PRODUCTION OF MONOCLONAL ANTIPLATELET ANTIBODIES BY THE HYBRIDOMA TECHNIQUE

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SYNOPSIS

The preparation of eight monoclonal antibodies which bind to human platelets is described. These antibodies bind to human platelets as measured by indirect immunofluorescence, exhibiting 50 — 80% platelet fluorescence. Characterization of the monoclonal antiplatelet antibody showed it to be a homogeneous preparation of IgG2b.

INTRODUCTION

Idiopathic or immune thrombocytopenic purpura (ITP) is an auto-immune disorder not uncommon in Asian populations (1). The destruction of platelets in a majority of patients with this disease is thought to be mediated by an auto-reactive antiplatelet antibody (APA). However, despite the identification and characterization of APA (2) we still have little information as to its origin, specificity of action and true role in the pathogenesis of ITP. Most importantly, although some degree of antibody specificity has been implied for APA, a specific antigen, group of antigens, or component of the platelet membrane to which APA may bind has not been identified (2). The recent development of hybridoma technology (3,4) to produce monoclonal, homogeneous and highly specific antibody preparations may provide a new approach to this problem of the antigenic specificity of APA and to the characterization of platelet membrane glycoproteins in general (5). We report here the production of a homogeneous, monoclonal APA to human platelets by the hybridoma technique.

MATERIALS AND METHODS

ICR strain laboratory mice were injected intraperitoneally with human platelets (isolated by standard procedures from 10 mls of blood from healthy donors) on days 0, 7, 14, 21, 28 and 32. Freunds incomplete adjuvant was incorporated with the first two doses. On day 35 a spleen cell suspension was prepared from immunized mice and then fused with mouse myeloma cells (P3-NS1-Ag4-1) using polythylene glycol according to standard procedures (4). Fusion mixtures were cultured in 96 well microtitre plates in the presence of selective HAT medium (hypoxanthine, aminopterin, thymidine) which allows only hybrid cells to grow. Supernatants from hybrids cells were assayed for the presence of APA after 2 weeks of culture. APA was assayed by indirect immunofluorescence as reported previously (6). Positive hybrids were then cloned by limiting dilution (4) and the APA characterized by agarose gel immunodiffusion against monospecific antisera.

TABLE 1 PRODUCTION OF MONOCLONAL APA BY THE HYBRIDOMA TECHNIQUE

Days of immunization	No. of wells			% positive wells
	screened	containing hybrids	containing APA Forming hybrids+	
0*, 7*, 14, 21, 28, 32	660	63	14	22

Freunds incomplete adjuvant included
50 — 80% platelet fluorescene. Negative wells gave fluorescence values of 5 — 7%.

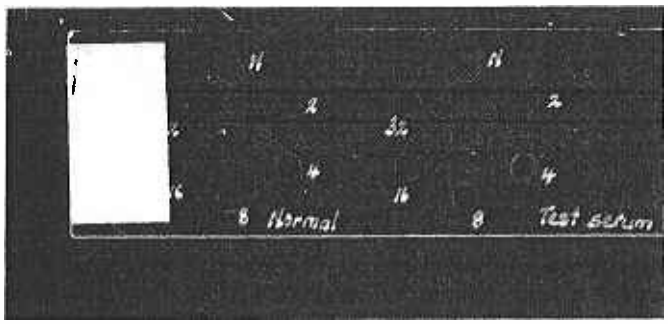

Fig. 1 Agarose gel immunodiffusion of monoclonal APA (Test serum — culture supernatant), normal mouse serum (Normal) against rat anti-mouse IgG (in centre well). N = neat sera; numbers refer to dilutions of sera (1 in 2, 1 in 4, etc.).

RESULTS

Of the 660 wells plated out from fusion mixtures, 63 hybrids (9.5%) were detected (Table I). Fourteen out of these 63 hybrids (22%) were positive for APA and exhibited strong fluorescence (50 — 80% platelet fluorescence) (Table I). No reaction was detected with human red blood cells, lymphocytes or granulocytes. Positive hybrids were then cloned, allowed to grow to the required density and supernatants tested again for APA. Although some of the original clones lost APA — producing ability during the cloning steps, eight cultures have remained stable and were subsequently transferred to tissue culture flasks. Characterization of the monoclonal APA showed it to be a homogeneous antibody of class IgG (Fig. 1), subclass IgG2b.

DISCUSSION

The development of hybridoma technology has enabled, for the first time, the production of antibody preparations of unprecedented homogeneity and specificity and, theoretically at least, in unlimited quantities. It has been widely used in the study of human cell surface antigens, viral and parasite antigens (7) and may have important applications in the investigation of a variety of human autoimmune diseases (8), particularly for obtaining pathogenic antibodies of specificities which may not be detectable in serum because of quantitative binding to target cells. In ITP patients with high affinity APA, for example, most or all of the immunoglobulin may be adherent to the platelet with only undetectable amounts remaining in the serum (2). A homogeneous and specific APA preparation could be used to isolate and individually study platelet membrane glycoproteins and resolve the complexity and functions of individual components (5). It could also be utilized in studies into the true role of APA in the pathogenesis of ITP e.g. in identifying platelet surface antigen(s) to which the APA binds and subsequent events affecting both platelet function and integrity. These studies are currently in progress in our laboratory. In other studies, monoclonal antibodies have been produced to glycoprotein I and glycoprotein II/III of human platelets (7). Since they specifically fail to bind to platelets in patients lacking these glycoproteins, they may be useful in identifying glycoproteins absent in certain genetic disorders such as Bernard-Soulier syndrome and Glanzmann's thrombasthenia. Furthermore, it may also become possible to detect many minor glycoproteins which may also have important physiological roles and thus enable the diagnosis of more subtle abnormalities to be made.

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