[CANCER RESEARCH 43, 6109–6112, December 1983]

Meeting Report

Third Annual Sapporo Cancer Seminar[1]

Biological Responses in Cancer Chemotherapy

The third annual Sapporo Cancer Seminar focused primarily on the immunomodulating effects of anticancer agents. The Organizing Committee was chaired by Y. Sakurai (Toyko, Japan) and consisted of A. Fefer (Seattle, Wash.), H. Kobayashi (Sapporo, Japan), E. Mihich (Buffalo, N. Y.), and T. Wada (Sapporo, Japan).

The seminar opened with 2 major lectures, one by H. Umezawa (Tokyo, Japan) and one by A. Fefer, which dealt with screening for new immunomodulating agents and with approaches in adoptive chemoimmunotherapy, respectively. As discussed by Umezawa, success has been achieved in identifying low-molecular-weight immunomodifiers produced by microorganisms through the use of primary screening of bacterial products against a panel of enzymes known to be located at the cell plasma membrane. Several of the compounds found active on aminopeptidases, phosphatases, or esterases were also found in subsequent, more detailed studies to affect immune functions; in most cases, they augmented the responses through stimulation of macrophage cytotoxic functions or inhibition of suppressor cell functions. Bacterial products such as Bestatin, arphanemine, amastatin, and forphenicine are examples of immunoaugmenting agents initially selected by the enzyme screen. It became apparent that this screen selected biologically active agents and had the propensity to identify potentially immunomodulating compounds, perhaps related to the membrane location of the enzymes used, but, despite this, there was no evidence that the activity of the agents on the immune system was mechanistically or directly related to the enzyme inhibitions seen. It also became evident, as subsequently stressed with many other examples throughout the workshop, that an agent active on the immune system may have a multiplicity of effects and that it is important, but often difficult, to relate specific functional changes to the overall immunomodulating actions of a compound.

While the lecture by Umezawa stressed the continuing need to develop more effective new agents, that by Fefer emphasized the opportunities offered by treatments based on the adoptive transfer of lymphoid cells. The emphasis was on the use of immune T-cells in conjunction with other types of antitumor treatments which could reduce tumor burden and, possibly, related suppressor factors. The modalities of adoptive transfer of cells and related treatments have been greatly improved based on the knowledge acquired on the functions and interactions of T-cells, of related lymphokines, and of the usage of chemotherapeutic agents. Using murine leukemia models, it was demonstrated that appropriate treatments with CY[2] plus the transfer of immune syngeneic lymphocytes exert curative effects not achievable with either alone. A number of important principles were derived from studies with the murine model which may eventually be applicable to studies in humans. For example, it was shown that the functions transferred are immunospecific; that, following transfer, a few weeks are required for the antitumor effects to be obtained with cells immunized *in vivo*; that the effectors are X-ray sensitive; that the cells transferred are not cytotoxic *in vitro*; and that they do not have the CTL phenotype but rather the Lyt 1^+ phenotype characteristic of the T-helper or -amplifier cells. If secondary *in vitro* sensitization is carried out prior to cell transfer, the response in recipients is augmented, but culture-expanded T-suppressor cells may exert antagonistic effects; the nature of the effector cells involved is not yet clear in this case. T-cells from immune mice maintained in long-term culture in the presence of IL2 are also effective upon transfer to leukemic mice, but the mechanism of this action seems different from that of noncultured cells. In fact, these cells have CTL phenotype characteristics and, in contrast to the noncultured cells, their action in recipient hosts is augmented and prolonged as a result of the administration of IL2; indeed, these cells might also be effective against weakly immunogenic tumors. This information indicates not only that the functions involved and the kinetics of development of the adoptive responses are different, depending on the pattern of development of the immunized cells to be transferred, but also that, under certain circumstances, the effectiveness of adoptive cell transfers may be augmented by the administration of lymphokines to recipients. The effectiveness of allogeneic cell transfer was also discussed, and an expectation was expressed that this approach may become useful after an appropriate solution to the additional problems posed was found.

Major consideration in the seminar was given to studies of the interactions between tumor, host defenses, and anticancer drugs, with particular emphasis on interferences with suppressor functions and augmentation of effector functions. The development of suppressor function in the spleen of C3H/He mice, consequent to the growth of methylcholanthrene-induced fibrosarcoma and related to cells of the monocyte-macrophage type, was discussed by N. Ishida (Sendai, Japan). He reported on the presence in serum of an immunosuppressive acidic protein which correlates with the growth of the tumor and the evidence of suppressor function. This suppressor function was reduced by indomethacin, consistent with a role of macrophage-derived prostaglandins in inhibiting T-cell effector functions.

As discussed by H. T. Wepsic (Chicago, Ill.), immunization with BCG cell walls enhanced the growth of syngeneic or allogeneic tumors in inbred ACI rats through the induction of suppressor functions of cells of the monocytic-macrophage type. Also in this model system, indomethacin reduced the enhancement of tumor growth and the suppression of Con-A mitogenesis, as did treatments with busulfan or mitomycin C given 5 days prior to BCG treatment. The same patterns of reversal by mito-

[1] The Third Annual Sapporo Cancer Seminar was held in Sapporo, Japan, July 14 to 16, 1983, under the sponsorship of the Cancer Seminar Foundation. The chairman of the meeting was T. Yamazaki (Sapporo, Japan).

[2] The abbreviations used are: CY, cyclophosphamide; IL2, Interleukin 2; TAM, tumor-associated macrophages; N-CWS, *Nocardia rubra* cell wall skeleton; BMT, bone marrow transplantation.

Received August 23, 1983; accepted September 8, 1983.

mycin C of suppressor-induced enhancement of tumor growth were noted in animals bearing Morris hepatoma 3924a.

The effects of certain drugs on immunological mechanisms in guinea pigs and mice were reviewed by J. L. Turk (London, England). It is apparent that CY and other agents, such as cyclosporin A or bleomycin, can alter the balance of effector to suppressor function depending on the relationships between time and dose of drug and the status and type of immunogical response studied. For instance, CY, when given before a variety of antigens, augments cell-mediated immune responses; when given at the time of immunization, it also reverses immunological tolerance and blocks antigenic competition. It can also allow the development of a latent antibody response to a carrier protein coated with a maximum number of haptens while, under different conditions, it inhibits antibody response and also abolishes immunological memory. Both T- and non-T-suppressor cells can be affected by CY. The hypothesis discussed was that, essentially, the drug is active on rapidly proliferating cells, and its effects on immunomodulation seem to depend in part on which type of cell has a rapid rate of turnover at the time the system is exposed to the drug. While a basically antiproliferative mechanism may be primarily involved in the action of CY, other mechanisms may play a role in that of other agents. For instance, bleomycin caused greater augmentation of immune responses against Meth A tumors in mice when given on Day $^{+}2$ than on Day 0 with respect to tumor implantation, consistent with the possibility that stimulatory effects similar to those reported by Mihich for Adriamycin, rather than an antisuppressor action, may be responsible for the effects seen. Again, the concept of multiplicity of drug effects was supported by the data discussed.

The therapeutic relevance of immunomodulation by CY was discussed by S. Dray (Chicago, Ill.). In BALB/c mice bearing MOPC-315 plasmacytomas, a low dose of CY (15 mg/kg) was curative if treatment was given at Day 10 to Day 16 after tumor inoculation, but not if given on Day 4. Time after tumor transplantation is needed for the effect to be seen and is apparently related to the requirement that a T-effector cell response be developed or under development at the time of CY administration. The therapeutic effect seems related to a drug-induced decrease of suppressor cells of the monocyte-macrophage type. Mice cured with low-dose CY, but not those cured with high-dose CY (200 mg/kg), were resistant to further tumor challenge; consistent with this, the adoptive transfer of cells from mice treated with low-dose CY had antitumor effects in contrast to the lack of effect of cells from mice treated with the high dose. Studies with melphalan indicated that this agent had effects analogous to those of CY.

Based on the availability of 4-hydroperoxycyclophosphamide, a chemically synthesized precursor from which 4-hydroxycyclophosphamide (an active form of CY) is spontaneously formed in aqueous solution, and of monoclonal antibodies to T-cell subsets, *in vitro* functional assays were set up by H. Ozer (Buffalo, N. Y.) to characterize the differentiation and regulatory interactions of human T-cells in mediating both humoral and cellular immune responses. It was found that CY selectively affects T-cell subsets within the OKT4 phenotype and specifically distinguishes inducer and suppressor cells regulating polyclonal B-cell differentiation. It appears that, in this system, CY blocks the differentiation of suppressor-precusor cells while sparing differentiated suppressor or cytotoxic cells. The data are consistent with the suggestion that low doses of CY block T-cell suppression by interfering with the $OKT4^{+}8^{-}$ inducer T-subset, regulating the differentiation of suppressor and cytotoxic precursors to mature effector cells.

In WKA rats given implantations of KMT-17 syngeneic tumor, F. Sendo (Yamagata, Japan) and M. Hosokawa (Sapporo, Japan) made essentially consistent observations using busulfan and bleomycin, respectively. Treatment with the drug prior to tumor implantation caused a time-dependent augmentation of the immune response to tumor, which was T-cell mediated and appeared related to an inhibition of suppressor cells or their precursors. In both cases, the drug-related augmentation of the antitumor response could apparently be reduced after the adoptive transfer of thymus cells from tumor-bearing rats not treated with the drug but not after transfer of cells from drug-treated donors; the mechanism and significance of this unexpected but consistent finding remain to be determined. In the same system, cooperative interactions were noted between drug treatment and immunization with X-irradiated tumor cells, these also being reduced by the adoptive transfer of thymic cells. With busulfan, similar phenomena were also observed in mice given implantations of Meth A tumor.

The immunomodulating effects of Aclacinomycin, oxanosine, and anthramycin-type antitumor antibiotics were discussed by M. Ishizuka (Tokyo, Japan), and those of Adriamycin were discussed by Mihich. Aclacinomycin was found to augment DTH and anti-SRBC antibody production. Suppressor cell functions, postulated to be macrophage-associated, were reduced by this antibiotic both in the anti-SRBC antibody and a tumor system. Oxanosine also prevented the generation of suppressor functions for DTH and appeared to activate both macrophages and T-cell effectors in tumor-bearing mice, with maximum effects being seen when treatment was given 6 to 12 days after tumor implantation. Neothramycin and mazethramycin augmented anti-SRBC antibody formation *in vivo* and *in vitro* and activated macrophages as indicated by phagocytosis and superoxide anion production; inhibition of S-180 tumor was maximal when the antibiotic was given 5 days before tumor implantation. Adriamycin was found to augment or inhibit humoral and cellular responses of C57BL/6 mice against allogeneic P815 tumor, depending on time of administration and response characteristics. The augmenting effects on cellular responses appeared to be related to augmented differentiation of nonadherent silica-insensitive macrophage precursors and also to inhibition of an adherent down-regulating T-cell; increased IL2 production may also play a role in the augmenting effects of this antibiotic. Inhibition of NK cell activity appeared related to increased production of prostaglandins by macrophages and was reduced by indomethacin.

The augmentation of therapeutic responses against leukemia L1210 by 6-mercaptopurine in conjunction with concanavalin A-bound L1210 cells used in vaccination was discussed by T. Kataoka (Tokyo, Japan). The augmenting effects of 6-mercaptopurine were optimal upon delayed treatment after tumor implantation, and they were shown to correlate with increases in antitumor T-cells in spleen and peritoneal cavity and with a decrease in peritoneal cells with suppressor function. These suppressor cells were silica sensitive; were resistant to anti-Lyt-1,2 or X-irradiation; were adherent to plastic; were Ia-negative; and were sensitive to 6-mercaptopurine. Such effects were not obtained with CY or 5-fluorouracil.

The immunosuppressive potential of a drug like 5-fluorouracil

was noted by H. Ishitsuka (Kamakura, Japan), who indicated the possibility of preventing such effects with thymosin-α_1, both when immunosuppression was reflected in the onset of lethal opportunistic infections and when it was instrumental in increasing tumor metastasis. The intriguing observation was reported that the preventive action of thymosin-α_1 could be transferred to immunosuppressed recipients by spleen cells deprived of T-cells but not by those deprived of NK cells.

The interactions between certain drugs and macrophages or polymorphonuclear leukocytes in affecting tumor cells were discussed by M. Yamazaki (Kanagawa, Japan). It was found with a MM46 tumor in the C3H/He mouse system that tumor cell cytolysis *in vitro* by polymorphonuclear leukocytes was dependent on the concurrent presence of a drug like actinomycin D, this drug-polymorphonuclear leukocyte interaction being of somewhat general significance as it appeared to occur with several agents. Other compounds, such as vincristine, augmented PE cell cytotoxicity against the syngeneic tumor studied, consistent with reports by others on the activation of cells of the monocyte-macrophage type by several anticancer agents.

The importance of assessing the diversity of functions of TAMs at different sites of tumor growth was emphasized by A. Mantovani (Milan, Italy). It was found that hydrocortisone reduced the level of TAM and growth of primary tumors (e.g., of MFS6 sarcoma, 3LL Lewis lung carcinoma, and M109 ovarian tumor) but augmented their metastasis. The antitumor effects were T- and NK-cell-independent and also were unrelated to the expression of high-affinity binding sites for glucocorticoids in the tumor cells. The possibility was discussed that TAM may exert tumor-favoring effects other than those related to host-defense functions. In poorly immunogenic tumors, however, TAMs are at low levels, and their tumor growth-promoting function may have reduced significance.

In concluding the part of the seminar that was specifically focused on immunomodulation by anticancer drugs, F. Spreafico (Milan, Italy) stressed the fact that different anticancer agents, even those having putatively similar modes of action, may differ widely in their interactions with cells of the immune system. Conversely, immune cells of the same lineage, or immune cells of the same type residing in different locations, may also vary widely in their responses to the same drug. An awareness of this heterogeneity is essential in interpreting the validity of the models used, the effects observed, and the generality of their significance.

Two reports were concerned with the value of local immunotherapy with N-CWS given, in combination with mitomycin C, to rats (T. Ogura, Osaka, Japan) or, alone, to humans (K. Yasumoto, Fukuoka, Japan). Antagonistic relationships between N-CWS and drug were noted to occur *in vitro*. After i.p. treatment of rats, however, drug and N-CWS exhibited marked synergism in causing increases in number of tumoricidal, adherent, phagocytic, and carageenan-sensitive peritoneal cells; tumoricidal macrophages were also stimulated by mitomycin C alone.

In 190 patients with lung cancer, a randomized trial of intrapleural N-CWS followed by intradermal N-CWS was performed between 1977 and 1981; survival, remission duration, and rate of recurrence were measured. Patients were divided into operable and nonoperable groups, and recurrence was classified as local *versus* distant metastasis in the resection groups. In the operable patients treated with N-CWS, increased survival rate and remission duration with decreased recurrence rate were noted. For instance, rates of distant recurrence were 34.1 and 17.6%, respectively, in untreated and N-CWS groups; corresponding rates of local recurrence were 13.6 and 0%. No serious side effects were noted.

The seminar ended with a review by Fefer of the current state of the art and achievements of BMT in the treatment of hematological cancers in conjunction with radiochemotherapy. In summary, more than 100 patients were treated with supralethal chemoradiotherapy and BMT from a normal, genetically identical twin: 25 to 50% of patients with acute leukemia or lymphoma were apparently cured; 12 of 12 patients with chronic granulocytic leukemia had a complete remission with disappearance of the Ph^1 chromosome, and 10 were alive, with 8 in complete remission, 3 to 7 years after BMT. The major problem in these groups of patients was neoplastic recurrence. With BMT from HLA-matched siblings, about 15% of patients with refractory acute leukemia were apparently cured, 25 to 70% of patients with acute nonlymphocytic leukemia given BMT in first complete remission were apparently cured, and 60 to 70% of patients with chronic granulocytic leukemia had long-term tumor-free survival. The advantage of performing BMT in patients with minimum residual disease was stressed. Major problems still to be overcome are leukemic relapse, idiopathic or cytomegalovirus pneumonia, acute or chronic graft *versus* host disease, and venocclusive disease of the liver.

The examples of immunomodulation by anticancer drugs discussed at the seminar allow several conclusions to be tentatively reached. It became apparant that different types of anticancer agents, classifiable as alkylating agents, antibiotics, and antimetabolites, may exert immunomodulating actions which are expressed in augmentation and/or inhibition of different responses or response components, depending on the model system used and the relationships between pharmacological and immunological characteristics of the interacting factors involved. The data discussed pointed toward suppressor function, either macrophage or T-cell related, as a frequent target of the action of antitumor agents, the modification of which could result in a therapeutically favorable imbalance of the immune system. An additional suggestion was that anticancer agents may interact cooperatively, sometimes synergistically, with the therapeutic effects of active or adoptive immunotherapy, particularly through a reduction of suppressor functions. The possibility of dissecting the immune system with a drug, even within the same serological specification of cell subsets, was substantiated and should be emphasized. The multiplicity of drug effects and the heterogeneity of the immune system with respect to sensitivity to drugs were apparent throughout the seminar and underscored the opportunities for selective interventions with drugs, but also emphasized the difficulties in attributing generalized significance to a phenomenon based on findings, the validity of which may be restricted to very specific models and/or conditions. Despite these difficulties, it became apparent that immunomodulation by anticancer drugs is likely to provide profitable leads toward the therapeutic exploitation of agents heretofore used primarily only for their antiproliferative and cytotoxic actions.

While this seminar put into focus this potentially useful aspect of cancer chemotherapy, it also brought up several additional questions. For instance, it should be possible to increase the production of lymphokines by the use of drugs and, thus, to modify favorably the antitumor host response. It should be possible to augment the antitumor responses of the host by

affecting T-helper other accessory cells. The immunomodulation by anticancer drugs may be primarily due to elimination of cell types and functions or also due in some cases to augmented differentiation of precursors of accessory or effector cells. Advantages may be obtained by combining drug-induced immunomodulation with active immunotherapy or the adoptive transfer of immune cells. An effective clinical implementation of immunotherapy and immunomodulation seems to be dependent on a knowledge of the factors specifying immunoregulation in humans; it is not clear to what extent *in vitro* or *ex vivo* models with human cells would provide valid information on the status of the immune response and its regulation. Some of the questions raised during this interesting seminar clearly deserve further investigation toward the optimal exploitation of this novel area of cancer therapeutics.

E. Mihich[3]
Roswell Park Memorial Institute
Buffalo, New York 14263

Y. Sakurai
Japanese Foundation for Cancer Research
Tokyo, Japan

H. Kobayashi
Hokkaido University School of Medicine
Sapporo, Japan

[3] To whom requests for reprints should be addressed.