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Production and characterisation of monoclonal antibodies to human pepsin

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Background: An indirect sandwich Enzyme Linked ImmunoSorbant Assay (ELISA) has been described to detect pepsin using polyclonal antibodies to human pepsin 3b (rabbit anti-HU3 peptide and goat anti-human pepsin 3b). This is been used to evaluate the presence of pepsin as a marker of gastric reflux in throat sputum of LPR patients [1]. This methodology has been advocated as a diagnostic test for extra-oesophageal reflux. Monoclonal antibody technology produces antibodies with high specificity of binding to the target antigen, homogeneity of the preparation and can be produced in unlimited quantities. These are qualities that are necessary for the development of a clinically useful reproducible diagnostic test. In addition, they enable a lateral flow test format (dipstick) to be developed. Here we discuss the production of two monoclonal antibodies (mAb) to human pepsin 3 and the characterisation of the resulting ELISA system.

Methods: Monoclonal Antibody Development:

Two mAbs were produced by immunisation of BALB/c mice with two human pepsin antigens:

Splenocytes were harvested and fused with mouse myeloma cells to produce immortal somatic cell hybrids (hybridomas). Hybridomas were screened for Ig production and for specific recognition of the target antigen. The selected positive cells were twice single-cell cloned and the antibody secreted into the ascite fluid was purified. mAb VDE1.1 was used as capture antibody. mAb 3D8 labelled directly with horse-radish peroxidase (HRP) for use as detection antibody. Both mAbs were IgG1 subtype.

Direct Sandwich ELISA:

A direct sandwich ELISA (using standard methodology) was evaluated to detect human pepsins (3 complex, 1 complex and gastricsin) and porcine pepsins (porcine pepsin A and porcine pepsinogen). HRP reactivity was detected using TMB/H2O2 and absorbance read at 414 nm.

Results: The direct sandwich ELISA using two new mAbs was able to detect human pepsin 3 complex with a sensitivity of 1 ng/ml. The response was almost linear up to

2 µg/ml. There was strong cross reactivity to human pepsin 1 complex but not to gastricsin (pepsin 5). There was cross reactivity to both the porcine pepsin antigens.

Conclusions: The two mouse mAbs against human pepsin 3 have been used to develop an ELISA method to detect pepsin which will be reproducible due to there being a consitent long-term supply of specific antibodies. The sensitivity is 1 ng/ml and a range of different isoforms of pepsin can be detected as they have a conserved amino acid structure which features the HU3 peptide sequence (or a homologous version as in the case of porcine pepsins). In contrast, gastricsin which has a distinct amino acid sequence is not detected. The ability to detect several isoforms of pepsin increases its suitability as a diagnostic test for reflux disease using the presence of pepsin from gastric juice as a marker.

References:

1. Knight et al. Laryngoscope 2005; 15: 1473-1478