THE USE OF MOUSE-HUMAN AND HUMAN-HUMAN HYBRIDOMAS IN HUMAN GENETICS AND IMMUNOLOGY

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INTRODUCTION

In order to investigate the molecular basis of human immunodeficiencies and to understand the immunological basis of human autoimmune diseases, it is important to be able to clone the genes for human immunoglobulin chains and the autoantibodies responsible for the pathogenesis of the autoimmune diseases respectively.

We have recently found that mouse x human hybridomas produce more human immunoglobulin chains than their human cell parents(1). Since this is the result of an increase in production of human immunoglobulin specific mRNAs in the hybridomas(1), we have predicted that it should be possible to clone human immunoglobulin specific cDNAs that have been transcribed from partially purified immunoglobulin mRNAs derived from the mouse x human hybridomas. Therefore we have purified human immunoglobulin specific mRNAs from hybrid cells and have characterized their specific cloned cDNAs by the hybrid selection and positive translation method(2) and by DNA sequencing(3). In addition we have used a human B cell line derived from a patient with multiple myeloma (GM1500) to obtain mutants deficient in hypoxanthine phosphoribosyltransferase (HPRT). Such mutants, named GM1500-6TG-Al 1 and -Al 2 were found to be deficient in HPRT and to die in HAT selective medium. The HPRT deficient GM1500 cells were found to secrete human IgG (λ2, κ) as the parental GM1500 cells(1, 4). We have then attempted to hybridize the human GM1500 HPRT mutant cells with human lymphocytes secreting specific antibodies to determine the feasibility to produce human x human hybridomas secreting human monoclonal antibodies. We have chosen to hybridize the GM1500 cells with peripheral lymphocytes derived from a patient with very high titers of antibodies against measles virus. This patient was
a 19 years old female suffering from subacute sclerosing panencephalitis (SSPE), who had first developed SSPE at age 9. The disease lasted for 2 years, leading ultimately to recovery with the persistence of only some residual symptoms. At the age of 18 years, she married and she became pregnant, then in the fourth month of pregnancy SSPE recurred with great severity. Measles virus was isolated from a brain fragment obtained from the patient. Serum from her, diluted 1:10^6, bound measles infected cells.

**METHODS.**

**Cell lines and hybrid production.** Human lymphoblastoid (GM607, GM1056, GM923) or myeloma (GM1500) cells were obtained from the Human Genetic Mutant Cell Repository (Camden, NJ). These cells were maintained in RPMI-1640 supplemented with 10% fetal calf serum under standard conditions. Normal human peripheral blood lymphocytes were obtained from healthy donors. All somatic cell hybrids were produced with mouse BALB/c P3x63Ag8 cells deficient in hypoxanthine phosphoribosyltransferase derived from the MOPC 21 plasmacytoma that secretes IgG1 κ or the nonsecreting subline(⁵). Hybrid cells were selected, maintained, and characterized as to their human isotype secretion (H and L chains) as described(⁴) as indicated in the legend of Table 1.

**Purification of H and L mRNAs.** Minimally degraded H and L chain mRNAs were prepared from all B cell lines and hybrids by the following method. Pelleted cells were lysed for 10 min in 5 volumes of cold 50 mM Tris-HCl, pH 7.4/25 mM NaCl/5 mM magnesium acetate/10 mM 2-mercaptoethanol/30% (wt/vol) sucrose containing polyvinyl sulfate at 20 μg/ml and 0.8% Nonidet P-40, followed by centrifugation at 15,000 x g for 15 min at 10°C to remove nuclei and mitochondria. The supernatant was adjusted to 1.5% NaDodSO₄ and immediately an equal volume of redistilled phenol/chloroform/isoamyl alcohol, 1:1:0.01 (vol/vol), saturated with 10 mM Tris-HCl, pH 7.4/1 mM EDTA/0.1 M NaCl/1.5% NaDodSO₄ was added. The solution was mixed for 10 min and centrifuged at 10,000 x g for 15 min at 20°C. The aqueous phase was precipitated with 3 vol of ethanol at -20°C after three extractions. The total cytoplasmic RNA was subjected to two rounds of oligo (dT)-cellulose chromatography with heating at 70°C for 5 min prior to the second round(⁶). The polyadenylated RNA was then fractionated by neutral 5-25% sucrose gradient centrifugation(⁷). Gradient fractions enriched in H and L chain mRNAs based on in vitro translation criteria(⁸) and immunoprecipitation(⁴) were centrifuged repeatedly until in vitro translation indicated substantial purification.

**Synthesis of double-stranded (ds) cDNA.** Two to 3 μg of human μ poly (A)+ mRNA from GM607 or a somatic cell hybrid αD-DH11-BC11 was primed with oligo (dT) (P.L. Biochemicals) at 50 μg/ml and subjected to reverse transcription essentially as described(⁹), with 900-1500 units of avian myeloblastosis virus.