and to compare this timecourse with that of other events associated with muscle damage.

Eight healthy University students (seven female, one male) volunteered for this study and signed the appropriate informed consent documents. Each volunteer performed 70 maximal eccentric contractions of biceps and triceps of one arm to induce muscle damage. Venous blood samples were taken and muscle soreness was assessed by questionnaire before the exercise and at 24 h intervals for 5 days afterwards. Total serum CK activity was measured for each sample by the method of Szasz et al. (1976) using a Sigma test kit (Sigma Chemical Co., St. Louis, MO 63178, U.S.A.). EDTA was added to aliquots of serum from each sample to prevent further interconversion of CK-MM isoforms (Chapelle et al., 1981).

Creatine kinase isoforms were separated by flat-bed isoelectric focusing performed on an LKB-Multiphor electrofocusing unit using LKB Ampholine PAG plates pH 3.5–9.5 (LKB instruments, Gaithersburg MD 20877, U.S.A.). The gels were focused for 2 h at 20 W constant power. The isoforms were visualized by overlaying the PAG plate with an agarose gel soaked in CK indicator solution. The gel and plate were incubated together for 20 min at 30°C, after which the agarose plate was removed and fluorescent bands were measured by a scanning densitometer. This procedure is a modification of that of Clarkson et al. (1987).

Total CK activity demonstrated a continuous increase through days 1–5. Soreness was maximal 2 days post-exercise and decreased thereafter (Table 1).

Isoelectric focusing revealed five CK-MM isoforms. Four of these showed an increase in a time-dependent sequence going from MM1 (maximum recorded on day 3) to MM4 (maximum recorded on day 5). The fifth isoform, which was cathodal to MM1 on the gel, was apparent only in the post-exercise samples, and then only in small quantities. The MM1:MM3 ratio followed a similar time course to soreness, being maximal 2 days post-exercise. By day 5 post-exercise the MM1:MM3 ratio had returned to normal, although total serum CK activity was still highly elevated.

The number and pattern of isoforms observed here are identical to those reported by Chapelle (1984) in patients with myocardial infarction. An increase in the ratio of CK-MM1:MM3 appears to indicate new release of CK (Morelli, 1984). Similarities in the timecourses of increase of the MM1:MM3 ratio and of muscle soreness may indicate that the initial alteration in muscle membrane permeability resulting in CK release also results in muscle soreness.


Received 15 June 1987

A new monoclonal antibody to human prostatic acid phosphatase suitable for immunohistochemistry in formalin-fixed paraffin-embedded tissue sections

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Prostatic acid phosphatase (EC 3.1.3.2, orthophosphoric monoester phosphohydrolase) is produced in large amounts in the adult prostate. The structure and activity in vitro of the enzyme is well characterized (Luchter-Wasyl & Ostrowski, 1974; Derechin et al., 1971; Abdul-Fadl & King, 1949). Elevated levels of the enzyme are associated with metastatic prostatic carcinoma and serum assay of tannic acid labile acid phosphatase is used in clinical diagnosis. In addition, prostatic acid phosphatase (PAP) can be demonstrated histochemically and immunocytochemically in sections of biopsy or autopsy material. Polyclonal antibodies have been used to demonstrate the prostatic origin of secondary deposits. The aim of this study was to produce a monoclonal antibody that could be used for detecting PAP in routinely processed formalin-fixed, paraffin-wax-embedded sections.

Abbreviation used: PAP, prostatic acid phosphatase; ELISA, enzyme-linked immunosorbent assay; SDS-PAGE, SDS-polyacrylamide gel electrophoresis.

PAP was purified from human seminal plasma by ammonium sulphate precipitation, affinity chromatography on a concanavalin A-Sepharose CL-4B column (Pharamacia Ltd, Milton Keynes, U.K.) followed by ion-exchange chromatography on a DEAE Sephadex column (Pharamacia Ltd, Milton Keynes, U.K.). Fractions were assayed for acid phosphatase activity by the method of van Etten (1977) and positive fractions were pooled, dialysed against water and lyophilized.

Swiss A2G mice were immunized with this preparation. Serum was tested immunocytochemically on routinely processed sections of benign prostate using an indirect immunoperoxidase technique (Heyderman et al., 1985). Positive animals were boosted intravenously before fusion and spleen cells were fused with the mouse myeloma cell line P3XAg8.653. Supernatants were screened primarily by an enzyme-linked immunosorbent assay (ELISA) using microtitre wells coated with the PAP preparation and positive supernatants were further tested immunocytochemically. Cells which gave positive immunocytochemical staining were subcultured into 96-well plates at an average density of 0.5 cells/well and the supernatant tested after 2 weeks as above. This was repeated a further two times, and cells from the supernatant giving the strongest immunocytochemical staining (PASE/4L1), were grown in flasks.

Table 1. Total serum CK activity, soreness and CK-MM1:MM3 ratio following a bout of maximal eccentric exercise

<table>
<thead>
<tr>
<th>Time</th>
<th>CK (unit/l)</th>
<th>Soreness (on scale 2–20)</th>
<th>MM1:MM3 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>50 ± 3</td>
<td>2.0</td>
<td>0.89 ± 0.06</td>
</tr>
<tr>
<td>Day 1</td>
<td>193 ± 75*</td>
<td>8.5 ± 0.7*</td>
<td>1.26 ± 0.14</td>
</tr>
<tr>
<td>Day 2</td>
<td>998 ± 622*</td>
<td>10.9 ± 0.9*</td>
<td>1.62 ± 0.24*</td>
</tr>
<tr>
<td>Day 3</td>
<td>2422 ± 998*</td>
<td>10.6 ± 1.0*</td>
<td>1.38 ± 0.09*</td>
</tr>
<tr>
<td>Day 4</td>
<td>3601 ± 1365*</td>
<td>7.0 ± 0.8*</td>
<td>0.75 ± 0.09</td>
</tr>
<tr>
<td>Day 5</td>
<td>4706 ± 1845*</td>
<td>5.5 ± 0.8*</td>
<td>0.80 ± 0.09</td>
</tr>
</tbody>
</table>
Collagen metabolism during compensatory lung growth after partial pneumonectomy

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After unilateral pneumonectomy in rats, a rapid compensatory growth of the remaining lung occurs. This involves the rapid production of various proteins, including those of the connective tissue matrix. Collagen is the major connective tissue protein and is important for the maintenance of lung structure and function. It has been shown previously that there is an increase in collagen content of the remaining lung (Cowan & Crystal, 1975; Watkins et al., 1985) due, at least in part, to an increase in collagen synthesis (Cowan and Crystal, 1975). However, the collagen content is regulated by a balance between synthetic and degradative processes which are particularly active in normal lung tissue (Laurent, 1987; McAnulty & Laurent, 1987). There is no information on the role of collagen degradation during compensatory lung growth after partial pneumonectomy. In this study we have used in vivo techniques to investigate the relative roles of collagen synthesis and degradation which result in an increased deposition of collagen in the lungs of rats after partial pneumonectomy.

Left pneumonectomy was performed on male Lewis rats weighing approximately 200 g. They were anaesthetised with 1 ml/kg body weight of Hypnorm intramuscularly [fentanyl citrate (0.315 mg/ml)/fluanisone (10 mg/ml)], intubated and ventilated. The chest was opened at the fifth intercostal space, the lung exposed, tied at the hilum and excised. The chest was closed and sutured in layers, and negative pressure in the thorax re-established. Control animals were operated in a similar way except the left lung was not removed. Rates of collagen synthesis and degradation were estimated in vivo in groups of at least five at 2, 6, 14 and 28 days after operation. Rats were injected intravenously with phosphate-buffered saline containing 1.4-C-proline (50 μCi/100 g body weight) and unlabelled proline (160 mg/100 g body weight). Animals were killed 30 min later, the lung removed and frozen in liquid nitrogen. Collagen content and rates of synthesis and degradation were estimated as described previously (Laurent et al., 1981; Laurent, 1982; Lauren & McAnulty, 1983; McAnulty & Laurent, 1987).

Table I shows the changes in collagen content and metabolism in the remaining lung after pneumonectomy. The collagen content was significantly increased by 14 days after operation and continued to increase until 28 days, when the content was about 70% above that of controls. Collagen synthesis rates were increased at all times, peaking at 6 days post-pneumonectomy when the rate was two to three times that in the sham-operated controls. The synthesis rate declined thereafter towards normal. The proportion of newly synthesized collagen degraded rapidly showed a trend