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Targeting of cancer cells with monoclonal antibodies specific for C3b(i)

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Abstract Purpose: The goal of this research is to determine the feasibility of an immunotherapeutic approach based on the use of monoclonal antibodies (mAb) to target complement activation fragments on opsonized cancer cells. Methods: We investigated whether treatment of LNCaP and C4-2 human prostate cancer cell lines with normal human serum would allow for deposition of sufficient amounts of the complement-activation protein C3b and its fragments [collectively referred to as C3b(i)] such that these proteins could serve as cancer-cell-associated antigens for targeting by mAb. Radioimmunoassays, flow cytometry, and magnetic purging with specific immunomagnetic beads were used for the analyses. Results: In vitro opsonization of human prostate cancer cells with normal human serum resulted in deposition of C3b(i) in sufficient quantity (approx. 100,000 molecules/cell) for the cells to be targeted in a variety of protocols. We found that 51Cr-labeled and C3b(i)-opsonized cancer cells could be specifically purged at high efficiency (95%–99%) using anti-C3b(i) mAb covalently coupled to magnetic beads. Flow-cytometry experiments indicated that most normal white cells were not removed under similar conditions. Opsonization of cancer cells with sera from men with prostate cancer led to lower levels of cell-associated IgM and, subsequently, lower amounts of C3b(i) deposited than in normal subjects. Prototype experiments suggested that this deficiency could be corrected by addition of IgM from normal donor plasma. Conclusion: mAb directed against complement-activation products may provide new opportunities to deliver diagnostic and therapeutic agents selectively to cancer cells and tumor deposits. These opportunities may include ex vivo purging of C3b(i)-opsonized cancer cells prior to autologous bone marrow or stem cell transplantation.

Key words Complement · Tumor antigen · Monoclonal antibody · Prostate cancer · Purging

Introduction

Many treatment modalities currently under investigation for cancer depend upon tissue-specific delivery of antineoplastic agents. One immunotherapeutic approach involves conjugating cytotoxic agents to mAb specific for a particular cancer cell epitope. In this manner, the agents can be delivered at a high therapeutic dose directly, and selectively, to the tumor site, thereby minimizing injury to healthy tissue [1, 20, 41]. This method first requires the identification of a target antigen for each cancer type and, second, the development of high-affinity mAb specific for the target antigen with minimal cross-reactivity with normal cells. A more widely applicable approach would be to identify a ubiquitous antigenic site, present on virtually all cancer cells, and then to develop a panel of mAb specific for this antigen.

Human cancer cells are characterized by abnormalities in the glycosylation patterns of their cell-surface
proteins and lipids [4, 22, 52, 53]. Natural IgM antibodies to these epitopes are present in the circulation, and the interaction of such IgM antibodies with these cancer cell surface antigens leads to activation of complement and covalent coupling of complement activation products [C3b and its fragments, collectively referred to as C3b(i)] to the tumor cells [8, 26, 38, 43, 62–65]. Although relatively large amounts of C3b(i) can be deposited on cancer cells, the concomitant expression of high levels of membrane-associated complement control proteins (e.g., decay-accelerating factor, membrane cofactor protein and, in particular, CD59) usually prevents complement-mediated lysis [5, 17, 18, 27, 28, 30, 34]. However, we hypothesized that C3b(i) deposited on a cancer cell surface could function as a tumor-associated antigen and be utilized as a target for directing mAb-based therapeutic and diagnostic agents to neoplastic lesions.

We focused our studies on the LNCaP and lineage-derived C4-2 human prostate cancer cell lines. The LNCaP/C4-2 progression model recapitulates progression of human neoplastic prostate disease from an androgen-responsive and minimally metastatic (LNCaP cells) to an androgen-refractory (defined as being able to proliferate in castrated hosts) and highly aggressive phenotype (C4-2 subline) [25, 45, 57]. We found that opsonization with normal human serum (NHS) led to a high level of C3b(i) deposition on both LNCaP and C4-2 cells, and that IgM in the serum played a major role in this reaction. Sera from men with prostate cancer promoted less C3b(i) deposition, and this effect appeared to be due to decreased levels of tumor-specific IgM in their sera. Prototype in vitro studies have allowed us to identify several candidate mAb specific for C3b(i), which may be useful in a variety of immune-specific targeting modalities for complement-opsonized cancer cells. Our results suggest that opsonization of tumors or cancer cells with normal human serum will facilitate the use of mAb specific for C3b(i) in immunotherapeutic approaches to the treatment of cancer.

### Materials and methods

**Cell lines and serum specimens**

LNCaP and its lineage-derived C4-2 cell line were obtained as described previously [57]. These cell lines represent corresponding androgen-dependent (LNCaP) and androgen-independent (C4-2) human prostate cancer cell lines defined by their ability to grow in adult male and castrated male mice respectively. These cell lines were maintained in T medium with 5% heat-inactivated fetal bovine serum (FBS; Gibco, Grand Island, N.Y.). Cultures were maintained at 37 °C in humidified 5% CO2, and split or harvested at 80%–90% confluence. Cells were collected using either phosphate-buffered saline (PBS) with 2.5 mM ethylenedinitrilotetra-acetic acid (EDTA; Sigma, St. Louis, Mo.) or trypsin (Gibco, Grand Island, N.Y.) diluted 1:10 in PBS. Samples were then washed twice in PBS by centrifugation of 450 g for 2 min and resuspended at a final concentration of (0.2–1) x 10^7 cells/ml in PBS with 1% bovine serum albumin (BSA/PBS).

Blood samples were obtained with written informed consent from normal male and female volunteers (University of Virginia, Charlottesville, Va.) and from men being followed for prostate disease (University of Virginia and Eastern Virginia Medical School, Norfolk, Va.). Serum was obtained from clotted blood, and plasma was isolated from blood samples anti-coagulated with EDTA. Prostate disease patients had pathological documentation of either benign or neoplastic prostate disease.

**Monoclonal antibodies**

IgG1 mAb 7C12, 2H11, and 8E11, specific for C3b(i); IgG1 mAb HB57, specific for human IgM; and IgG2a mAb 7G9, specific for human complement receptor 1 (CR1), have been previously reported [11, 54, 59] and were used in parallel with isotype-matched controls. Anti-C3b(i) mAb 3E7 (IgG1), which bound to a different epitope and was not blocked by the other anti-C3b(i) mAb, was prepared by our previously described methods [59]. The specificity of mAb 3E7 for C3b(i) was confirmed by indirect flow cytometry as follows. Both mAb 3E7 and 7C12 bound to serum-opsonized pig erythrocytes, as revealed by fluorescein-isothiocyanate (FITC)-labeled anti-(mouse IgG). In the presence of 5 μg/ml purified, heat-treated (56 °C) C3 (Quidel, San Diego, Calif.), binding of the mAb to opsonized pig erythrocytes was reduced by 88% and 62% respectively (results not shown). Radiolabeling of mAb with 125I was performed by the iodogen procedure [10, 12]. A bispecific mAb complex (a heteropolymer) was prepared by cross-linking mAb 3E7 with mAb 7G9 [49, 56].

**Flow cytometry and radioimmunoassays**

Opsonized cancer cells were probed with FITC-labeled goat anti-human IgM Fc5μ (Pierce, Rockford, Ill.), FITC-labeled goat anti-human IgG Fc (Accurate, Westbury, N.Y.), or a cocktail of the anti-C3b(i) mAb 7C12 and 8E11 (typically 200 ng each mAb/10^6 cells) followed by a secondary FITC-labeled goat anti-(mouse IgG) (Sigma, St. Louis, Mo.). All incubations were at 37 °C for 20 min in BSA/PBS. Controls included non-opsonized cells and irrelevant isotype-matched mAb. In selected cases, cells were stained with propidium iodide (Sigma, St. Louis, Mo.) used at a final concentration of 2 μg/ml in BSA/PBS for 5 min, in the dark, on ice, to ascertain IgM or C3b-opsonization of the viable cell populations only. Viability was usually above 75%. One- or two-color fluorescence analysis was performed with CellQuest software on a FACSCalibur flow cytometer (Becton Dickinson, San Jose, Calif.).

Studies of the binding of 125I-labeled anti-C3b(i) and anti-human IgM mAb to cancer cells followed previously published procedures [10, 54]. Briefly, after opsonization, 5 x 10^5 cancer cells were incubated for 20–30 min with 125I-labeled mAb 7C12, 8E11, 3E7, or HB57 (final concentrations between 0.1 μg/ml and 10 μg/ml) or matched-isotype controls. As a separate control for