Letters to the Editor

Peripheral white cell counts in Australian aboriginals

Bain et al have commented on the lowered white cell count and absolute neutrophil count in blacks of African and West Indian origin compared with north European whites, Indians, and Orientals. Other groups of blacks of different ethnic origin may not show this difference.

We have recently studied the aboriginal community of Yirrkala (population 1022) in North East Arnhemland in the Northern Territory of Australia. The total white cell counts (×10⁹/l) in apparently well adult men and women (aged 12 or over) are shown in Table 1.

Table 1 Total white cell counts (×10⁹/l) in apparently well adult aboriginals

<table>
<thead>
<tr>
<th>Sex</th>
<th>No</th>
<th>White cell count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range</td>
</tr>
<tr>
<td>M</td>
<td>131</td>
<td>6–14</td>
</tr>
<tr>
<td>F</td>
<td>191</td>
<td>5–19</td>
</tr>
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</table>

The mean percentage differential counts in random groups of men and women were calculated (Table 2). All these were apparently clinically well, but some showed a usually minor haemoglobin or leucocyte abnormality. Thirty five subjects who were well and had “normal” Caucasian values for haemoglobin and white cell count were also examined.

These figures are significantly different from Caucasian percentages only with regard to eosinophils. In the “normal” group, only nine subjects had absolute eosinophil counts of <500 × 10⁹/l, 18 had

References

500–1000, and eight >1000. Our subjects are members of a well established aboriginal community whose floral and footwear habits have not changed substantially over the years. Intermittent random sampling of stools has shown a high prevalence of stool parasites, especially of hookworm. Malaria, however, was eradicated from this community in the late fifties.

Although our investigation was not as rigorously controlled as that of Bain et al., these figures and those of others such as Vaterlaws et al. working with Papua New Guinea highlanders suggest that blacks other than those of African origin have total white cell and neutrophil counts which are more akin to Caucasian rather than black African values.

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References


Two further alkaline phosphatase staining methods for immunohistochemistry

Alkaline phosphatase labelled antibodies may be used as alternative reagents to peroxidase-antiperoxidase (PAP) reagents in the immunohistochemical method of Sternberger. Visualisation of alkaline phosphatase can be achieved by various azo coupling reactions. We report two novel staining protocols for alkaline phosphatase, one resulting in a green and the other a brown-black final reaction product. The new methods are described in relation to the staining of human synovium.

Material and methods

Human synovial tissue was fixed in 4% non-buffered formalin. The specimens were processed by routine methods and embedded in paraplast plus.

All chemicals used were of analytical grade and were obtained from Sigma (München, FRG). Antibodies, if not stated otherwise, were provided by Dakopatt (Bohringer, FRG).

IMMUNOCHEMISTRY

An indirect alkaline phosphatase method was compared with a standard PAP technique. The first antibody was a heavy chain specific rabbit antihuman immunoglobulin diluted 1/50 (final protein concentration 0.2 mg/ml; alkaline phosphatase activity/slide = 50 units).

Alkaline phosphatase was coupled to a swine antirabbit IgG by the method of Müller.

Standard staining methods for alkaline phosphatase were employed. The final reaction products comprised: fast blue BB combined with naphthol-AS-MX-phosphate and fast red ITR with the coupling reagent naphthol-AS-TR-phosphate.

PAP staining was performed by standard methods. The first antibody was used at the same dilution as in the alkaline phosphatase methods. The bridging antibody was a swine antirabbit IgG diluted 1/100 (protein concentration 0.1 mg/ml) followed by rabbit PAP complex diluted 1/100. The final reaction substrates used were amino-9-ethyelcarbozole, diaminobenzidine, 4 chlor-naphtol, or Hancer Yates reagent.

Novel alkaline phosphatase techniques

1 Fifteen milligrams of naphthol-AS-GR-phosphate was dissolved in 25 ml 25% sodium 5, 5-diethylbarbiturate (pH 9.2-9.4), 0.2 ml 10% MgCl₂ was added to the incubation medium. Twenty five milligrams of varimine blue was added to this solution, which was filtered and used immediately after preparation. Incubation time was 15–30 min at 20–22°C. A one minute wash was performed in 1% acetic acid before counterstaining.

2 A second staining protocol originally used by McGadey for demonstrating alkaline phosphatase in enzyme histochemistry was adapted for this study. Incubation medium consisted of 3 mg neotetranitro-blue-tetrazolium (TNBT) and 5-diethylbarbiturate (pH 9.2–9.4) dissolved in 10 ml 0.2 M TRIS HCL (pH 9, 2–9, 4) to which 2 mg 5-bromo-4-chloro-3-indoxylphosphate toluidine salt was added. Incubation time was 30 min at 20–22°C.

Results

By the standard alkaline phosphatase methods either blue or red final reaction products are achieved. The blue final reaction product of haematoxylin counterstaining, whereas the fast red ITR is well differentiated against the blue background. The azo dye coupling method described here for alkaline phosphatase based on varimine blue salt and naphthol-AS-GR-phosphate yields a distinct green product, which, as far as we know, has not yet been used in immunohistochemistry or enzyme histochemistry.

Methodological comparison of five different immunohistochemical results

<table>
<thead>
<tr>
<th>Method</th>
<th>Peroxidase-antiperoxidase</th>
<th>Alkaline phosphatase</th>
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<tbody>
<tr>
<td>Number of incubation steps</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Colour of reaction product</td>
<td>Red</td>
<td>Blue</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>Very good</td>
<td>Good</td>
</tr>
<tr>
<td>Background staining</td>
<td>Minimal</td>
<td>Slight</td>
</tr>
<tr>
<td>Counterstaining with haematoxylin possible</td>
<td>+</td>
<td>-</td>
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