AN ASSAY FOR CRYPTIC TUMOR ANTIGENS
IN SERA OF WOMEN WITH BREAST CANCER

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The utility of circulating mucins as breast cancer markers was first proposed by Ceriani, et al. (1) and was rapidly confirmed and expanded by many other laboratories, including those of Chu, Taylor-Papadimitriou, Hilgers and Kufe (2-5). The antibodies used in mucin assays measure epitopes expressed on or cross-reactive with the human milk-fat globule membrane (HMFG) antigens. Assays using antibodies developed in many of the above mentioned laboratories are now commercially available, and have been used with some success in monitoring breast cancer patients.

Immunoassays for these markers measure epitopes at least partly composed of oligosaccharides, which are linked to the protein core through serine or threonine in O-glycosidic linkages (6). The oligosaccharide chains are expressed in multiple copies per glycoprotein molecule (7), and recent evidence indicates that the peptide core to which the carbohydrate chains are attached also has a repetitive structure (8). Immunoassays for these molecules can be problematic because of the high epitope density which may be present on some but not all molecules, and the variability of carbohydrate chain synthesis from individual to individual. In addition, the high negative charge and water of hydration of these molecules, properties associated with the presence of sialic acid on many, but not all of the carbohydrate chains (6-8), may affect assay performance. In some cases, the sialic acid is an important part of the epitope recognized by tumor specific antibodies (9), and in other cases it is not (10,11).

We have constructed an assay for a mucin-like glycoprotein which we believe has utility in monitoring breast cancer patients' response to therapy. In addition, we have devised a method of modifying the behavior of these glycoproteins in the assay system, resulting in improved assay performance and specificity without adding steps to the assay. The assay utilizes sialidase (C. Perfringens) to expose tumor-associated antigens that are cryptic in some, but not all, patients' sera because of sialylation of the epitopes. The presence of cryptic cell surface antigens, some of them tumor-associated, has been reported by several laboratories, notably of Burger, Springer and Feizi (12-14).

We present here some data on a cryptic tumor antigen assay for HMFG associated epitopes generated in our laboratories. A mouse monoclonal to
the paragloboside family of oligosaccharides, M85/34, is used as the catcher and the peroxidase conjugated probe is the breast cancer MAb (F36/22) generated in T.M. Chu's laboratory at Roswell Park Memorial Institute (11, 20).

MATERIALS

Defatted human milk fat globule membrane preparations were provided by R. Ceriani, John Muir Cancer and Aging Research Institute, Walnut Creek, CA. Sera from women judged to be normals by personal histories and physical examination by Dr. K. McCarty, Jr., were used to establish the normal cutoff for the assay. Other sera were from various sources and were acquired by the Abbott Laboratories serum bank. NCI blind panel specimens were obtained from Dr. R. Aamodt, Biological Markers Program, National Cancer Institute, Bethesda, MD. Glycolipid preparations were provided by Samar Kundu, Abbott Laboratories. Anti-sera to paragloboside was obtained from Dainabot, Ltd.

![Western blot of HMFG, developed with MAb MA85/34 (Lane 2) or control mouse IgM (Lane 1), or with MAb F36/22 (Lane 4) or control mouse IgG (Lane 3).](image)

Fig. 1. Western blot of HMFG, developed with MAb MA85/34 (Lane 2) or control mouse IgM (Lane 1), or with MAb F36/22 (Lane 4) or control mouse IgG (Lane 3).