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POSTER PRESENTATION

## MONOCLONAL ANTIBODIES AS RADIOPHARMACEUTICALS

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INTRODUCTION: Since of the development of hybridoma technology (Köhler & Milstein, 1975) monoclonal antibodies have found an increasing number of applications and uses. The availability of monoclonal antibodies to tumour associated antigens having a high degree of specificity has contributed to more extensive "in vivo" use of radiopharmaceutical. The intact IgG molecule is the easiest to use but because of its size and its ability to bind to Fc receptors, it may not be the ideal radiopharmaceutical. The fragments of the IgG molecule lacking the Fc portion may be the most appropriate form to use. The aim of this study was to develop an "experimental protocol" to optimize the immunochemical procedures to obtain an adequate immunological reagents from biologic preparations, such as anti-CEA 4C11 IgG<sub>2a</sub> subclass.

MATERIAL AND METHODS: The ascitic fluid from BALB/c mouse carrying the anti-CEA 4C11 monoclonal antibody IgG<sub>2a</sub> subclass was generously provided by Ludwig Institute/Brazil.

PURIFICATION OF IgG<sub>2a</sub> FROM ASCITIC FLUID: The intact IgG<sub>2a</sub> was isolated by affinity chromatography Protein A-Sepharose according to Ey et al, 1978. Mouse IgG<sub>2a</sub> binds to Protein A and is eluted from the column at pH 4.5. The column effluent is monitored by measuring the absorbance at 280nm.

FRAGMENTATION OF IgG<sub>2a</sub> (Lamoyi & Nisonoff, 1983). The

bivalent  $F(ab')_2$  fragments are prepared by incubating the purified  $IgG_{2a}$  with pepsin at pH 4.2 for 24 hours at 37°C. The protein to enzyme ratio is 20:1. The  $F(ab')_2$  fragments are separated from small amounts of undegraded  $IgG_{2a}$  and Fc portions by Protein A-Sepharose. The column effluent is monitored at 280nm. The purity of  $IgG_{2a}$  and their fragments are determined by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE). It is carried out in 10% gels by the method of Laemmli, 1970.

RESULTS: The concentrations of  $IgG_{2a}$  and  $F(ab')_2$  fragments were determined by optical density at 280nm  $1\% = 14$ . The final yield from purified  $IgG_{2a}$  to purified  $F(ab')_2$  fragments was an average of 10% of the starting material. Our future work will investigate the optimal conditions to radiolabeling of the  $F(ab')_2$  fragments for immunoscintigraphy.

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