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[16.15–16.45]

Innate immunity and cancer: Genetics, specificity and function of natural monoclonal IgM antibodies

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Innate immune mechanisms are crucial for first defense and initiation of secondary responses to "non-self" structures. This has been shown for bacterial antigens, but the recognition and defence-activity against malignant cells is still obscure. By using human hybridoma-technology we have isolated hundreds of human monoclonal antibodies from different patients with different tumours. All of these antibodies are of IgM type, none of them was an affinity-maturated IgG or IgA type. We have sequenced several of these antibodies and have characterised binding pattern and determined origin and genetics. It was found that all IgM antibodies are not or only less mutated (germ-line coded), and bind to carbohydrates on modified tumour-specific receptors (DAF, CFR-1). The degree of cross-reactivity to other tumours correlates with the grade of mutations in coding regions. By using an anti-idiotypic antibody, we could show, that the IgM-producing cells are CD5 positive. The striking similarities between the humoral response to bacteria and tumor cells, presented here, makes it likely that the same or similar recognition and defense mechanisms are used. Origin, reactivity pattern and genetics of these real tumor-specific antibodies are close to that what we know from the defense to bacterial structures and it is likely that innate imunity is not only resposible for recognition and elimination of bacterial structure but also for removal of malignant cells.

[16.45–17.05]

A human monoclonal antibody (HMMC-1) that selectively recognizes müllerian duct-related carcinomas

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Introduction: We report the establishment of a human monoclonal antibody using cross-bred Trans-Chromosomic (TC) mouse that specifically recognizes human müllerian duct-related carcinomas. We also report a preliminary analysis of the carbohydrate structure of the carcinogenesis-related antigen moiety recognized by this monoclonal antibody in these malignancies.

Methods: A human monoclonal antibody was raised against the human uterine endometrial cancer cell line SNG-S. The monoclonal antibody was established using hybridoma of myeloma cells and spleen cells from cross-bred TC mouse that were developed to produce a complete human immunoglobulin. Specificity of the antibody was confirmed by both ELISA and immunohistochemistry. The carbohydrate antigen recognized by this antibody was analyzed by a series of exoglycosidase digestions and immunoblotting experiments.

Results: Our newly established human monoclonal antibody termed HMMC-1 (of IgM subclass) was found to specifically recognize m¤„llerian duct-related carci-

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nomas. Immunohistochemical staining revealed that the reactivity of HMMC-1 was positive in 54.6% of uterine endometrial adenocarcinoma specimens, 76.9% of uterine cervical adenocarcinoma specimens and 42.4% of epithelial ovarian cancer specimens. Furthermore, this monoclonal antibody does not react with either proliferative or secretory phase normal endometrium or normal uterine cervical samples. Although slight positivity was observed in the epithelium of the collecting duct in the kidney and gallbladder, no staining was observed in a wide variety of other normal and malignant tissues. Carbohydrate analysis determined that the epitope structure for this antibody is a mucin-type O-linked carbohydrate structure.

Conclusions: These findings demonstrate that this new human monoclonal antibody reacts selectively with müllerian duct-related carcinomas, including both endometrial adenocarcinoma and uterine cervical adenocarcinoma cells. More importantly, this antibody produced by TC mice is a human antibody, not a chimeric-human antibody. These characteristics implicate a possible future diagnostic as well as therapeutic role using this antibody.

Keywords: human monoclonal antibody, Trans-Chromosomic mouse, müllerian duct-related carcinoma

[17.05–17.25]

Tissue-array indicates specificity of B-CLL IgM. Preliminary results

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Background: Over the past decade, there has been considerable interest in and controversy about the mechanisms behind the monoclonal B cell expansion found in B-type chronic lymphocytic leukemia (B-CLL). In particular, two questions have been stressed: whether B-CLL cells have experienced antigenic stimulation and whether antigenic experience, if encountered, influences the development and diversification of these cells. Since the majority of B-CLL belongs to CD5+ B cell compartment, autoantigens, and in particular, T-independent autoantigens may play the main role in both processes. To gain insight into the potential role of B-CLL cell stimulation by autoantigens, we analyzed the specificity of IgM secreted by B-CLL cells.

Previous results: Rosén and his coworkers (Wendel-Hansen et al., 1994) obtained three immortalized lines of B-CLL cells transformed by Epstein-Barr virus (EBV): I83-E95 (IgM isotype and VH3-30 family, mutated), 232 B4-CLL (IgM,?) and WaC3, CD5+ (IgM,? and VH3-30 family, mutated).

Method development: Cells were expanded and cultivated in RPMI 1640 medium with 10% of fetal calf serum and antibiotics at 37°C in CO_2 incubator. Two cell lines, I83-E95 and WaC3, CD5+ secreted $\sim$ 500–1000 ng of IgM per ml of culture medium as determined by IgM ELISA. 232 B4-CLL cell line grew very rapidly, but did not secrete any IgM even after stimulation by EBV, PMA or PMA/Ionomycin. IgM was purified from culture media of I83-E95 and WaC3, CD5+ cell. IgM specificity was investigated by conventional autoantibody screening in immunofluorescence followed by tissue-microarrays (SuperBioChips Labs). IgM from TJ99D cell line supernatant, specific for the Po-protein/myelin (Kvanström M. et al., 2002), was used as positive control and culture medium from 232 B4-CLL cells was used as negative control.

To determine the IgM reactivity in the tissue microarray we used different slides: BA3 (24 species: 12 normal and 12 tumor specimems), BB4 (60 species of tumor tissue) and AA8 (60 species of normal tissue) were used. The treatment of slides was done according to a modification of the tissue-array method. Briefly: slides were de-parafinized and hydrated, immersed in boiling 0.002 M citrate pH 6.0, then left for 20 min at room temperature, quenched in 0.3% hydrogen peroxide and incubated with $\sim$ 15 ml of different supernatants or biotinylated IgMs. (Biotinylation was performed according to Pierce manufacturor's protocol; the efficiency of biotinylation was checked by ELISA using HRP-streptavidin). A monoclonal anti-μ HRP-conjugate (1:10,000) or HRP-streptavidin was used for detection followed by DAB substrate development. Slides were counter-stained in Meyer's hematoxylin, dehydrated and mounted in Permount.

Results: The IgM B-CLL antibodies were screened in immunofluorescence against a panel of autoantigens. IgM from I83-E95 showed strong reactivity against smooth muscle, the other clones did not reveal any specific pattern. The tissue arrays showed that I83-E95 and WaC3, CD5+ IgM in addition reacted with spleen and lymph node. The detailed epitope analysis is currently undertaken.

Conclusion: The use of tissue arrays is a powerful tool in analysis of the antigenic specificity of IgM derived form B-CLL clones. These results may cast new

light on the role of antigenic selection in the induction and expansion of leukemic clones.

[17.25–17.55]

The Use of Novel Fusion Proteins as Targeting Molecules in Pretarget® radioimmunotherapy (RIT)

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Pretarget® RIT is a 3-step therapeutic approach that has the potential to increase the dose of radionuclide delivered to tumor sites while limiting radiation exposure to normal tissues by delaying injection of the radionuclide moiety until after optimal targeting of tumor sites. We have recently examined the use of two novel genetically engineered fusion proteins as the targeting molecules. They were generated by construction of a single chain antibody to a single chain of streptavidin (SA), produced using an E. coli fermentation process. Stable tetramers are formed which are composed of 4 identical subunits, each containing a single chain antibody fragment genetically fused to a SA subunit. We have used an anti-CD20 fusion protein (B9E9, m.w. $=$ 173,688 D) for a Phase I lymphoma trial, and an anti-TAG72 fusion protein (CC49, m.w. $=$ 175,808 D for a Phase I adenocarcinoma trial, using the fusion proteins as the targeting molecule. This is followed by a synthetic biotin-N-acetylgalactosamine clearing agent (sCA) to clear unbound circulating fusion protein, and then a synthetic small molecule DOTA-Biotin, to which the radionuclides ^{90}Y and ^{111}In are chelated. We were particularly interested in the behavior of these novel components in patients. These dose optimization studies determined the optimal doses and schedule of administration of the 3 components.

Methods: Nine pts with TAG-72+ metastatic colorectal cancer and 15 pts with CD20+ B-cell NHL were enrolled in two studies. Patients received 160 or 320 mg/m^2 of the fusion proteins. The sCA was then administered IV either 48 or 72 hrs later, at a dose of 45 mg/m^2. ^{90}Y/^{111}In-DOTA-Biotin, was then administered IV 24 hrs after the sCA at a DOTA-Biotin dose of either 0.65 or 1.3 mg/m^2. All pts in the colorectal trial received 5 mCi of ^{111}In labeled DOTA-Biotin for imaging/dosimetry purposes and pts # 4-9 received 10 mCi/m^2 of ^{90}Y as well. Patients in the lymphoma trial received 5 mCi of ^{111}In labeled DOTA-Biotin for imaging/dosimetry purposes, and 15 mCi/m^2 of ^{90}Y.

Results: The mean serum $T_{\frac{1}{2}}$ for the CC49Fusion protein was 27 $\pm$ 5 hrs while the B9E9Fusion protein was 25 $\pm$ 5.8 hrs. Greater than 95% of the circulating fusion proteins were eliminated from the circulation within 6 hrs of sCA administration. Following radiolabeled DOTA-Biotin administration, rapid tumor localization occurred in the first 2 hrs with little or no normal tissue localization. Less than 10% of the injected dose remained in the circulation after 24 hrs (elimination occurring in the urine). The predicted marrow doses from circulating ^{90}Y-DOTA-Biotin (median 0.21 cGy/mCi in the CC49 study, and 0.25 cGy/mCi in the B9E9 study) were lower than the marrow doses delivered from directly radiolabeled antibodies. The mean tumor: whole body ratios were $>$ 40:1 in both studies, which compare favorably to other forms of RIT. No infusion-related toxicities were noted.

Conclusion: The two fusion protein targeting molecules have similar plasma pharmacokinetics with plasma half-lives shorter than most murine or recombinant monoclonal antibodies but long enough to get optimal tumor targeting in a 48 hour interval. The dosimetry estimates indicate that patients will tolerate higher doses of ^{90}Y (higher MTD than directly labeled antibodies) with resultant improved tumor radiation dose. These studies support further escalation of the ^{90}Y-DOTA-Biotin dose with both of these fusion proteins. Pretarget® RIT may represent a promising new therapeutic approach for pts with TAG-72+ or CD20+ malignancies.

Disclosure statement: This study was sponsored by NeoRx Corp. Hazel Breitz and Robert Sims are employees of NeoRx Corp. Albert LoBuglio is a consultant to and has received support for clinical trials from NeoRx Corporation.

Keywords: Radioimmunotherapy, pretarget therapy, Anti-TAG72 and anti-CD20 antibody