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Characteristics and application of monoclonal antibody to progesterone

I. Production of monoclonal antibody to progesterone

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Progesterone의 단크론성 항체에 관한 특성 및 활용에 관한 연구

I. 단크론성 항체의 생산

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초록 : 11α-hydroxyprogesterone hemisuccinate-BSA를 항원으로 하여 항원량을 50 μg/head 및 100 μg/head의 2 group(세마리씩)으로 나누어 BALB/c mouse에 면역접종한 결과 후자에서 항체가의 상승이 확인되었다. 이와 동시에 항원(20 μg)과 adjuvant의 비율을 1 : 9로 하여 장기 접종한 결과는 100 μg투여시 보다 항체가가 낮았다.

항체가가 확인된 clone의 culture에 의해 progesterone 단일크론 hybridoma를 생산해 이의 supernatant에 대한 분석을 실시한 결과 immunoglobulin class는 IgM이었다. progesterone 이외의 다른 steroids와의 교차반응은 매우 낮았다.

Key words: monoclonal antibody, progesterone, radioimmunoassay, cattle.

Introduction

We have used the modified method of Kähler and Milstein[1] to generate hybrid cell lines producing antibodies to progesterone. It was hoped to select monoclonal antibodies with a significantly higher degree of specificity than has so far been reported for antisera obtained by conventional methods.[2] In this conventional method, the antibody showed not only for the progesterone but for the bridge through which it is attached to the carrier protein.[3,4] Therefore, in an attempt to increase the yield of high affinity monoclonal antibodies from a single fusion experiment, we have exploited the cloning feature of the monoclonal antibody technique and have immunized mice that at least two high affinity antibody might be obtained from the cell line. It would be very available in unlimited supply as reagents with constant binding properties and in saving time, labour and materials. In this study, in an attempt to develope a simple technology that dose not require any other materials and to standardize immunoassay using progesterone monoclonal antibody, an experiment was undertaken to produce an antibody to progesterone.

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Materials and Methods

Reagents: [1, 2, 6, 7-^{3}H(N)] progesterone(90~115 Ci/m mol) was purchased from New England Nuclear, USA. Activated charcoal, hypoxanthine and thymidine were obtained from Sigma Chemical Co, USA, pristane(2, 6, 10, 14-tetramethylepentadecane) from Aldrich Chemicals, USA and fetal calf serum and RPMI 1640 from Gibco Laboratories, USA.

Immunization: The antigen of 11α-hydroxyprogesterone hemisuccinate conjugated to bovine albumin (Sigma Chem Co, USA) was dissolved in 0.9% physiological saline solution and mixed with an equal volume of Freund's complete adjuvant (Difco Lab, USA) and 50 and 100 μg of antigen were given as an intraperitoneal injection to each 3 female BALB/C mice.

At two week intervals, booster injections were given as described above but using incomplete instead of complete Freund's adjuvant.

When the antisera attained greater than 50% binding of [^{3}H] progesterone at a 1 : 400 serum dilution, Both groups of the mice were given an intraperitoneal injection of 100 and 150 μg above antigen in phosphate-buffered saline 3 days before cell fusion, respectively.

Cell fusion: The cell line used for fusion was p3-x63-Ag8-ui(P3U1). Cells were grown under 5% CO_2 and 95% air in RPMI 1640 medium supplemented with 10% fetal calf serum. Cell fusion was based on the method of White et al.[5] Polyethlene glycol 4,000 (BDH Chem, Canada) was used as fusing agent. Spleen cells (1×10^8) were fused with 1×10^7 P3U1 myeloma cells. Suspensions of the cells after fusion (10^7 myeloma cells per 10^8 spleen cell) were plated into four 24-well tissue culture plates containing 1 ml feeder layers of spleen cells (10^7/ml) from a nonimmunized BALB/c mouse.

Cloning of hybrid cells: After 14~21 days colonies of hybrid cells showing antibody activity were subcultured in 2 ml wells using selective growth medium without methotrexate. Supernatants of the media were tested for antibody production after 10~14 days, and positive cultures were cloned immediately in soft agar. The clones stopped producing antibody or showed very low binding acivities were not used. Hybrids were repeatedly cloned until to establish stable antibody-secreting clones.

Detection of antibody activity: The screening of hybrid cells for progesterone antibody activity was carried out using conventional radioimmunoassay.[6] After addition of tritiated progesterone to aliquots of cell supernatant, 250 μl from large wells and 50 μl from small wells, incubation volumes were adjusted to 500 μl. Following incubation, addition of dextran coated charcoal and centrifugation, radioactivity was measured in the decanted charcoal-free supernatant(200 μl). In addition, antibody activity assay was also performed by enzyme linked immunosorbent assay.

Determination of immunoglobulin class and specificity: The immunoglobulin class was determined by using mouse monoclonal antibody isotyping kit(Amersham, USA).

The cross-reaction of the antibody was determined as the ratio of the mass of binding of [^{3}H]-progesterone by 50% according to Abraham.[7]

Results and Discussion

The growth of hybrid cells was observed in 50~65% of the wells following fusion and 5~15% of progesterone antibody producing clones were obtained from these fusions. The more positive clones were resulted when the higher level(100 μg) rather than the lower level(50 μg) of 11α-hydroxyprogesterone hemisuccinate conjugated to bovine serum albumin was used. However, one ninth volume of 11α-immunogen was emulsified with Freund's complete adjuvant and 0.1ml of it(containing 20 μg antigen) was injected, antibody activity was lower than that of the 100 μg antigen levels.

Two stable monoclonal antibodies to progesterone were isolated from 2 independent hybridizations. Preliminary assays showed that the sensitivity and cross-reactivity of the antibodies from the two positive stable clones were indistinguishable. Therefore, only one cell subculture was used to establish a cloned hybrid cell line producing antibody against progesterone.

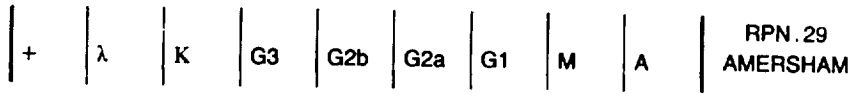

Fig 1. Immunoglobulin class pattern of hybridoma cloned supernatant.

Table 1. Cross-reaction and 50% binding(B-50%) of the monoclonal antibody

Steroid	B-50%	Cross-reaction (%)
Progesterone	85pg	100
Pregnenolone	5.5μg	0.002
Testosterone	73ng	0.116
Estrone	>100μg	<0.0001
Estradiol-17β	45μg	<0.0001
Aldosterone	1.05μg	0.008
Hydrocortisone(cortisol)	1.2μg	0.007
Cortisone	1.00μg	0.009
Corticosterone	8.7ng	0.997
11α-deoxycorticosterone	3.9ng	2.179

Cross-reaction of the monoclonal antibody raised aganist the 11α-immunogen is shown Table 1. Cross-reaction with structurally related other steroids ranged from 0.01 to 2%.

High cross-reaction with progesterone(100%) and low cross-reaction with testosterone(0.1%), DOC (2.18%) and corticosterone(0.98%) confirmed that it was elicited in specific response to the 11α-linked progesterone antigen.[6] The immunoglobulin classification of monoclonal antibody produced was IgM (Fig 1).

Detailed characterization of monoclonal antibody including affinity constant and assay system were not carried out.

In particular, the high cross-reaction of the antibody with progesterone(100%) reveals that it has more specific affinity to progesterone than to other steroids.

Summary

Monoclonal antibody to progesterone was produced using the antigen 11α-hydroxyprogesterone hemisuccinate conjugated to bovine serum albumin. Hybridomas secreting antibody to progesterone were detected by radioimmunoassay and enzyme-linked immunosorbent assay and cloned in soft agar. Two stable monoclonal antibodies which were highly specific to progesterone were obtained, so it may be advantageously used to study on several physiological functions of progesterone including immunological reserch.

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