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MONOCLONAL ANTIBODY-DEFINED CIRCULATING HUMAN TUMOR-ASSOCIATED ANTIGEN WITH EPITOPE SHARED BY CYTOKERATINS

M.Hollmann, H.J.Staab, E.Spindler, M.Sproll, F.A.Anderer+, H.P.Fortmeyer*

Friedrich-Miescher-Laboratorium der Max-Planck-Gesellschaft,
7400 Tübingen, Spemannstrasse 37-39 , Fed. Rep. Germany

*Klinikum der Universität Frankfurt, Tierversuchsanlage,
6000 Frankfurt a.M., Fed.Rep.Germany

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Summary: Sera of human colonic carcinoma xenografted rnu/nu rats were used to immunize rnu/+rats in order to obtain an immune response against circulating human tumor-associated components. After fusion of rat spleen cells with mouse myeloma cells monoclonal antibody MAB 108 could be established which reacted with two 40 and 45 kD cytokeratins as well as with vimentin, with a soluble 37 kD protein apparently derived from the 45 kD protein and with a 37 kD protein released by tumor cells. The MAB 108-specific epitope was also detected in tissue polypeptide antigen (TPA), a human tumor-associated antigen originally described by Björklund et al. (22).

Human circulating tumor-associated antigens (TAA) deserve special interest since they can be of great prognostic and diagnostic value in the management of cancer patients and can be monitored by non-invasive techniques. TAA are generally prepared from tumor tissue using methods potentially involving secondary alterations of their physical and chemical structure. Furthermore, different biological processing of tissue type antigens and circulation type antigens may lead to additional structural differences. The fact that human malignant tumors can be heterotransplanted into athymic mice and rats maintaining their growth characteristics and ability to release human TAA into the circulation (1,2) opened up the possibility to use these sera as source of circulating human TAA for the immunization of immunocompetent (nu/+) litter-mates or (+/+) mice of the mother-strain in order to obtain an antibody response against circulating human TAA (3,4). In the present report

+To whom reprint requests should be adressed.

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we describe antigens defined by a monoclonal antibody which was derived from a (rnu/+) rat immunized with the serum of human colonic tumor-bearing (rnu/nu) rats.

MATERIALS AND METHODS

Tumor cells and animals: The Slu-1 tumor cell line, exhibiting a high CEA release, was established from a liver metastasis of a patient with a primary adenocarcinoma of the sigmoid colon. Wistar (rnu/nu) rats were xenografted each with 10^7 SLu-1 cells. Tumor growth was monitored by determinations of circulating human CEA and ß2m. Sera were collected during logarithmic growth in intervals of 3-4 days. The resulting serum pool (137 ng CEA/ml) was used to immunize a Wistar (rnu/+) rat with dosages of 250µl.
Fusion and screening: Splenocytes of the immunized rat were fused with P3-X63-AG 8.653 mouse myeloma cells (5) as described by Galfré et al. (6). After fusion, cells were plated with feeder peritoneal cells in 1000 microwells using RPMI-HAT medium (7). Supernatants of growing colonies were used for ELISA on formaldehyde-fixed adhesive HT-29 cells, another human colon carcinoma cell line (8). ELISA-positive supernatants were further screened by indirect immunofluorescence techniques using cryostat sections of HT-29 tumors.
Characterization of antigens: One- and two-dimensional gel electrophoresis were performed according to (9)and(10) respectively, immunotransfer as described in (11). For antigen detection on nitrocellulose recommendations (12, 13) were used to modify the method of Towbin et al. (14). Intermediate filaments were prepared as described by Franke et al. (15) and purified by preparative gel electrophoresis with electroelution of protein bands (16). Silver staining was performed as proposed by Wray et al. (17) and protein was estimated by the method of Peterson (18). For determination of TPA a commercially available TPA-RIA kit (Sangtec Medical AB, Bromma, Sweden) was used.

RESULTS

Sera of Wistar (rnu/nu) rats xenografted with human SLu-1 colonic carcinoma cells were used for immunization of Wistar (rnu/+) rats to obtain a humoral immune response against the circulating human components. After fusion of mouse myeloma cells with splenocytes of an immunized rat, screening for monoclonal antibodies was first carried out with HT-29 cells, another human colonic carcinoma cell line, using an ELISA technique. From 554 primary clones 71 showed positive reaction but no reaction with STU mouse cells. Only 8/71 clones were positive in an immunofluorescence assay performed with cryostat sections of HT-29 tumors. The monoclonal antibodies (IgM) of clone MAB 108 showed cytoplasmic staining of unfixed cryostat sections of human colonic and mammary carcinoma xenografts and human tumors of the gastrointestinal tract. This type of fluorescence was also observed with some embryonic as

well as some normal human epithelial tissues. Treatment of sections with 2% formaldehyde for more than 1 min reduced the reaction markedly maintaining positive reactions in tumor tissue only. The staining pattern of monolayer cells and its sensitivity to pretreatment of cells with colchicine indicated involvement of intermediate filaments.

After gel electrophoresis of a lysate of HT-29 cells prepared by rapid immersion of cells in hot SDS buffer (Fig.1, A1) and immunotransfer of the proteins to nitrocellulose strips, two proteins of 40 and 45 kD carrying the MAB 108 epitope could be detected (Fig.1, B1) (19). When cells were ultrasonicated in the absence of SDS a third MAB 108-positive protein with 37 kD was detected (Fig.1, B2). This protein appeared to be a product of proteolytic degradation since in ultrasonicates kept at 4°C for 7 days the 45 kD component had disappeared in favor of the 37 kD component (Fig.1, B3). The 37 kD protein was not structurally bound, it could be also detected in the 120 000 x g supernatant (Fig.1, B4) whereas the 40 kD and 45 kD components

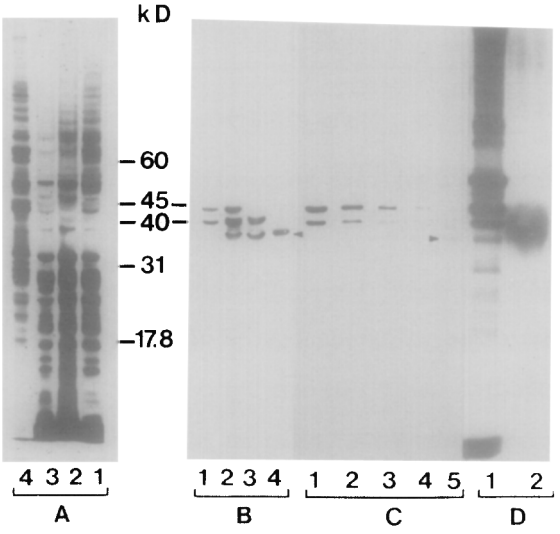

Figure 1 A: Silver stained SDS-gel of (1) HT-29 SDS homogenate (22 µg), (2) HT-29 ultrasonicate 120 000 x g sediment (30 µg), (3) HT-29 ultrasonicate kept at 4°C for 7 days (30 µg), (4) HT-29 ultrasonicate 120 000 x g supernatant (7,5 µg); B: Antigen detection with MAB 108 after immunotransfer of A; C: MAB 108 immunodetection of purified intermediate filaments on blot (1) 5 µg; (2) 2 µg; (3) 1 µg; (4) 0,5 µg; (5) HT-29 spent medium (150 µg protein, FBS-free); D: Autoradiogram of 14-C-labeled crude intermediate filaments (1) and commercially available 125-J-TPA (Sangtec) (2). Arrows mark 37 kD proteins.

were exclusively found in the sediment. Both proteins showed different peptide patterns after V8-protease treatment and were constituents of purified intermediate filaments of HT-29 cells (Fig. 1,C1-4).This was confirmed by two-dimensional gel electrophoretic analysis which resulted in a typical cytokeratin pattern as described by Moll et al. (20), the 40 and 45 kD proteins being cytokeratins No. 19 and 18, respectively, in the Moll nomenclature. Using intermediate filaments purified from HeLa cells or purified vimentin of mouse ascites cells, reactions of MAB 108 with vimentin could be demonstrated thus supporting reports (21) on the existence of crossreactive epitopes on both types of intermediate filaments.

To answer the question which of the proteins carrying the MAB 108 epitope are released by human colonic tumor cells, FBS-free spent medium of HT-29 cells was concentrated 1:850 and subjected to SDS gel electrophoresis and transfer to nitrocellulose membranes using MAB 108 for antigen detection. Only one major band with 37 kD was detected accounting for about 0.1-0.3% of the material applied to gel electrophoresis (Fig.1, C5) which is possibly identical with the 37 kD component formed during and after ultrasonication of cells.

Tissue polypeptide antigen (TPA), a tumor-associated antigen originally described by Björklund et al. (22), exhibits a variety of properties which have led to speculations that TPA may be related to components of intermediate filaments (23-26). Therefore, we investigated whether TPA expresses the MAB 108 epitope performing competition experiments with MAB 108 as well as with MAB 108 antigens as competitors in a commercially available radioimmunoassay for TPA determination. As shown in Table 1, MAB 108 from hybridoma cell supernatant or from ascitic fluid increased binding of labeled TPA. Furthermore, purified intermediate filaments or their isolated 40 kD component competed effectively for TPA-binding antibodies thus decreasing binding of labeled TPA. However, most effective competition was exhibited by the FBS-free spent medium of HT-29 cell cultures. The 125-J-TPA standard of the TPA-

TABLE 1

Competition of MAB 108 (A) and MAB 108 antigens (B) for TPA and anti-TPA using a commercially available TPA-RIA kit (Sangtec)

	Additions to standard TPA-RIA	relative amount of labeled TPA bound
A	- (standard only)	100
	hybridoma cell supernatant dil. 1:10;1:100	126; 101
	ascitic fluid dil. 1:10;1:100;1:1000;1:10 000	162; 138; 127; 108
B	HT-29 cell sonicate 300 ng; 60 ng (protein *)	49; 68
	purified intermediate filaments 250; 50; 10 ng *	34; 65; 91
	purified 40 kD-component 250 ng; 50 ng; 10 ng *	43; 71; 92
	HT-29 spent medium (FBS-free) 1250; 250; 50 ng *	17; 27; 54

RIA kit revealed a relatively broad electrophoretic band between 37-45 kD with maximum intensity at 37 kD (Fig.1, D2).

Tumor transplantation experiments with HT-29 and SLu-1 tumor cells in (nu/nu) mice showed that neither pre- and posttreatment with MAB 108 nor transplantation of tumor cells in mixtures with MAB 108 led to tumor protection indicating that MAB 108 antigens do not represent target antigens for tumor cell killing.

DISCUSSION

The data presented by us indicate that the MAB 108 epitope (or cross-reacting variants) is expressed by two cytokeratins with 40 and 45 kD as well as by vimentin and by tissue polypeptide antigen (TPA) widely referred to as a tumor marker. The immunizing procedure by which the MAB 108-specific response was induced is expected to be specific for circulating tumor-associated antigens. However, it is unlikely that the generally insoluble 40 and 45 kD cytokeratines were the response-inducing circulating antigens. The formation of a soluble, relatively stable, proteolytic 37 kD fragment in cell sonicates and the release of a soluble 37 kD protein into the medium of tumor cell

cultures indicate a possible pathway for a MAB 108 epitope-carrying circulating antigen. The molecular size of these components is comparable to that of TPA, all three are soluble, express the MAB 108 epitope and are possibly one and the same protein. The question whether the 37 kD protein is actively released into the circulation or only during tumor necrosis remains to be answered.

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