

**PRODUCTION AND CHARACTERISATION
OF
MONOCLONAL ANTIBODIES
TO
PARATHYROID HORMONE RELATED
PROTEIN**

BY

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ABSTRACT

The production and characterisation of monoclonal antibodies to the human parathyroid hormone related protein1-86 (PTHrP1-86) was described. Commercially prepared synthetic human PTHrP1-86 peptide was purchased from Bachem chemicals. Five BALB/c mice were immunised using the PTHrP1-86 antigen. Efficiency of immunisation was monitored using the titre of an antibody by a radioimmunoassay (RIA) based method. For this purpose the peptide was labelled with ^{125}I using N-Bromosuccinimide and Lactoperoxidase techniques and the labelled peptide was purified with the aid of a C_{18} Sep-Pak cartridge and an ultrogel column.

The solid phase Lactoperoxidase technique was found to be a suitable and successful method for iodination of PTHrP1-86 with ^{125}I iodine. Highest purification efficiency was achieved with the aid of an ultrogel column. Four out of the five BALB/c mice immunised, responded well to the PTHrP peptide. Three days after the final boost, spleens were removed from the two mice giving the highest antibody titre, splenocytes isolated and fused with the myeloma cells using 50% polyethylene glycol (PEG) 1500. These two cell fusions produced specific hybrids and were screened to determine which hybridoma produced antibodies with the desired specificity. Twenty four monoclonal antibodies belonging to IgG_1 , IgG_3 and IgM isotypes were identified. Aspirated ascitic fluid with high concentration of monoclonal antibody was collected for the development of an IRMA for the detection of PTHrP1-86, as a tumour marker in human blood.