[CANCER RESEARCH 46, 2193-2196, April 1986]

Meeting Report

Fifth Annual Sapporo Cancer Seminar
Monoclonal Antibodies—Progress In Cancer Immunobiology and Clinical Application[1]

This meeting addressed primarily the impact of monoclonal antibodies on progress in cancer biology, as well as the diagnosis and therapy of human neoplasms. The organizing committee was chaired by A. Yachi (Sapporo, Japan) and consisted of M. I. Greene (Boston, MA), Y. Hashimoto (Sendai, Japan), K. Kikuchi (Sapporo, Japan), R. A. Reisfeld (La Jolla, CA), and T. Takahashi (Nagoya, Japan).

The meeting opened with a lecture by Reisfeld that dealt with the impact of monoclonal antibodies on the development of new approaches for monitoring and therapy of cancer. A most constructive advance has already been made in the biological analyses of human tumors that has led to the discovery of a variety of new tumor markers for monitoring cancer progression. It is apparent that monoclonal antibodies will be more likely to make their most decisive impact on cancer therapy by initiating the development of new therapeutic modalities. In this regard, monoclonal antibodies directed to chemically defined surface markers on human malignant melanoma cells, *i.e.*, proteoglycan and disialoganglioside GD_3, can "arm" effector cells to effectively destroy established human melanoma tumors in athymic (*nu/nu*) mice. Destruction of established human lung adenocarcinoma tumors also can be achieved in nude mice when a monoclonal antibody directed to a cell surface glycoprotein (p40[2]) is covalently conjugated with methotrexate and subsequently injected i.v. These animal experiments have led to currently ongoing clinical Phase I trials at Scripps Clinic, La Jolla, CA, that test this treatment modality in patients with lung adenocarcinoma. After briefly describing the use of a monoclonal antibody to G_{D2} in a serum assay to monitor recurrence of neuroblastoma tumors in children following remission, Reisfeld stressed what became a theme repeated by several other lecturers during this conference: multiple treatment modalities with multiple antibodies directed to different antigenic structures on human tumor cells are the most likely requirement to achieve a real impact on tumor destruction.

From several papers dealing with the merits of glycolipids and glycoproteins as tumor markers, it became apparent that there are several requirements for optimal reactivity of monoclonal antibodies with glycolipid antigens at the surface of tumor cells. These include density and consequent antigen clustering, concentration of coexisting glycoproteins that can affect crypticity of antigens with shorter carbohydrate chains, and length and composition of fatty acids on the ceramide chains. The most frequent tumor-associated glycolipid markers on the most commonly occurring cancers appear to be modified blood group related carbohydrates. Also, pure glycolipids are most effective as immunogens to clearly distinguish intricate differences in carbohydrate epitopes that define unique tumor-related antigenic structures, as discussed by B. Fenderson (Seattle, WA). In this regard, effective monoclonal antibodies to general human carcinoma carbohydrate markers were produced with synthetic immunogens, *i.e.*, synthetically prepared glycolipids and neoglycoproteins containing immunodominant T or Tn carbohydrates with either α or β linkages placed on a human serum albumin backbone. Such monoclonal antibodies detect in some cases differentiation antigens that signal premalignant conditions. An additional advantage of this approach is the relative abundance of the synthetic immunogens that can mimic an antibody response to large, highly complex mucin-like molecules that are most difficult to isolate in any quantity. B. M. Longenecker (Edmonton, Alberta, Canada) noted that monoclonal antibodies to such structures may prove most useful for new immunodiagnostic approaches, including tumor imaging. In a methylcholanthrene-induced rat fibrosarcoma system, tumor-associated glycosphingolipids were also found to be antigenic in a syngeneic rat system. Such antigen structures included globotriosyl- and lactoneotetraosylceramide; however, only naturally existing antibodies to the latter antigen were cytotoxic against cells of the immunizing cell line, according to M. Naiki (Sapporo, Japan).

Several presentations dealt with B-cell cancers and their immune responses. An autocrine mechanism exists in B-cell growth as indicated by a monoclonal antibody (B_1H_5) that recognizes receptors for a B-cell growth factor. The simultaneous expression of the genes for this factor and its receptors is thought to be responsible for immortalization of B-cells, T. Kishimoto (Osaka, Japan) observed. A whole battery of monoclonal antibodies was shown to distinguish a number of differentiation antigens selectively expressed on human B-cells. These include antigens on small resting B-cells, or on mitogen- and interleukin 2-activated B-cells. This battery of monoclonal antibodies appears useful for the classification and diagnosis of human B-cell cancers, noted Y. Ishii (Sapporo, Japan). A detailed molecular analysis of an idiotype from a patient with B-cell lymphoma whose tumor manifested resistance to the therapeutic effects of anti-idiotype antibody indicated that tumor cell populations arose that retained surface immunoglobulin but were completely unreactive with the anti-idiotype antibody. A study of the immunoglobulin genes in this patient's tumor indicated that these tumor cell subpopulations were derived from the same original clone of neoplastic B-cells. The idiotypic variables observed were found to be the result of somatic mutation, a fact that was clearly demonstrated by cloning and sequencing the immunoglobulin variable regions of individual B-cells obtained from pre- and posttherapy biopsies. It is considered possible that the antibody therapy may have exerted a strong selective force against tumor cells expressing the idiotypic determinant since all these mutations became apparent during a short time span and in the context of partial tumor response to the therapy. Data from these studies led to the conclusion that multiple anti-idiotype antibodies may be necessary to identify all cells of a malignant clone. Moreover, S. Levy (Stanford, CA) pointed out, treatment with more than one monoclonal antibody may be required to prevent escape from anti-idiotype therapy in some B-cell lymphoma patients. In further studies of lymphoid cancers dealing with autologous bone marrow transplantation, three monoclonal antibodies reactive with null type, acute lymphoblastic leukemia cells proved

Received 12/26/85; accepted 1/6/86.

[1] The Fifth Annual Sapporo Cancer Seminar was held in Sapporo, Japan, on July 11 to 13, 1985.

[2] The abbreviations used are: p40, M_r 40,000 protein (other proteins are similarly designated; PCNA, proliferating cell nuclear antigen; CEA, carcinoembryonic antigen; NCA, nonspecific cross-reacting antigen; Mab, monoclonal antibody; HMW-MAA, high molecular weight melanoma-associated antigen; AFP, α-fetoprotein; PAP, pokeweed antiviral protein.

useful for the detection of leukemic cells in the bone marrow of 3 of 5 patients. Treatment of these 3 patients' bone marrow with these antibodies and complement did result in good preservation of hematopoietic stem cells and prompt recovery of WBC and platelets. Two of these three patients remained in remission for 2 and 14 months, respectively, Y. Morishima (Nagoya, Japan) reported.

The combined use of complement and two antibodies with different specificities, *i.e.*, J-2 and J-5 (Calla), enhances *in vitro* elimination of tumor cells. Clinical studies of 33 patients with relapsed acute lymphocytic leukemia indicated that one-third remained in remission for several years, another one-third suffered from early relapse, while one-third of the patients died within 3 months after autologous bone marrow transplantation. Data from 8 patients who received autologous bone marrow transplants showed that the risk of graft *versus* host disease could be reduced considerably when mature T-cells are eliminated with anti-T12 monoclonal antibody and complement. After a period of weeks there was complete immunological reconstitution of mature T-cells, J. Ritz (Boston, MA) observed. Data presented on a PCNA indicated it to be a good antigenic marker for blast transformation in cancer. An autoantibody to PCNA that reacts with proliferating cells actually appears in 3% of patients with systemic lupus erythematosus, especially those in blast transformation. PCNA appears in the nucleolus during late G_1 and early S phase of the cell cycle and the autoantibody directed to it as well as a monoclonal antibody produced against it could be used successfully to detect blast crisis in patients with myeloid leukemia. PCNA, once purified ~1400-fold from calf thymus, was shown to be a single chain protein of M_r 36,000 by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and an immunoblotting enzyme-linked immunosorbent assay developed for rapid detection of autoantibody to PCNA in human serum showed a correlation with events associated with cell proliferation and blast transformation, according to R. M. Nakamura (La Jolla, CA).

A discussion of the microencapsulating technology used commercially for the large scale production of monoclonal antibodies revealed some interesting data on such reagents currently in use in therapy trials for B-cell lymphoma and lung carcinoma. In the first case, an IgM/K idiotype-secreting human × human hybridoma line (7D7-Gg) produced 0.9–1.2 mg idiotype IgM/ml of intracapsular fluid which in turn was purified >90% by anion exchange chromatography and then used to generate anti-idiotype secreting cell lines. Two such murine anti-idiotypic lines produced after 21 days in fermentation vessels from 12–20 g intracapsular antibody per 4.4-liter batch. The murine hybridoma cell line (KS 1/4) secreting an IgG2α monoclonal anti-lung carcinoma antibody produced after an 18-day culture 8 g of ~80% pure antibody per 4-liter batch. This antibody was purified to 98% by anion exchange chromatography. Both of these antibodies met all Food and Drug Administration requirements for pharmaceutical GNP preparations. The main advantage of this technique over the murine ascites approach for mass production of antibodies is the fact that one can produce hundreds of grams and even kilogram quantities of monoclonal antibody of high purity with the microencapsulation technology, a feat that is practically impossible to achieve with the "mouse ascites method," A. P. Jarvis (Needham Heights, MA) stated.

A session on the molecular analysis and clinical application of monoclonal antibodies revealed that an adenocarcinoma-associated antigen (YH 206), characterized with a monoclonal antibody, appears to be well expressed in neoplastic tissues of patients with adenocarcinomas of the lung, stomach, and pancreas. This antigen is found only sparsely in some normal tissues but is widely distributed in fetal tissues. The antigen epitope seems to be carbohydrate in nature since periodate oxidation of tissue sections abolishes reactivity with Mab YH206. The carbohydrate epitope may be partly cryptic because neuraminidase treatment of tissues unmasks additional reactive antigenic sites. The YH 206 antigen detected in the spent medium of human lung adenocarcinoma cells (A 549) has a molecular weight of >330,000 as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and "western blotting" and appears to be a useful marker for "monitoring" since passive hemagglutination assays of cancer patients' serum revealed a positive frequency of 45–73% in patients with lung and gastrointestinal cancer *versus* only 10 and 13% in normal individuals and patients with benign diseases, as noted by K. Imai (Sapporo, Japan). A massive and detailed analysis of the antigenic heterogeneity of CEA indicated that 120 hybridoma clones could be subdivided into 5 major reactivity groups. Based on changes in antigen reactivity after treatments with neuraminidase and periodate, epitopes recognized by antibodies of groups I, II, III, and V appear to be protein in nature whereas group IV antibodies recognize a carbohydrate epitope. The reactions of the sera of patients with colorectal cancer with antibodies of group IV further documented the intense antigenic heterogeneity of the CEA molecule as antigens of diverse molecular weights could be detected in sera and saliva, Y. Matsuoka (Fukuoka, Japan) noted. An additional focus was placed on the heterogeneity of the CEA molecule by a detailed molecular analysis of this and related antigens with monoclonal antibodies. Whereas monospecific, polyclonal immune sera had characterized CEA in the past as a glycoprotein of M_r 180,000 ± 20,000, monoclonal antibodies now indicate 2 molecular species with a molecular weight difference of ~20,000. The principal cross-reactive antigen of CEA, NCA, that polyclonal anti-CEA antisera indicated in the past to be a M_r 60,000 molecule has now been resolved by Mabs into 3 molecular forms, with molecular weights of 50,000 to 60,000, 75,000, and 100,000. Although CEA and NCA can be clearly distinguished from one another, the exact molecular relationship between CEA and its numerous related antigens as well as possible organ-specific differences can be elucidated only after gene cloning and DNA hybridization experiments. Ongoing studies in several laboratories have thus far not yielded an authentic CEA complementary DNA clone, S. von Kleist (Freiburg, Federal Republic of Germany) reported.

The discussion of a different topical area of work, namely radioimaging of metastases in patients with malignant melanoma, focused on a useful tumor marker, *i.e.*, HMW-MAA, with a restricted tissue distribution on only melanoma, nevi, and some skin carcinomas. Because of its high affinity, the antibody localizes quickly and can thus be used together with radioisotopes with short half-lives. When 42 patients with melanoma in stages III and IV were injected with $F(ab')_2$ fragments of the anti-HMW-MAA Mab, radioactivity (^{131}I, ^{123}I, ^{111}In, ^{99m}Tc) accumulated in metastases within minutes following injection, reacting maximally between 6 and 72 h. The accumulation of radioactivity in the metastatic lesions appears to be specific since there is a correlation between the results of immunostaining of lesions and immunoscintigraphy and also because distribution of the Mab in tumors and adjacent tissues is clearly distinct from that of ^{99m}Tc-pertechnate or radiolabeled antibodies directed to irrelevant antigens. A number of factors were discussed by S. Ferrone (Valhalla, NY) that distinctly

influence the outcome of immunoscintography such as size, anatomic site, degree and vascularization of lesions, accessibility of tumors to antibody, and level of expression of the "HMW-MAA" on melanoma cells. Several monoclonal antibodies directed to tumor-associated carbohydrate antigens are currently in use for the immunohistochemical and serological diagnosis of gastric cancers and breast cancer. One Mab (NCC-ST-439), reacting with a sialyl-sugar residue distinct from sialyl-Lewis^a or sialyl-Lewis^x, was found useful for the histological examination of bile duct cancers that are often highly differentiated and thus difficult to diagnose correctly. Also, a high molecular weight antigen (>1 × 10^6), detected by NCC-ST-439 in the serum of cancer patients, could be used in a solid phase inhibition assay to positively indicate 69% of gastric cancer and 58% of breast cancer cases with a low rate of false positives in patients with benign diseases, according to S. Hirohashi (Tokyo, Japan).

A session dealing with various therapeutic approaches to cancer using murine monoclonal antibodies was highlighted by a detailed analysis of results from clinical trials of anti-idiotype therapy for β-lymphocytic cancer. All but 1 of 11 patients treated received extensive prior treatment with conventional lymphoma therapy. Ten patients received only one antibody whereas one patient received 3 anti-idiotype antibodies concurrently. None of the patients showed detectable serum paraproteins. However, plasmapheresis had to be used in six patients to temporarily reduce serum idiotype protein levels that ranged from 1 to 200 μg/ml. Another problem ensuing from the presence of serum idiotype protein was the increased requirement for mouse antibody to achieve effective tumor penetration. The occurrence of an immune response to mouse immunoglobulin in 5 of 11 patients was considered a serious obstacle to therapy since further infusion of antibody failed to reach the tumor or to induce tumor regression. In fact, such additional antibody infusions in these specific cases were associated with toxicity. Although the one initial patient remains in remission 42 months after receiving the anti-idiotype treatment, 5 of 10 additional patients had objective remissions that were not complete and of relatively short duration. Five patients did not show any improvement. The cautious and objective analyses of these data led to the conclusion that although this type of therapy for B-cell cancers shows promise, it will require basic studies of the mechanism(s) responsible for the antitumor effects to significantly improve the clinical results (Levy).

Data from animal model systems designed to evaluate the utility of a murine monoclonal antibody (MB3.6; IgG3) directed to the disialoganglioside G_{D3} indicate that this abundantly expressed molecule on human melanoma cells serves as a relevant target antigen for antibody-mediated tumor cytolysis *in vitro* and *in vivo*. Either complement-dependent cytotoxicity or antibody-directed cellular cytotoxicity, both mediated by this IgG3 type antibody, caused cytolysis *in vitro*. A minimal expression of G_{D3} on the cell surface appears a requirement since neither of the two mechanisms was operative on a melanoma cell line that expressed only 13% G_{D3} by fluorescence-activated cell sorter analysis. Although i.v. injection of MB3.6 into nude mice 24 h after inoculation of human melanoma tumor cells could suppress tumor growth, established (7 days) tumors in such animals regressed dramatically only when a single i.v. injection of "armed effector cells," *i.e.*, 20 × 10^6 mouse splenocytes plus 400 μg MB3.6, was administered i.v. into tumor-bearing nude mice. Since MB3.6 can cause specific detachment of human melanoma cells from fibronectin in the absence of either effector cells or complement, D. A. Cheresh (La Jolla, CA) suggested that anti-G_{D3} antibodies are involved in melanoma-cell substratum and/or growth factor interactions that may be important in tumor metastasis. Continuing discussions focused on the effects of anti-G_{D3} antibodies on human melanoma tumor growth in clinical trials. Thus, some marked regression of metastases were observed when R_{24}, an IgG3 mouse monoclonal antibody to G_{D3}, was injected i.v. into 12 patients with metastatic melanoma at 3 dose levels: 8, 80, or 240 mg/m^2, over a period of 2 weeks. Peak antibody levels detected in the serum were dose related, ranging from <0.1 to 20 μg/ml. Patients treated at doses >80 mg/m^2 exhibited inflammatory reactions around tumor sites. Tumor biopsies taken during and after antibody treatment revealed infiltration of lymphocytes and mast cells as well as mast cell degranulation and complement deposition, suggesting possible complement-mediated inflammatory reactions. Most important, 3 of 12 patients treated showed major tumor regression. In an objective analyses of the data obtained thus far, a number of potential limitations of this type of treatment were pointed out. Nevertheless, A. N. Houghton (New York, NY) pointed out, these data are certainly most encouraging and suggest that treatment of some solid tumors with certain specific mouse monoclonal antibodies could be optimized to the point where such reagents may indeed become a major adjunct to tumor therapy.

Another theme of potential monoclonal antibody-mediated tumor therapy dealt with the effectiveness of antibody-drug conjugates. Specifically, 15–35 mol of daunomycin, Adriamycin, and mitomycin C were covalently coupled via intermediate carriers (dextran, poly-L-glutamic acid, serum albumin) per molecule of monoclonal and polyclonal antibodies directed to AFP. These conjugates exhibited cytotoxic activity *in vitro* and delayed development of AFP-producing tumors in rats or mice more effectively than drug or antibody alone or a conjugate of drug with normal immunoglobulin. In these trials, drug conjugates with either monoclonal or polyclonal anti-AFP antibodies proved equally effective, Y. Tsukada (Sapporo, Japan) reported. Further discussions focused on tumor therapy with monoclonal antibodies conjugated with such immunotoxins as PAP, examining particularly its effectiveness in catalytically inactivating mammalian ribosomes and thereby inhibiting protein synthesis of tumor cells. In this case PAP, a nonglycosylated, homologous single chain protein (M_r 30,000), when covalently linked via a disulfide bond by SPDP to a monoclonal anti T-cell antibody (31-E6), effectively inhibited the growth of murine T-cell leukemia cells that were injected s.c. into mice and followed shortly by an injection of immunotoxin. Whereas the divalent fragment of antibody 31-E6 alone failed to protect the mice against leukemia, the same fragment when coupled to PAP was able to protect these mice against death, indicating the protective effect of this immunotoxin, L. Houston (Emeryville, CA) stated. The discussion of new modalities for tumor therapy closed with the presentation of data on the characterization several anti-human tumor monoclonal antibodies and the presentation of potential strategies for their application to tumor therapy. These involved multilamellar liposomes as carriers, where subunits of a monoclonal IgM antibody directed to mouse mammary carcinoma were coupled to a liposome containing on its inside actinomycin D at a concentration of 1 μg/liposome. *In vivo* tests in a syngeneic mouse mammary carcinoma model indicated that such "chemoimmunoliposomes" could protect the mice against a challenge of 5 × 10^6 tumor cells when injected 1 day after tumor cell inoculation. The growth of established mammary carcinomas could at best be suppressed by ~50%. Problems, such as phagocyte trapping of

liposomes, were limited by using liposomes of neutral charge. Hashimoto emphasized that this approach, although potentially useful, still has to overcome such problems as adequate internalization into tumors, *versus* that into RES organs and sufficient number of antigenic sites on the tumor cell surface.

The final session of the Cancer Seminar dealt with virus and oncogenes and highlighted the use of monoclonal anti-receptor antibodies as specific vaccines and as probes for cell surface receptors. In the model system used, syngeneic monoclonal anti-idiotypic antibodies that recognize the mammalian receptor of reovirus type 3 were applied to isolate reovirus receptors by immunoprecipitation from panels of normal and transformed cells. These receptors appear identical on all cells analyzed thus far, exhibiting a molecular weight of 67,000 and a range of pI values from 5.8 to 6.0. Most strikingly, there is a structural homology between reovirus receptors and β-adrenergic receptors indicating that the latter may serve as reovirus binding proteins on the cell surface. Anti-idiotypic antibodies also precipitate β-adrenergic ligands bound to the cell surface. Syngeneic monoclonal anti-idiotypic antibodies were found to be useful as vaccines for the induction of anti-reovirus type 3 specific T-cell responses and for the production of anti-reovirus type 3 neutralizing antibodies. Although β-adrenergic receptors apparently comprise the primary cellular targets of reovirus type 3, it is not yet established whether the virus utilizes these receptors to gain entry into cells and to initiate the replicate cycle. Another question that needs to be answered, according to M. I. Greene (Philadelphia, PA), is whether reovirus binding acts on β receptors in an agonist or antagonist fashion.

Further discussion in this section focused on four monoclonal antibodies reactive with *ras* oncogene, specifically with $p21^{ras}$. The immunogen to produce these antibodies was derived from *Escherichia coli* expressing the *ras* gene product. Characteristically, all the monoclonal antibodies complexed $p21^{ras}$ in immunoblots, bound specifically to guanine nucleotides, and localized at the inner surface of membranes when assayed by immunofluorescence microscopy. There is an indication that Mabs to such oncogene products as $p21^{ras}$ may find some clinical application in cancer since these antibodies stained only malignant melanoma lesions by direct immunofluorescence but were nonreactive with benign nevi, N. Kuzumaki (Sapporo, Japan) reported. Finally, data were presented indicating that monoclonal antibodies to a product of the *neu* oncogene, *i.e.*, a M_r 185,000 cell surface protein (p185) encoded by it, can rapidly and reversibly down modulate cell surface and total p185 expression of NIH 3T3 cells transformed with *neu* oncogene. Actually, the monoclonal antibody to p185 causes the *neu*-transformed cells to revert to a nontransformed phenotype, as indicated by anchorage-independent growth. Greene remarked that cross-linking experiments with monovalent fragments of anti-p185 antibody indicate that aggregation and cross-linking of p185 is important in both its modulation and the reversion of the transformed phenotype.

It became abundantly clear from the data presented and from ensuing discussions during this Cancer Seminar, that murine monoclonal antibodies constitute most effective probes for the molecular dissection of complex biological processes involved in human cancer. It is also clear that these reagents have produced some promising results and that they may eventually become highly useful as adjuncts for cancer diagnosis and therapy. Obviously, much more basic and clinical research is required to fully realize this goal in the near future.

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