Table 2. Quantitative immunofluorescence analysis of 1 x 10⁶ EAT cells

<table>
<thead>
<tr>
<th>Absorbing tissue</th>
<th>Serum . . .</th>
<th>'RantiEAT'</th>
<th>'RantiMFT'</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.700</td>
<td>1.550</td>
<td>2.650</td>
</tr>
<tr>
<td>EAT cells</td>
<td>0.760</td>
<td>0.820</td>
<td>1.410</td>
</tr>
<tr>
<td>Adult mouse liver and kidney</td>
<td>—</td>
<td>1.800</td>
<td>3.750</td>
</tr>
</tbody>
</table>

Development of a radiochemical assay for glycyl-leucine dipeptidase in human B- and T-lymphocytes

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Recent technical advances in the isolation of human lymphocytes have made it possible to obtain preparations enriched in B- or T-cells (Wybran, 1973; Pellegrino et al., 1976). This in turn facilitates studies on the comparative enzymology of both B- and T-lymphocytes, which may be relevant to some leukemic states. Studies on lysosomal enzymes, including acid phosphatase, β-glucuronidase (Meusers et al., 1976) and N-acetyl-β-glucosaminidase (Crockard et al., 1979) in B- and T-cell-enriched fractions have already been carried out. Haschen & King (1966) found increased activities of the cytoplasmic enzyme N-glycyl-L-leucine dipeptidase (EC 3.4.13.1) in leucocytes from patients with chronic lymphatic leukaemia. The purpose of the present investigation was to measure the same activity in the fractionated lymphocytes and also to improve the assay by using a radioactive substrate, [2-³H]N-glycyl-L-leucine, since cellular constituents interfere with assays using ninhydrin.

The substrate preparation was obtained as follows: glycine, containing [2-³H]glycine as tracer, at a final specific radioactivity of 2 mCi/mmol was converted into the N-benzylxycarbonyl derivative and L-leucine to its benzyl ester. After condensation with 1-(3-dimethylaminopropyl)-3-ethylcarboxyamide hydrochloride (Aldrich Chemicals Ltd. Gillingham, U.K.) both blocking groups were removed simultaneously from the conjugate (1.71 g) by hydrogenation with a Pd/charcoal catalyst (170mg). The yield in each of the first three steps was over 90% and the final yield was over 57%.

Cells (1 x 10⁶ - 2.5 x 10⁷) were homogenized in 0.25 m-sucrose (0.5 ml) at 0°C. Insoluble proteins were removed by centrifugation at 100,000 g. The cell extract was diluted with 0.25 m-sucrose. Assay mixtures contained diluted cell extract (100 μl) and a solution (100 μl) of dipeptide in 0.06 m-KH₂PO₄/K₂HPO₄ buffer, pH 8.1, containing 0.25 m-sucrose. Portions of 25 μl were removed at intervals during incubation (20 min) at 25°C. Proteins were precipitated by addition of an equal volume of a 2% (w/v) solution of ice-cold trichloroacetic acid. The proportion of substrate hydrolysed was determined by subjecting samples (5 - 1 μl) of the supernatant to t.l.c. on cellulose (0.1 mm) precoated on plastic sheets (20 cm x 20 cm; Polygram Cel 300; Macherey-Nagel and Co., Düren, Germany), with a 2% aprotic solvent system as solvent. The substrate was visualized by spraying the plates with 5% (w/v) trichloroacetic acid in methanol, followed by exposure to iodine vapor.


Fig. 1. Hydrolysis of N-glycyl-L-leucine by dipeptidase from B-cell-enriched lymphocytes

The cells (1.75 x 10⁷) were extracted as described in the text and the extract was diluted 1:3.5 before incubation with a final concentration of 0.6 mm-substrate as described in the text. A curve-fitting procedure using orthogonal polynomials (Elmore et al., 1963) was employed to estimate the initial velocity.

1980
A comparison of α-L-fucosidase activity in normal and chronic-lymphocytic-leukaemia lymphocytes

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α-L-Fucosidase (EC 3.2.1.51) has been detected in most mammalian tissues, including lymphoid cells. Its activity has been demonstrated in peripheral-blood lymphocytes from normal donors (Tanaka, 1979) and patients with fucosidosis (Beratis et al., 1975). In the present study the activity and characteristics of the enzyme were investigated in normal unfractionated lymphocytes, in enriched B- and T-lymphocyte subpopulations and in lymphocytes from patients with B-cell chronic lymphocytic leukaemia.

Mononuclear cells were isolated from fresh human blood by density-gradient centrifugation on Ficoll-Paque medium (Pharmacia, Sweden), as described by Böyum (1974). Monocytes were removed by adherence to plastic Petri dishes as described by Beutler et al. (1976). The cell suspension was incubated with sheep erythrocytes, which form rosettes with T-lymphocytes, and the mixture was recentrifuged as above (Pellegrino, 1976). Recovered T-cells were freed from the erythrocytes by hypotonic lysis. B-cells were recovered from the interface of the Ficoll and suspension medium. Cell enrichment was quantified by using immunological marker tests (Jondal et al., 1972; Seligmann et al., 1973).

A typical assay of enzyme activity from a B-enriched cell preparation is shown in Fig. 1. The suspension had a specific activity of 8.4 x 10⁻²⁴ units/10⁶ cells. Controlling activities from unfraccionated lymphocytes and from the T-cell enriched preparation from the same subject were 2.2 x 10⁻³ units/10⁶ cells and 4.3 x 10⁻³ units/10⁶ cells respectively.

After these preliminary assays, a survey of enzyme activities in cell fractions from normal subjects and patients with chronic lymphatic leukaemia is required.

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