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PRODUCTION OF A MONOCLONAL ANTI-11-DEOXYCORTISOL ANTIBODY

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The production of a monoclonal antibody to 11-deoxycortisol is described. Spleen cells from BALB/c mice immunized with 11-deoxycortisol linked to bovine serum albumin (BSA) were fused with P3-NS1/1-Ag4-1 myeloma cells. A monoclonal antibody which had a high binding affinity for 11-deoxycortisol (K_a= 2 x 10^{10} M^{-1}) was obtained from cloned hybridomas in culture medium. Cross-reaction studies showed that the antibody is specific to 11-deoxycortisol.

KEYWORDS ——— monoclonal antibody production; 11-deoxycortisol; monoclonal anti-11-deoxycortisol antibody; radioimmunoassay; antibody specificity; cross-reaction

Recently, the novel hybridization technique developed by Köhler and Milstein[1] has been shown to be useful for the preparation of monoclonal antibodies to steroid hormones.[2] In general, monoclonal antibodies offer many advantages, such as high specificity and availability in large quantities, compared to conventional polyclonal antisera. However, production of a monoclonal antibody specific to a steroid is not always easy, since steroid-specific efficiencies in the cell fusion experiments are low, and the antibody specificity is influenced by the position on the steroid molecule used for conjugation to a carrier protein.

Immunoassays of 11-deoxycortisol in human plasma are useful in the metyrapone test,[3] an assessment of pituitary-adrenal reserve. In a previous paper of this series, we reported on the specificity of the anti-11-deoxycortisol antisera elicited in rabbits by immunization with haptenic derivatives linked through C-4 to BSA.[4] The present paper describes the production of a monoclonal antibody specific to 11-deoxycortisol.

The steroid derivative used as a hapten was 4-(2-carboxyethylthio)-11-deoxycortisol. Female BALB/c mice (2 months old) were immunized by an intraperitoneal injection of the haptenized BSA (50 μg/mouse) with complete Freund's adjuvant. Booster immunizations were given at two weekly intervals. Three days before the fusion experiment, the mice were given a booster injection of the immunogen in saline. Spleen cells were then isolated and fused with P3-NS1/1-Ag4-1 myeloma cells by using 45% polyethylene glycol. The hybridomas were cultured in a selec-

Table I. Cross-Reaction of Monoclonal Antibody

Steroid	% Cross-reaction
11-Deoxycortisol	100
Cortisol	0.2
Cortisone	0.4
Corticosterone	0.02
11-Deoxycorticosterone	7
17α-Hydroxyprogesterone	7
Progesterone	0.2

tive medium and antibody-secreting hybridomas were cloned by limiting dilution.

The screening of hybridomas for 11-deoxycortisol antibody activity was carried out using the radioimmunoassay procedure[4]: [^{3}H]-11-deoxycortisol was used as a labeled antigen, and the bound and free fractions were separated by a dextran-coated charcoal method.

A monoclonal antibody (IgG_1,κ) showing a satisfactory titer was obtained by mass growth of a selected hybridoma line in culture. The dose-response curve was constructed by incubating 10-500 pg of unlabeled 11-deoxycortisol and a fixed amount of the labeled antigen. The antibody had an association constant of 2×10^{10} M^{-1}, as calculated by the Scatchard analysis.[5]

The specificity of the anti-11-deoxycortisol antibody was assessed by ascertaining the ability of various steroids to compete with the labeled antigen for binding to the antibody.[6] The cross-reactions of the antibody with six kinds of related steroids are listed in Table I. The results obtained with cortisol and cortisone show that this antibody is capable of discriminating the functional groups at the 11-position. Thus, it is evident that the antibody is reasonably specific. Development of immunoassays using this monoclonal antibody for plasma 11-deoxycortisol is being conducted in these laboratories.

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