¹indium granulocyte scanning assesses spontaneous bowel inflammation, it is not influenced by delays in the generation of chemotactic factors. Furthermore, accumulation is monitored dynamically minute by minute. Thus ¹¹¹indium granulocytes are more specific than the skin window technique for examining intravascular granulocyte margination and extravascular migration, the first two steps necessary in the natural defence mechanism dealing with foreign material.

Although ¹¹¹indium granulocyte scanning offers clear advantages over existing techniques available to examine granulocyte migration, there are important theoretical and practical limitations. Dynamic scanning is also a 'leading front' measurement and so may have a problem in common with *in vitro* assays. The linearity of the accumulation in inflamed bowel during the 40 min imaging period

suggests that it is assessing a representative granulocyte population. A major limitation of this technique is that only patients with active Crohn's disease can be investigated, as those with inactive disease will not accumulate granulocytes. Thus the technique could fail to detect a subtle granulocyte migration defect if it was present only in Crohn's disease in remission and which in theory could allow the initiation of a chain of events leading to a normal acute inflammatory response, ultimately assessed by ¹¹¹indium granulocyte scanning. There is no evidence, however, either from *in vitro* or skin window studies that the migration defect is confined to quiescent disease. The technique is not applicable to other conditions where defective granulocyte chemotaxis has been demonstrated – for example, Hodgkin's disease,¹⁷ sarcoidosis¹⁸ and alcoholic cirrhosis¹⁹ unless there is a fortuitous complicating abscess. It is, therefore, very difficult to establish a normal range of values.

The results from the study suggests that in most patients with Crohn's disease in relapse there is rapid migration of granulocytes to inflamed bowel and provides no evidence to support impaired granulocyte function in this condition.

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