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PROGRESS IN NUCLEOMEDICAL UTILIZATION OF MONOCLONAL ANTIBODY (MoAb)

2 In vitro utilization

c. thyroglobulin

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The purpose of this study was to study the molecular structure of human thyroglobulin (Tg) and to develop Tg RIA which can measure Tg in the presence of autoantibody against Tg (Auto Ab) using MoAb against Tg.

Six MoAb were prepared using hybridoma cells of mouse myeloma cells and spleen cells and the binding sites of these MoAb on the surface of Tg molecule were investigated using radioimmune blocking assay. Normal Tg coated wells were incubated with MoAb and an inhibitor, such as various iodine content Tg, rat Tg and T₄, and inhibitions of binding of MoAb to Tg by these inhibitors were examined. The results of this inhibition study showed that T₄ presents on the surface of Tg molecule and that iodine related portions of human Tg share antigenicity with rat Tg and that iodine not related portions do not.

In order to develop Tg RIA able to measure Tg in the presence of Auto Ab, only one MoAb which does not cross react with Auto Ab and has a high binding affinity for and specificity for human Tg could be obtained after making hybridoma 5

times and screening approximately 20,000 wells. Using this MoAb, a RIA for Tg has been developed employing sandwich method. The 96 well plates were coated with MoAb and serum or standard samples were added after blocking these wells with bovine albumin solution and ¹²⁵I MoAb was added after washing the wells. The sensitivity of this method to detect serum Tg was as low as 4ng and Auto Ab did not interfere with standard curves. Dilution curves of serum Tg with Auto Ab showed a straight line and the recovery rate of added Tg into Auto Ab positive serum was about 90%. The concentrations of serum Tg measured by a conventional RIA were compared with those by this method. A good correlation was observed in Auto Ab negative samples, but the serum Tg concentrations by the conventional RIA were clearly lower than those by this method in Auto Ab positive samples. These results suggest that the measurement of Tg in the presence of Auto Ab can be possible in this method. In summary, MoAb is very useful clinically and laboratorily.

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CURRENT STATUS OF RADIOIMMUNODIAGNOSIS IN THE UNITED STATES. Gerald L. DeNardo and Kiyoshi Koizumi. University of California, Davis Medical Center, Sacramento, CA and Kanazawa University School of Medicine, Kanazawa.

The number of presentations concerned with monoclonal antibodies substantially increased and reached 65 in number, or about 10% of the total presentations, at the recent meeting of the Society of Nuclear Medicine in Houston, 1985. Anti melanoma, colon cancer, ovarian cancer, prostatic cancer, B-cell lymphoma, cutaneous T-cell lymphoma, and soft tissue sarcoma antibodies were reported for clinical application. The detection ratio with these antibodies varied from 40-90%. An interesting approach, immunophosphorimetry, was described and showed promising results. While no severe side-effects have been reported to date regardless of the route of administration, a significant number (22 of 47, 47%) of patients that received intact murine IgG developed a positive antimouse antibody test. Two groups reported regression of T-cell lymphoma or renal cell carcinoma when treated with I-131 labeled specific monoclonal antibody (Mab). Techniques of radio-labeling were extensively discussed at this meeting. Antibodies were found to have better in vitro and in vivo behavior when iodinated at less than 1 to 10 or conjugated with DTPA at less than 5 to 1. Newer chelating agents such as BrEDTA, heterobifunctional reagents and so forth were described.

Pharmacokinetic studies revealed that the biodistribution of some MABs is dose dependent, that is, larger doses of intact antibody delay the blood disappearance. Differences in the biodistribution of In-111 labeled and iodide labeled antibodies were described. The uptake in the liver and tumor were higher with In-111 labeled Mab.

In our lab at UC Davis, we have evaluated the pharmacokinetics of several different intact MABs and their fragments. We found that the pharmacokinetics of an anti B-cell lymphoma Mab (Lym-1) was profoundly altered in patients by the amount of antibody administered. The antibody was labeled with I-123 by chloramine-T. Blood and urine samples were obtained, quantitated and analyzed by HPLC (TSK 3000). Profound differences in the biodistribution of intact IgG, F(ab')₂ and Fab were observed. Multicompartmental modeling was applied to analyze the pharmacokinetics of these different circumstances. A nonlinear, saturable antibody processor was demonstrated. Simulation curves of blood disappearance for different amounts of administered antibody clearly showed dose dependency. Recently several MABs labeled with In-111 have become available in kit form (Hybitech Inc.) and have been used in clinical trials. Anti melanoma or prostatic cancer MABs have shown very promising results in the imaging of patients.