Monocytes in inflammatory bowel disease: monocyte and serum lysosomal enzyme activity

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Summary

1. The activity of a specific lysosomal marker enzyme N'acetyl-β-D-glucosaminidase has been determined fluorimetrically in the monocytes and in the serum of patients with Crohn's disease and ulcerative colitis and compared with results obtained from healthy and disease control subjects.

2. Enzyme activities were measured in a monocyte-enriched suspension from a Ficoll–Triosil gradient and in an adherent monocyte preparation.

3. The results indicate that enzyme activity is greater in both monocytes and sera of patients with Crohn’s disease and ulcerative colitis than in those from control subjects (P < 0.01).

4. Enzyme activity within monocytes correlated with disease activity (P < 0.05).

5. Lysosomal enzymes may contribute to the pathogenesis of the mucosal inflammation in inflammatory bowel disease.

Key words: N'acetyl-β-D-glucosaminidase, Crohn's disease, monocytes, ulcerative colitis.

Introduction

Absolute numbers of monocytes are increased in patients with both Crohn's disease and ulcerative colitis (Thayer, Charland & Field, 1976; A. S. Mee, J. Berney & D. J. Jewell, unpublished work) and there is evidence that monocyte turnover is increased in patients with Crohn's disease (Meuret, Bitzi & Hammer, 1978). These cells also show increased phagocytosis suggesting that they are circulating in an activated state (Mee, Szwatakowski & Jewell, 1978). One of the consequences of monocyte activation is increase in lysosomal enzyme activity (Unanue, 1976) and release of these acid hydrolases may contribute to tissue inflammation.

We have therefore measured the activity of a specific lysosomal enzyme, N'acetyl-β-D-glucosaminidase, within the monocytes and in the serum of patients with both Crohn's disease and ulcerative colitis.

Methods

Patients studied

The activity of N'acetyl-β-D-glucosaminidase was estimated in the monocytes of patients with Crohn’s disease and ulcerative colitis and compared with that found in normal healthy controls.

A mononuclear cell population was used to study enzyme activity in 28 patients with Crohn’s disease, 28 patients with ulcerative colitis and 34 normal controls.

A purified monocyte suspension was used to estimate enzyme activity in the monocytes of 21 patients with Crohn’s disease, 19 patients with ulcerative colitis and 21 normal controls.

Serum lysosomal enzyme activity was measured in 51 patients with Crohn’s disease, 40 patients with ulcerative colitis, 63 normal healthy controls and 31 disease controls. The age and sex distribution for these different groups is given in Table 1.
The diagnosis of Crohn's disease and ulcerative colitis was established on standard clinical, histological and radiological criteria.

For patients with Crohn's disease, disease activity was graded according to the criteria of de Dombal, Burton, Clamp & Goligher (1974) and for patients with ulcerative colitis according to the criteria of Truelove & Witts (1955).

The disease control group consisted of patients with either peptic ulceration or the irritable bowel syndrome, neither of which is thought to be immunologically mediated.

**Mononuclear cell preparation**

Twenty millilitres of heparinized venous blood (5 μg of heparin/ml) were layered on to 8 ml of Ficoll–Triosil (Ficoll, Pharmacia Fine Chemicals, Uppsala, Sweden; Triosil, Nyegaard and Co., Oslo, Norway) in siliconized glass centrifuge tubes and centrifuged at 400 g for 30 min at 12°C. The cells at the interface were removed with a siliconized Pasteur pipette, washed twice with Hanks' balanced salt solution (Gibco Biocult, Glasgow, Scotland, U.K.) and resuspended in the same medium.

**Adherent cell preparations**

The mononuclear cell suspension obtained by centrifugation over the Ficoll–Triosil gradient was washed twice in Hanks' balanced salt solution before resuspending in Hanks' balanced salt solution/10% foetal calf serum (Flow Laboratories, Irvine, Scotland, U.K.) containing heparin (10 μg/ml). This suspension was placed in a 10 cm plastic Petri dish and incubated for 90 min at 37°C in an atmosphere of CO₂/O₂ (5 : 95, v/v). After this time the supernatant medium was decanted and the adherent cells were washed twice with warm Hanks' balanced salt solution. Ten millilitres of cold EDTA in phosphate-buffered saline (150 mmol/l), pH 7.2, were added to the cells in the Petri dish and left for 15 min at 4°C before all the cells were gently removed with a rubber policeman and a siliconized Pasteur pipette. The cells were spun at 400 g for 7 min before finally resuspending in 2 ml of Eagle's minimal essential medium containing penicillin (200 μg/ml) and streptomycin (100 mg/ml) (Wellcome Research Laboratories, Beckenham, Kent, U.K.).

To ensure that the enzyme activity present within the mononuclear cell population was originating from the monocytes, enzyme activity was also determined in isolated populations of both lymphocytes and platelets.

**Lymphocyte isolation**

Heparinized venous blood (20 ml) was mixed with 6 ml of 5% dextran in sodium chloride solution (saline: 150 mmol/l). To this was added 400 mg of Carbonyl iron powder (G.A.F., Great Britain, Ltd, Manchester, U.K.). The mixture was agitated at 37°C in a water bath for 10 min. This was then allowed to sediment for 25 min, after which the plasma was centrifuged over a Ficoll–Triosil gradient as described previously.

**Platelet isolation**

Twelve packs of outdated pooled platelets were obtained from the Blood Transfusion Laboratory. The platelets were counted in a Coulter counter.

For all techniques except the platelet isolation, cell numbers in the final suspension were counted in a standard Neubauer counting chamber. In all cases cell viability was assessed with 0.5% trypan blue exclusion. Identification of the percentage of those cells which were monocytes was performed by staining for peroxidase activity as described by Druguet & Pepys (1977).

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Mononuclear cells</th>
<th>Plastic adherent cells</th>
<th>Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Sex</td>
<td>Mean age (years) (with range)</td>
</tr>
<tr>
<td>Crohn's disease</td>
<td>28</td>
<td>8 M</td>
<td>39-1 (15-91)</td>
</tr>
<tr>
<td></td>
<td>20 F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>28</td>
<td>16 M</td>
<td>36-8 (17-81)</td>
</tr>
<tr>
<td></td>
<td>12 F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy controls</td>
<td>34</td>
<td>22 M</td>
<td>29-1 (20-47)</td>
</tr>
<tr>
<td>Disease controls</td>
<td>34</td>
<td>12 F</td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE 2. Percentages of monocytes obtained with different methods of isolation
Mean values ± SEM are shown.

<table>
<thead>
<tr>
<th>Method</th>
<th>Monocytes obtained (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy controls</td>
</tr>
<tr>
<td>Ficoll-Triosil</td>
<td>32.3 ± 1.9</td>
</tr>
<tr>
<td>Sedimentation</td>
<td></td>
</tr>
<tr>
<td>Plastic adherence</td>
<td>82.0 ± 1.7</td>
</tr>
</tbody>
</table>

The percentage of monocytes obtained by each method is given in Table 2.

For enzyme activity cell numbers were adjusted to 1 x 10⁷ cells/ml of suspending medium, except for the platelet suspension, which was adjusted to 1 x 10⁷ cells/ml. All cell suspensions were then centrifuged at 400 g for 7 min and the resulting cell pellet was lysed with 0.1% Triton X 100 in sucrose solution (0.25 mol/l). This solution was then examined under a light microscope to ensure complete cell lysis.

**Serum**

All sera used were separated within 4 h of venesection and stored at -20°C. The serum was diluted 1:10 in saline before enzyme assay.

**Enzyme assay**

The activity of N’-acetyl-β-D-glucosaminidase (EC 3.2.1.30) in all lysed cell preparations was assayed by using a fluorogenically labelled substrate as described by Kolodny & Mumford (1976). Briefly, 0.1 ml of cell solution equivalent to 1 x 10⁴ cells (1 x 10⁶ platelets), or 0.1 ml of diluted serum, was incubated with 0.1 ml of substrate solution (4-methylumbelliferyl-2-acetamido-2-deoxy-β-D-glucopyranoside, 5 mmol/l) and 0.1 ml of citrate/phosphate buffer, pH 4.25, for 30 min, when the reaction was stopped by the addition of 1.7 ml of glycine carbonate buffer, pH 10.5. The amount of fluorogenic label released by the enzyme was measured fluorimetrically with an Aminco SPF-125 spectrofluorimeter. Each sample was assayed in duplicate. For each assay a blank control was included in which the cell lysate was added after the addition of the glycine carbonate buffer.

Statistical analysis was by the one-way analysis of variance and simple linear regression.

**Results**

Results are represented as nmol of methylumbelliferone released from substrate by enzyme from 10⁴ cells/h (nmol h⁻¹ 10⁻⁴ cells).

![Fig. 1. Activity of N’-acetyl-β-D-glucosaminidase in mononuclear cell suspension from patients followed serially from relapse into remission.](image)

**Lymphocyte and platelet enzyme activity**

The activity of N’-acetyl-β-D-glucosaminidase in lymphocytes from healthy controls was 1.0 nmol h⁻¹ 10⁻⁴ cells (SEM 0.08; n = 8). For platelets, the enzyme activity was 0.019 nmol h⁻¹ 10⁻⁴ cells (SEM 0.19; n = 12).

**Enzyme activity in the monocyte-enriched population of mononuclear cells**

The mean activities of 4.55 nmol h⁻¹ 10⁻⁴ cells (SEM 0.28; n = 28) in patients with Crohn’s disease and 4.53 nmol h⁻¹ 10⁻⁴ cells (SEM 0.3; n = 28) in patients with ulcerative colitis were significantly greater than the activity found in healthy controls, which was 3.45 nmol h⁻¹ 10⁻⁴ cells (SEM 0.11; n = 34) (controls vs Crohn’s disease, P < 0.01; controls vs ulcerative colitis, P < 0.01). The enzyme activity in 14 patients with active Crohn’s disease was 5.1 nmol h⁻¹ 10⁻⁴ cells (SEM 0.4; n = 14), which was significantly higher than the activity of 3.96 nmol h⁻¹ 10⁻⁴ cells (SEM 0.35) in 14 patients with inactive disease (P < 0.05).

The activity in 12 patients with moderate or severe ulcerative colitis was 5.3 nmol h⁻¹ 10⁻⁴ cells (SEM 0.25; n = 12), again significantly higher than the activity of 3.96 nmol h⁻¹ 10⁻⁴ cells (SEM 0.46; n = 16) present in 16 patients with mild or inactive disease (P < 0.05).

This relationship of disease severity and enzyme activity was further shown by the results of serial studies in seven patients followed from relapse into remission (Fig. 1). There was a significant fall in enzyme activity (P < 0.05) to normal or near normal over a period of 2–4 weeks. That the fall in
enzyme activity was not related to steroid therapy per se is shown by the results of serial studies carried out on eight healthy controls taking prednisolone, 20 mg daily for 1 week. There was no consistent trend either upwards or downwards in enzyme activity before (3.99 ± 0.45 nmol h⁻¹ 10⁻⁴ cells) or after (3.91 ± 0.38 nmol h⁻¹ 10⁻⁴ cells) prednisolone.

**Enzyme activity in adherent cells**

The mean activity of 4.54 nmol h⁻¹ 10⁻⁴ cells (SEM 0.28; n = 21) in patients with Crohn’s disease and 4.99 nmol h⁻¹ 10⁻⁴ cells (SEM 0.45; n = 19) in patients with ulcerative colitis was significantly higher than the mean activity found in the healthy controls, which was 3.09 nmol h⁻¹ 10⁻⁴ cells (SEM 0.09; n = 21). Controls vs Crohn’s disease patients: P < 0.01; controls vs ulcerative colitis patients: P < 0.01.

The positive correlation between enzyme activity and disease severity was seen again with adherent cells. The mean activity in 10 patients with active Crohn’s disease was 4.9 nmol h⁻¹ 10⁻⁴ cells (SEM 0.58; n = 10) compared with 4.28 nmol h⁻¹ 10⁻⁴ cells (SEM 0.25; n = 11) in 11 patients whose disease was judged to be inactive. This difference failed, however, to reach statistical significance.

The enzyme activity in seven patients with ulcerative colitis who had moderate or severe disease was 6.59 nmol h⁻¹ 10⁻⁴ cells (SEM 0.75; n = 7), and in patients who had mild disease or were in remission it was 4.06 nmol h⁻¹ 10⁻⁴ cells (SEM 0.39; n = 12). This difference was highly significant (P < 0.005). These results are shown in Fig. 2.

**Serum enzyme activity**

Results of assays of serum lysosomal enzyme activity are expressed as nmol of substrate released h⁻¹ of incubation ml⁻¹ of serum (nmol h⁻¹ ml⁻¹). The mean activities of 1149 nmol h⁻¹ ml⁻¹ (SEM 42; n = 51) in patients with Crohn’s disease and 1135 nmol h⁻¹ ml⁻¹ (SEM 46; n = 40) in patients with ulcerative colitis were significantly greater than the activity found in healthy controls, which was 822 nmol h⁻¹ ml⁻¹ (SEM 22, n = 63) and in disease controls, which was 901 nmol h⁻¹ ml⁻¹ (SEM 45; n = 31) (healthy controls vs Crohn’s disease patients: P < 0.01; healthy controls vs ulcerative colitis patients: P < 0.01; disease controls vs Crohn’s disease patients: P < 0.01; disease controls vs ulcerative colitis patients: P < 0.01). These results are shown in Fig. 3.

No difference in serum enzyme activity was found between patients with ulcerative colitis and patients with Crohn’s disease. For both disease groups, there was no correlation between serum enzyme activity and the activity of the disease.
Likewise there was no correlation between serum enzyme activity and length of history, extent of disease or drug therapy at the time of the study.

In 18 patients with Crohn's disease in whom serum and monocyte estimations had been performed on the same blood sample, there was a strongly positive correlation between the results for adherent monocyte lysosomal enzyme activity and serum lysosomal enzyme activity \( (r = 0.57; P < 0.02) \). No such correlation was seen in the 13 patients with ulcerative colitis similarly studied \( (r = 0.4; P < 0.05) \).

Discussion

The results reported here indicate that the peripheral blood monocytes of patients with both Crohn's disease and ulcerative colitis have an increased content of the lysosomal marker enzyme \( N'\)-acetyl-\( \beta \)-d-glucosaminidase. This increase in enzyme activity is present when both mononuclear cells (approximately 36% monocytes) and plastic adherent cells (approximately 85% monocytes) are investigated. These results are in agreement with those found in a small number of patients by Ganguly, Kingham, Lloyd, Lloyd, Price, Triger & Wright (1978).

A slightly different pattern emerges when enzyme activity within the two different cell populations is looked at in relationship to disease severity. For the mononuclear cell population there is a significant correlation between lysosomal enzyme activity and disease severity. This relationship is strengthened by the finding that enzyme activity assessed in this population of cells falls when patients are followed serially (Fig. 1).

However, for plastic adherent cells, although enzyme activity was higher in those patients with both Crohn's disease and ulcerative colitis whose disease was active at the time of the study, this only reached statistical significance in the ulcerative colitis group. This lack of correlation between lysosomal enzyme activity and disease severity in patients with Crohn's disease may relate either to the difficulty in accurately assessing disease severity or may result from activated cells being more rapidly removed from the circulation. Evidence for this latter suggestion already exists (Meuret et al., 1978; A. S. Mee, J. Berney & D. P. Jewell, unpublished work). It is possible that extraneous sources of \( N'\)-acetyl-\( \beta \)-d-glucosaminidase might have influenced the results. However, even though contaminating lymphocytes and platelets known to contain the enzyme (Holmsen, Setkowsky, Lages, Day, Weiss & Scrutton, 1975; Beutler, Kuhl, Matsumoto & Pangalis, 1976) were present in the mononuclear cell suspension, the data show that enzyme activity present within these cells was small compared with that found in the monocytes of healthy controls.

The stimulus for the increase in monocyte lysosomal enzyme activity is unknown. Any activating agent may be implicated, e.g. lymphokines, complement activation products, phagocytosis of organisms or immune complexes. Many of these possible 'activating' factors have been described in patients with inflammatory bowel disease. It is likely that stimulatory lymphokines are released in the diseased mucosa since cell-mediated responses (Eckhardt, Heinisch & Meyer Zum Büschenfelde, 1976) and the presence of activated lymphocytes (Clancy, 1976) have been reported. Complement breakdown products have been found in the sera of these patients (Teisberg & Gjone, 1975) and circulating immune complexes have been demonstrated by use of various techniques (Hodgson, Potter & Jewell, 1977a; Fiasse, Lurhuma, Cambiaso, Masson & Dive, 1978; Nielsen, Binder, Daugherty & Svehag, 1978). The phagocytosis of a variety of candidate organisms, proposed as aetiological agents in inflammatory bowel disease, may induce lysosomal enzyme activity as has been described after the phagocytosis of other substances (Axline & Cohn, 1970).

The results for the activity of \( N'\)-acetyl-\( \beta \)-d-glucosaminidase within the serum of patients with inflammatory bowel disease reflect the increased activity of the enzyme within the monocytes of these patients with the exception that there is no correlation with disease severity. This lack of correlation may exist for a variety of reasons. Disease severity may be difficult to assess accurately in patients with Crohn's disease. Monocyte enzyme production and release may be modified if mechanisms for these processes are blocked by, for example, immune complexes. Holland, Felix, Alston, Hay & Nineham (1978) have demonstrated that in systemic lupus erythematosus serum activities of \( N'\)-acetyl-\( \beta \)-d-glucosaminidase correlate directly with the presence of circulating immune complexes, which may also occur in inflammatory bowel disease (Hodgson et al., 1977a).

In patients with Crohn's disease there was a positive correlation between monocyte and serum lysosomal enzyme activity. This was not so in patients with ulcerative colitis. This may possibly
reflect the small number of subjects in whom both assays were performed. However, N\textsuperscript{1}-acetyl-β-D-glucosaminidase is distributed throughout a variety of tissues containing lysosomes (Leaback, 1974) and serum enzyme activity may be derived from the very large numbers of polymorphonuclear leucocytes present within the inflamed colons of patients with ulcerative colitis.

Elevated serum activities of N\textsuperscript{1}-acetyl-β-D-glucosaminidase may be of pathogenetic significance since it is known that other lysosomal enzymes are capable of activating complement via both classical (Taubman & Lepow, 1971) and alternative pathways (Schorlemmer & Allison, 1976). Patients with both Crohn's disease and ulcerative colitis show evidence of complement activation, complement turnover is increased and their sera have higher titres of immunoconglutinins than those of controls (Hodson, Potter & Jewell, 1977b; Potter, Mee, Hodson & Jewell, 1979; Potter, Brown, Watson, Cavendish & Jewell, 1979).

Significantly elevated activities of the lysosomal marker enzyme N\textsuperscript{1}-acetyl-β-D-glucosaminidase have been demonstrated in the monocytes and sera of patients with both Crohn's disease and ulcerative colitis. Release of this increased amount of enzyme and its presence within the sera of these patients may have implications in the pathogenesis of the inflammation seen in these two diseases.

Acknowledgments

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References


