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NATURAL ANTIBODIES AS CONTAMINANTS OF HYBRIDOMA PRODUCTS

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Summary. This report cites an example of natural antibodies in mouse serum and ascites fluid which may contaminate hybridoma products and cause difficulties in interpreting their reactions. These natural antibodies react with determinants expressed on human foetal glycoproteins (extracted from meconium) and on other blood group precursor-like substances which express a number of tumour associated- and differentiation antigens. Variable amounts of these antibodies may be present in the ascites fluid of mice obtained by intraperitoneal injection of cloned hybridomas. Although in most cases the hybridoma antibodies will be used at dilutions beyond the endpoint of these contaminating antibodies, it is important to be aware of possible reactions due to natural and acquired antibodies with diverse specificities in evaluating the reactions of hybridoma products harvested from any type of serum containing medium.

There is much current interest in carbohydrate structures as differentiation and tumour-associated antigens (reviewed in refs. 1 and 2). Monoclonal antibodies have been of special value in singling out such carbohydrate determinants, among the vast array at the cell surface, during early embryogenesis, cellular differentiation[3-6] and oncogenesis[7,8]. It is now standard procedure to inject cloned hybridomas intraperitoneally into mice in order to obtain large amounts of 'immune' ascites fluids rich in the desired monoclonal antibodies. In the course of investigating possible carbohydrate specificities of several hybridoma antibodies against cell surface antigens we have observed that certain samples of 'immune' ascites react with human foetal glycoproteins extracted from meconium. With one hybridoma system we observed different reactions with two batches of ascites fluid containing the same monoclonal antibody. Further investigations were carried out and are the subject of this report in which we present evidence that these reactions did not involve the hybridoma antibody but were due to contamination with varying amounts of natural antibodies from serum, directed against

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determinants expressed on foetal glycoproteins and on blood group precursor-like substances.

MATERIAL AND METHODS

Serum from untreated (BALB/c x C3H)F_1 mice, two samples of ascites from mice of this strain bearing the cloned hybridoma H9/25, and a sample of culture supernatant containing H9/25 (IgM) antibody[9,10] were kindly provided by Dr. Cesar Milstein. IgM rich fraction, containing 6 mg protein/ml was isolated from ascites fluid by chromatography on Sephadex G-200.

Double antibody radioimmunoassays involving binding of mouse antibodies to ^{125}I-labelled glycoproteins extracted from human meconium (non-secretor type) and enriched for blood group precursor-like activity were performed as described previously[6]. Unless stated otherwise the assays were performed at 4°C. Details of reference blood group substances used as inhibitors, kindly provided by Drs. E.A. Kabat and W.M. Watkins, have also been given previously.[6] Mild acid hydrolysis of glycoproteins (200 µg/ml solutions in isotonic saline) was performed in the presence of 0.02 M H_2SO_4 at 100°C for 90 mins. The reaction was terminated by neutralization with two volumes of 0.02 M NaOH.

RESULTS

Initial studies were performed with a sample of ascites (sample a) containing the monoclonal antibody H9/25 which has previously been shown[9,10] to recognize a differentiation antigen on activated T and B lymphocytes. Binding was demonstrated by radioimmunoassay to ^{125}I-labelled glycoprotein-rich extract of human meconium (Fig. 1). The shape of the binding curve resembled those observed[11] with certain low affinity anti-carbohydrate antibodies such as those with anti-I and anti-i specificities. The binding was strongly inhibited by several samples of human meconium and by reference glycoproteins lacking in blood group ABH activities (referred to as blood group precursor-like subtances[12,13]) but there was negligible reaction with substances rich in blood group ABH activities (Fig. 2). These inhibition patterns resembled those seen with human monoclonal antibodies with I and i specificities[14,11] and with the hybridoma antibody, anti-SSEA-1 (Ref. 6). Unlike SSEA-1 this determinant is not destroyed by mild acid hydrolysis (Fig. 1) and is therefore unlikely to involve fucose or sialic acid residues. Cold agglutinins with anti-I and i specificities[15] were also ruled out since substantial binding to the ^{125}I-labelled reference glycoproteins occurred at 30-37°C and human erythrocytes from adults and neonates were not agglutinated at 4°C (results not shown).

In contrast to sample (a), a second sample, (b), from a different batch of ascites fluid containing antibody H9/25, which was also raised in

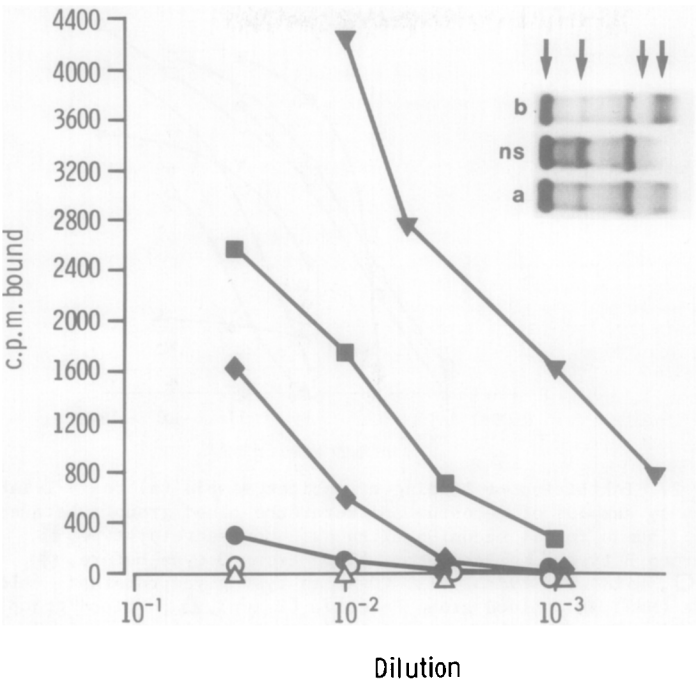

Fig. 1. Double antibody radioimmunoassays showing binding to ^{125}I-labelled glycoprotein rich extract of human foetal meconium (non-secretor type). Symbols: ascites samaples (a) and (b), ◆, ●; IgM rich fraction of ascites sample (b), ○; culture supernatant from H9/25 clone, △; normal serum from (BALB/c x C3H)F_1 and A/Sn mice, ■, ▼.

Inset: Cellulose acetate electrophoresis of normal (BALB/c x C3H)F_1 mouse serum (ns) and ascites samples (a and b). Arrows from left to right point to positions corresponding to serum albumin, α-globulins, normal gammaglobulins and H9/25 protein.

(BALB/c x C3H)F_1 mice, showed negligible binding to the radioiodinated foetal glycoproteins. Further, the IgM rich fraction isolated from this sample showed no binding (Fig. 1). A sample of culture supernatant from H9/25 clones also showed no detectable binding to this glycoprotein. Serum from untreated (BALB/c x C3H)F_1 mice was next tested; substantially higher binding was obtained than with ascites fluid sample (a). Cellulose acetate electrophoresis of the two samples of ascites fluid showed that sample (b) contained a higher concentration of monoclonal protein but a lower concentration of serum albumin and other serum components (Fig. 1 inset). Thus the reactions of ascites sample (a) in the radioimmunoassays were not associated with the hybridoma antibody but with other components in the ascites fluid which were more abundant in sample (a) than in sample (b).

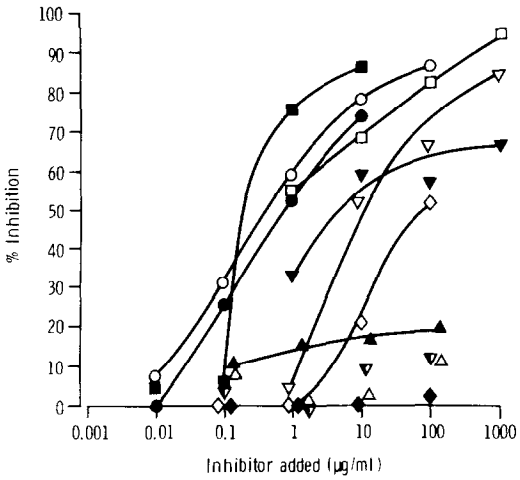

Fig. 2. Inhibition of binding of ascites sample (a) to ^{125}I-labelled meconium by samples of meconium and reference blood group substances. Symbols: human foetal meconium extracts:- non-secretor type, ■ ; secretor, blood group B type, □ ; secretor, blood group O type before, ● , and after, ○ , mild acid hydrolysis. Ovarian cyst glycoproteins:- blood group A-active (MSS), ◆ ; blood group B-active (Beach), △ ; blood group HLe^b-active (JS), ▼ ; blood group Le^aI-active (N-1), ▲ ; blood group ABH-inactive, precursor like, Ii-active glycoprotein (Fl) before, ▼ , and after, ▽ , mild acid hydrolysis; blood group precursor like, i-active glycoprotein '502', ◇ .

In studies to be described in detail elsewhere we have observed that sera from different strains of mice contain variable amounts of natural antibodies reacting with the glycoproteins from meconium. For example, in 6-7 month old A/Sn mice, the titres were 10 to 15 fold higher than those observed in (BALB/c x C3H)F_1 mice (Fig. 1). The relationship of these natural antibodies to those described previously that react with mouse lymphoma and other tumour cell lines and with neuraminidase treated normal lymphocytes[16-23] are currently under investigation.

DISCUSSION

These studies show that mouse sera contain natural antibodies reactive with human foetal glycoproteins and other blood group precursor-like substances which are known to express a number of tumour associated[24,25] and differentiation[1,5,6] antigens. Since substantial amounts of serum components are present in the ascites fluids of hybridoma bearing mice, the natural antibodies are also likely to be present and may cause difficulties in unravelling the determinants recognized by the hybridoma antibodies. The levels of these natural antibodies may vary in different batches of

ascites fluid raised in the same strain of mice. In the example cited, the ascites sample (b) contained a larger amount of hybridoma antibody than sample (a) but the amount of natural antibodies reacting with the foetal glycoproteins was very low; sample (b) contained lower levels of other serum components. A likely explanation for reduced levels of serum proteins in ascites sample (b), is that mice with extensive hybridoma load resemble debilitated patients with advanced myelomatosis who often have large amounts of monoclonal proteins but reduced levels of normal immunoglobulins and albumin in their serum[26]; the levels of the serum components in the ascites fluids will be correspondingly low.

Ascites fluids that contain monoclonal proteins of high affinity and very high titres of antibody activity, will generally be used at dilutions beyond the endpoints of the natural, contaminating antibodies. However, with anti-carbohydrate antibodies of low affinity such as human monoclonal anti-I and anti-i, the antibody titres associated with large M-bands are sometimes surprisingly low (unpublished observations) and the likelihood of encountering activities due to contaminating natural antibodies is very real. Our observations stress the need to consider natural antibodies, derived from serum, as possible contaminants not only in ascites fluids but also in culture media. Such antibodies may prove difficult to separate from the hybridoma antibodies, unless purified preparations of the relevant antigens are available for specific absorption and elution.

Hybridoma antibodies will be increasingly available for world-wide distribution and they will be used in diverse experimental systems many of which will differ from the antigen systems for which they were originally raised and standardised. For each new experimental system a minimum requirement will be, to have available as controls, samples of normal serum (or foetal calf serum in the case of culture supernatants) from mice injected with the hybridomas. An additional consideration which is harder to control is the possible triggering of auto-, iso- or other types of antibodies in the mice injected with hybridoma cells. In this instance the problems facing the research worker are similar to those usually encountered while using conventional antisera.

Because of their narrow specificities not only for restricted determinants but also for different facets of the same antigenic determinants, monoclonal antibodies toward the same antigens often show striking differences in their reaction patterns in various immunochemical and immunocytochemical assays. This has been clearly illustrated with monoclonal anti-carbohydrate antibodies[14,27-30]. The present studies indicate that the possible occurrence of natural or acquired antibodies should also be borne in mind in accounting for unexpected reactions when hybridoma products are used as immunochemical reagents.

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