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Immunomodulation of Neoplasia

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Cover illustration

Photomicrograph of normal peripheral blood (small cells) which form conjugates with the human NC tumor target BT-20 (human breast carcinoma). These cells are stained with Wrights Giemsa and show intimate association between the tumor and PBL. Photomicrograph provided through the courtesy of Drs. Antonella Stoppacciaro and Edmund C. Lattime, Laboratory of Cellular Immunology, Sloan-Kettering Cancer Center, New York, NY, USA.

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Cellular and Cytokine Immunotherapy of Cancer

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Paul Ehrlich [1] in 1909 recognized that an intact immune system was a requisite for maintenance of host resistance against neoplasia. This concept was restated as immunological surveillance by Lewis Thomas [2] in 1959 and further refined by Sir McFarlane Burnet [3] in 1970.

In 1953, Foley induced resistance in C3H/He mice to rechallenge with viable cells of the same chemically-induced tumor whose first or second transplant generation regressed following strangulation of the neoplasm in these same hosts [4, 5]. This demonstration of tumor graft rejection in inbred mice was followed in 1957 by Prehn's discovery of tumor-specific transplantation rejection in autochthonous immunized host mice [6].

The immune surveillance theory postulated that T lymphocytes recognize and monitor antigenic changes accompanying malignant transformation and eliminate transformed cells. Although a considerable amount of evidence supports immune surveillance, other data place its validity in doubt [7, 8]. Supporting evidence includes an increased incidence of virus-induced and chemically induced tumors in animals that have been neonatally thymectomized or treated with antilymphocytic serum or subjected to whole body irradiation and by the increased cancer incidence in individuals with suppressed immune reactivity. Although athymic nude mice, without functional T cells, support the growth of allogeneic or syngeneic tumors, they do not have an increased frequency of spontaneous tumors or show any special susceptibility to carcinogenic chemicals. Thus, the absence of T cells does not appear related to an increased tumor incidence. Nude mice appear to have a full complement of active natural killer (NK) cells, whose role in immune

surveillance is clouded by the failure of NK-deficient *beige* mice to show a increased susceptibility to chemically induced neoplasia. Yet another postu late suggests that the surveillance function delaying tumor developmer lacks immunologic specificity and depends not on lymphocytes but upo effector mechanisms that recognize the tumor phenotype [8].

Mitchison [9] demonstrated that immunity against tumor allograf could be transferred adoptively with lymphocytes. Immunity against syn geneic tumors was shown subsequently to be mediated through this mecha nism. Passive transfer is effective only when performed relatively early i hosts already challenged with tumor cells. Antitumor reactivity of immun lymphocytes is dependent principally upon T cytotoxic (Tc) cells that recog nize tumor-associated antigens in conjunction with major histocompatibilit complex (MHC) antigens in cell membranes.

With the emergence of the nature and structure of lymphoid cell recep tors capable of interacting with epitopes that include those on the surface of tumor cells, there has been a rapid accumulation of knowledge about th molecular products of lymphocyte activation. These messenger molecul termed cytokines or lymphokines include a variety of substances rangin from interferons (IFN) to the interleukins [10, 11].

Although much attention has been directed in the recent past to th interferons in cancer treatment, interleukin 2 (IL-2), the T cell growth facto is a focus of current investigation in tumor immunotherapy [12]. Cells recog nized to participate in immune reactivity against selected experimental tu mors include different classes of T, e.g. Tc (cytotoxic), and B lymphocyte NK cells, natural cytotoxic (NC) cells, macrophages, and lymphokine-act vated killer (LAK) cells generated by culture of host splenic or peripher blood lymphocytes with IL-2 [13, 14].

Experimental manipulation of the tumor-bearing host's reactivit against neoplasia has been investigated not only by the administration lymphokines alone, such as IL-2, but also by the in vitro treatment of tumo reactive host lymphoid cells with immunoregulatory molecules. Early tria employing IL-2 alone or in combination with adoptively transferred lym phoid cells have produced some remarkable antitumor effects but excessiv doses of IL-2 have been accompanied by systemic toxicity [13].

Investigations to elucidate the nature of these novel and innovativ developments in tumor immunity through immunomodulation constitu the subject matter of the present volume.

Successful resistance against the development of neoplasia may depen upon a variety of host immunologic and other factors. Tumors differ wi

espect to the type of host immune mechanisms required for their elimina-on. Cytotoxic T cell reactivity is classically MHC-restricted and represents n aspect of cell-specific immunity. By contrast, NK cells are non-MHC estricted. Whereas, immunization may increase T cell immunity, it does not acilitate NK cell function. Killer or K cells may react against tumor targets hrough binding of their surface Fc receptors with the Fc region of IgG anti-ody molecules bound through their Fab regions to epitopes on tumor cell urfaces. Thus, multiple mechanisms may be associated with successful mmunologic reactivity against tumors in the animal host [7, 15].

In their elegant description of host-tumor interactions in immune sur-eillance against cancer, Urban and Schreiber discuss UV light-induced neo-lasms in mice that are normally rejected by syngeneic hosts based on their igh antigenicity. Nevertheless, variants resulting from immune selection nd genetic instability of tumor cells may escape and continue to grow in otherwise healthy animals. The authors used this system to assay host mmune responsiveness to tumors and the tumor's response to host immune nechanisms.

Cytotoxic T lymphocytes (CTL) and activated macrophages were found o be effector cells capable of tumoricidal activity in the rejection of UV-nduced neoplasms. By contrast, NK cells were apparently unnecessary and neffective against this type of tumor. Observations that selection for resis-ance to macrophages and regressor-specific T lymphocytes support the con-cept that these particular cell types are critical for host tumor resistance. Tumor necrosis factor (TNF) was found to be a principal mediator of tumor cell injury mediated by macrophages. Suppression of T lymphocyte immuni-y observed in animals exposed to UV radiation or aging animals or tumor-bearing mice permits progression of tumor development. UV-induced immunosuppression is not tumor specific. It can be transferred passively by Lyt-2$^-$ T lymphocytes but has no effect on allogeneic or other forms of humoral immune reactivity. UV diminishes antitumor reactivity by nonim-mune lymphoid cells but is ineffective in suppressing specific antitumor reac-tivity induced by immune lymphoid cells.

They employed the 1591 tumor and cloned cytolytic T cell lines to inves-tigate reactivity of cytotoxic T lymphocytes for tumor-specific surface epi-topes. They identify tumor sites recognized by macrophages as well as an antigen reactive with helper T lymphocytes. Antigenic deletion of surface epitopes occurred in association with progression of tumor development. The first antigenic determinant to be deleted was that recognized by macrophages followed by the A antigen identified by T lymphocytes followed by the loss

of B antigen. By contrast, the epitope reactive with helper T lymphocytes was not deleted with selection and appeared even on metastatic variants of the 1591 tumor.

The authors point out that hierarchy in immune reactivity results in specific immunity only against the dominant tumor antigen which enables escape from immune reactivity by variant forms that have lost the dominant antigen. However, this does not prevent the induction of immunity against numerous other antigens on the tumor cell. Urban and Schreiber conclude that immunotherapy aimed at numerous separate epitopes on a tumor cell might be employed to immunize the host against progressive growth of tumor cell variants.

Fujiwara and Hamaoka discuss their novel approaches to the enhancement of cellular mechanisms of tumor-specific immunotherapy. Malignant transformation of cells may be associated with the appearance of tumor-associated antigens (TAA) or tumor-associated transplantation antigens (TATA), either of which may serve as targets of specific antibody or specific cellular immune responsiveness mounted by the host. Whereas, these neoantigens appear on chemically induced tumors in experimental animals, it is unknown whether or not they occur on all types of tumors. Even those neoplasms bearing TATA may fail to halt tumor progression because of weak immunogenicity of neoantigens. The authors' experiments were aimed at enhancing host immune responsiveness to tumors, investigating the types of immune reactivity induced by TATA and the effector mechanisms responsible for tumor cell injury and death. They investigated the mechanisms whereby tumor-specific Lyt-1^+2^- ($L3T4^+$) T lymphocytes recognize tumor antigens. Antigen-presenting cell (APC) cooperation is required for these T cells to recognize tumor antigens. The APC process tumor antigens and present them in conjunction with self-class II MHC molecules. Tumor-specific $L3T4^+$ T lymphocytes activate nonspecific effector cells leading to tumor cell injury as well as CTL precursors with tumor-specific reactivity. The authors suggest that these particular T cells' significance in antitumor immunity could be associated with their ability to produce substances requisite for activation of CTL, macrophages, LAK and B lymphocytes, all of which play a role in antitumor immunity.

Mechanisms of cellular interaction associated with tumor-specific immunotherapy were investigated employing models of Lyt-1^+2^- ($L3T4^+$) T lymphocyte reactivity against neoplasia. Tumor cells coupled with exogenous antigens were used as an immunogen to increase tumor-specific protective immunity in hosts previously primed with these antigens.

Tumor immunity was enhanced by immunization of hosts sensitized to BCG with tumor cells coupled to muramyl dipeptide (MDP) haptens. Tumor cell-MDP hapten conjugates were successfully used to inhibit tumor cell growth in a solid tumor mass or metastases in BCG-sensitized hosts. The authors cite their experimental results in support of a theoretical basis for enhancing tumor-specific immunity.

Greenberg and co-workers describe their innovative approach to therapy of disseminated tumors by adoptive transfer of specifically immune T lymphocytes into tumor-bearing hosts. Evidence continues to accumulate that human tumor cells may bear epitopes capable of stimulating the host immune response. Such TAA may be reactive with antibodies as well as with tumor-specific T lymphocytes. Although there are isolated reports of spontaneous regression of selected tumors or of their metastases, most neoplasms grow unabated in hosts with intact immune systems. Rather than indicating that a tumor is nonimmunogenic, progressive growth may be attributable to the inability of the immune response to keep pace with tumor cell proliferation resulting in a neoplastic mass too large to be eliminated by immune system cells alone. As the size of the neoplasm increases, the immune response may actually be suppressed. Tumor immunity is brief in animals carrying immunogenic tumors and may not be apparent if assayed after the tumor has become advanced. Tumor-specific T lymphocytes have been demonstrated in patients with many types of tumor, as numerous human neoplasms are capable of inducing T lymphocyte responsiveness.

Attempts to facilitate host immune responsiveness to tumors include the selective depletion of potential suppressor cells in vivo, sensitization to tumor antigens concentrated in a matrix that increases their immunogenicity compared to the tumor cells from which they were derived, and treatment of the host with immunologic adjuvants including purified cytokines.

Adoptive immunotherapy permits the infusion of effector T lymphocytes derived by expansion of the few tumor-reactive T cells isolated from the host by means of culture technology. The authors review their studies on the adoptive therapy of disseminated leukemias and lymphomas in mice. Mice with disseminated syngeneic tumors received donor lymphocytes from syngeneic mice specifically immunized against the tumor. This work demonstrates the possibilities that might be achieved by manipulation of autologous lymphocytes isolated from tumor-bearing hosts. Their investigations have centered around recognition and injury of tumor cells by the transferred lymphocytes and have led the authors to conclude that the infusion of great numbers of effector T cells into tumor-bearing animals may not result in swift

and total elimination of tumor cells. They review problems which require modification of the host-tumor relationship prior to adoptive immunotherapy in order to permit tumor immunity to be manifested after cell transfer.

Surgical reduction of a tumor mass may diminish suppressor T lymphocyte reactivity that was induced by the expanding tumor mass and led to diminished tumor immunity. Thus, alleviation of this suppressor mechanism could facilitate tumor immunity by adoptively transferred immune lymphoid cells.

Adoptive chemoimmunotherapy (ACIT) represents a modification of their basic technique and includes the treatment of mice with advanced tumors with cyclophosphamide (CY) prior to the adoptive transfer of immune T lymphocytes. CY facilitates expression of tumor immunity by abolishing T suppressor cells induced by the tumor and those in the host which would inhibit the immune reactivity of adoptively transferred T lymphocytes. The drug is also tumoricidal against tumor cells.

Investigating subpopulations of donor T lymphocytes mediating the therapeutic effect in adoptive immunotherapy, the authors demonstrated that transferred $L3T4^+$ T lymphocytes facilitated total eradication of disseminated leukemia in mice. This pointed to the significance of T cell-dependent mechanisms, exclusive of direct lysis by CTL, for the elimination of tumor cells. The $L3T4^+$ $Lyt2^-$ T cell subset recognizes antigens only in association with class II MHC antigens and includes helper T lymphocytes. $L3T4^+$ cells did not produce a cytolytic effect on target cells. However, CTL which produce significant therapeutic effects in several tumor models, could not be induced in culture in the absence of $L3T4^+$ T lymphocytes.

Besides direct cytolytic effects by adoptively transferred T cells, immune T lymphocytes may secrete lymphokines leading to activation of various effector cells, such as B lymphocytes that synthesize antibody that may lyse tumor cells directly or act through antibody-dependent cell-mediated cytotoxicity (ADCC). Lymphokines may also activate NK cells and stimulate LAK cells or induce macrophages to become tumoricidal.

Individual tumor antigens may selectively activate only one T lymphocyte subset that will be therapeutically effective against the tumor.

The successful application of adoptive immunotherapy to the treatment of human cancer would require improved methods to culture, identify and expand tumor-specific T cells isolated from tumor-infiltrating lymphocytes among other facilitating factors.

Wiltrout and Salup continue the discussion of adoptive immunotherapy combined with chemotherapy for treatment of cancer. In recent years, bio-

logical response modifiers (BRM) have been shown to stimulate tumoricidal action by NK cells, T lymphocytes and macrophages. Selected cell populations, isolated after sensitization can be passively transferred to a tumor-bearing host to provide adoptive immunotherapy (AIT). The discovery that IL-2 is able to facilitate T lymphocyte proliferation and to augment NK cell activity as well as induce the tumoricidal activity of LAK cells has further increased the attractiveness of this treatment approach. The development of large quantities of recombinant human IL-2 have made possible preclinical and clinical trials with AIT in cancer therapy. Either specifically sensitized T lymphocytes and exogenous IL-2 have been employed or nonsensitized lymphocytes have been cultured in IL-2 to render them tumoricidal. Antitumor activity has been shown with both protocols but results are often improved considerably by the combination of AIT with chemotherapy. The authors compare these approaches and stress the action of cytotoxic lymphocytes with broad specificity in ACIT, using mouse renal cancer as their primary model.

Specifically sensitized, CTL have proven effective for therapy of immunogenic tumors in mice by AIT. The therapeutic effects were enhanced when specifically sensitized CTL were employed in conjunction with CY for ACIT. The CY facilitates tumor debulking and elimination of suppressor T cells, which otherwise inhibit specific CTL antitumor activity. Allogeneic CTL reactive against minor histocompatibility antigens have also been employed for AIT treatment of murine tumors expressing minor histocompatibility antigens.

Future studies must be directed to the identification of tumor-specific antigens (TSA) on human tumors that stimulate T cell reactivity. Current efforts in many laboratories are aimed at isolating tumor-infiltrating lymphocytes (TIL) from such human tumors as carcinomas of the lung, colon, and breast with the possibility of culturing and expanding the cell populations in the presence of IL-2.

Unsensitized lymphocytes derived from murine spleen or blood, or from human peripheral blood can be activated by IL-2 in culture to become LAK cells with tumoricidal activity. NK cells have been shown to be precursors of LAK cells in both mouse and man. LAK cells have been used in conjunction with IL-2 administration in experimental therapy of tumors in mice and humans. Regrettably, considerable toxicity has resulted from the systemic injection of large doses of IL-2, including capillary leak syndrome in some patients. The relative size of tumors also limits the antitumor effectiveness of LAK cell therapy alone. AIT employing LAK cells is most effective when

the tumor burden is minimal suggesting that it might serve as a valuable adjunct to surgical debulking treatment or radiotherapy or chemotherapy.

Chemotherapy used in conjunction with AIT may facilitate AIT's antitumor effect by reducing the tumor mass and eliminating suppressor T cells.

Talmadge provides a lucid description of the therapeutic potential of cytokines, comparing preclinical and clinical studies. BRM can alter a host's biological reactivity to a neoplasm with therapeutic effects as a consequence. Some BRM, classified as biologics, include cytokines such as TNF, IFN, IL, and colony-stimulating factors (CSF). Techniques of genetic engineering have permitted the generation of recombinant cytokines in quantities sufficient to permit their investigation in experimental immunomodulatory and therapeutic trials. The author discusses experiments employing recombinant murine (rM) IFN-gamma, recombinant human (rH) and M TNF, rH IL-2, and rM CSF-GM. Talmadge and co-workers have examined the immunomodulatory and therapeutic actions of BRM and used their results to develop clinical and preclinical hypotheses.

Whereas immunotherapy appears promising in experimental situations where normal animals with a minimal tumor mass receive treatment, clinical trials may be disappointing because metastases have already taken place by the time the tumor is diagnosed. Thus, control of metastases is of greater significance than elimination of the primary tumor. Thus, animals with known metastatic tumors must be employed in protocols designed for the identification and investigation of BRM that may be clinically useful.

Recombinant human IL-2 therapy of either experimental or spontaneous metastases leads to a biphasic therapeutic response curve. Dose and administration schedule are determining factors in the OTP. Although IL-2 is therapeutically more effective when administered intraperitoneally than when injected intravenously, chronic administration of rH IL-2 is therapeutically far more effective in doses less than the MTD, than when administered only briefly.

As discussed earlier, antigenic autochthonous tumors may be induced by repeated exposure of mice to UV radiation. Whereas, normal syngeneic mice reject these tumors when transplanted, syngeneic mice rendered immunologically suppressed by UV radiation may experience progressive growth of the tumors upon passive transfer. This is partially attributable to tumor-specific suppressor T lymphocytes in these immunosuppressed hosts. The suppressor T cells are antigen specific and do not suppress other immune responses to either exogenous antigens or allogeneic grafts. Thus, the UV carcinogenesis

model represents an excellent mechanism to evaluate immunotherapeutic agents for the therapy of autochthonous neoplasms.

Although toxicity has been shown to accompany therapeutic activity of rH IL-2 when administered frequently at relatively high doses as well as in association with LAK cells or TIL, preclinical studies indicate that rH IL-2 may be therapeutically effective at lower doses that produce far less toxicity. These lower nontoxic doses show the greatest therapeutic activity when given by continuous infusion in conjunction with LAK cells.

The therapeutic action of recombinant mouse interferon (rM IFN) gamma in the treatment of metastatic disease is linked to its augmentation of CTL and macrophages. The treatment of spontaneous metastases by the administration of rH TNF reveals a significant therapeutic effect when administered i.v. but no beneficial action when given i.p. rH TNF produces a greater therapeutic effect on spontaneous metastases than on experimental ones. The therapeutic action of rH TNF is apparently due to its immunomodulatory and coagulative actions. Recombinant mouse colony-stimulating factor (CSF-gM) revealed no significant therapeutic effect when used alone, although rM CSF was able to function as a myelor restorative agent.

The therapeutic effects of cytokines may be relatively small and associated with chronic augmentation-activation to yield meaningful therapeutic actions. Treatment must be over a long period rather than short range. These substances may have a bell-shaped immunomodulatory and therapeutic response curve. It is anticipated that combination chemoimmunotherapy employing such BRM as CSF, IL and IFN may prove of considerable value in future trials.

The T cell growth factor, IL-2, is recognized as a significant immunomodulator of neoplasia. Rossio and Ruscetti provide a critical analysis of the role of IL-2, used either alone or in conjunction with other therapy, for the treatment of neoplastic disease. Its in vivo antitumor effects have been ascribed to four mechanisms. It is critical to the development of cytotoxic lymphocytes that produce injury and death of tumor cell targets. CD8 cytotoxic lymphocyte development in vitro is dependent upon T helper lymphocytes. IL-2 also facilitates the differentiation of normal blood lymphocytes into LAK cells, which have broad antitumor cytotoxic activity but are not injurious to normal cells. IL-2 activates NK cell proliferation and antitumor function. NK cells are active against many tumor types and are probably significant in immune surveillance. Some NK cells stimulated by IL-2 may develop into LAK cells. IL-2 is an important immunoregulator through the stimulation of secondary lymphokines, activation of T lymphocyte prolifer-

ation and its ability to control its own production through regulation of the IL-2 receptor. It is able to enhance CTL development in allograft and tumor models. Tumor immunotherapy models in mice and rats reveal that the administration of IL-2, under prescribed conditions, may effectively regulate tumor growth and metastases.

The inoculation of specifically immune CTL has long been known to be therapeutically effective in the treatment of tumors in animal model systems. When spleen cells are incubated in vitro with IL-2, LAK cells are generated which upon adoptive transfer to a tumor-bearing host, with or without the administration of supplemental IL-2, may demonstrate a significant antitumor effect. The activity is governed by many variables outlined by these and other authors in the present volume. Most types of immunotherapy are more effective against relatively small tumor masses than against a large neoplastic burden. If used alone, IL-2 requires relatively high doses for antitumor activity but is more effective if used in conjunction with LAK in AIT against tumors. IL-2 has been administered by bolus injection, continuous infusion, or direct inoculation into tumor sites. The maximum tolerated dose of this lymphokine has been used but excessive doses may produce toxic side effects. Delivery of the lymphokine is also critical, since IL-2 acts at close range rather than by circulating in the serum to distant sites of action. It is important in antitumor therapy to localize lymphokines to tumor sites where they may be effective. This is accomplished by either direct injection into the tumor mass or inoculation into lymph nodes or blood vessels linked to tumor sites or through coupling lymphokines to monoclonal antibodies against tumor.

Since relatively large and frequent doses of IL-2 are required for effective antitumor therapy, patients have experienced severe toxicities including fever, chills, fatigue, confusion, nausea, diarrhea, and many developed capillary leakage syndromes with weight gain associated with extravasated fluid and hypotension, cardiac arrythmias and renal dysfunction. Some also developed eosinophilia, among other abnormalities. Fortunately, the adverse effects were reversible upon cessation of IL-2 therapy.

IL-2 administration diminishes the number of LAK cell precursors in the peripheral blood, which is restored upon termination of IL-2 therapy. IL-2 alone can be safely administered to human subjects although it is apparently less effective than when used in conjunction with other treatment modalities such as adoptive LAK cell therapy, in which it is believed to maintain viability and activity of LAK cells in vivo. IL-2 has been effective for various types of tumors including ovarian carcinoma and bladder carcinoma.

Three separate protocols have been employed for LAK therapy. Rosenberg and co-workers prime patients by IL-2 injection followed by discontinuation to permit recovery of LAK precursors which are collected and cultured with IL-2 in vitro to permit LAK cell differentiation. These LAK cells are readministered together with IL-2. Other investigators have used variations of this approach such as continuous IL-2 infusion or local administration of LAK cells and IL-2 into a tumor site rather than systemic injection. The results of a number of these studies show considerable promise with various types of tumors.

Forni and co-workers provide a critical assessment of tumor immunotherapy by local injection of IL-2 and nonreactive lymphocytes. They present both experimental and clinical results. Tumor infiltrating lymphocytes (TIL), cyclosphosphamide and IL-2 have been shown to be effective in eradicating metastases in mice. As pointed out elsewhere in this volume, IL-2 not only induces tumor-specific Tc lymphocytes but facilitates their expansion in vitro and their survival in vivo. Thus, IL-2 and Tc lymphocytes may be useful in the elimination of tumor cells and metastases that remain following conventional cancer therapy. Problems include the fact that adoptively transferred Tc lymphocytes are effective principally in mice that are sublethally irradiated or suppressed.

Tumor-induced, T cell-mediated immunosuppression is a principal hindrance to specific adoptive immunotherapy. There is also the difficulty that relatively few of these specifically reactive Tc lymphocytes reach areas of tumor growth, representing a homing problem. This is due, in part, to the lack of specific antigens on spontaneous tumor cells that effectors might recognize.

Many different types of effector cells participate in cytolytic activity against tumors. These include not only T lymphocytes but also granulocytes, activated macrophages, NK cells and LAK cells. Lymphokines play a significant role through their activation of cells such as tumor cytolytic macrophages that inhibit metastases. NK cells do not recognize TSA and are not MHC restricted but play an important role in immune surveillance against primary tumors and in resistance to metastases. Both IL-2 and IFN can enhance their killing effect. IL-2 facilitates their expansion in culture. Adoptive transfer of NK cells in vivo inhibits blood-borne metastases. Future studies will be aimed at prolonging sustained NK activity.

As presented by various authors in this volume, LAK activity is effective against a relatively broad range of fresh tumor cells and differs from specific CTL effects. Whereas LAK cells are derived mostly from the NK cell popula-

tion, LAK activity is different from that of enhanced NK cells. LAK cell and IL-2 therapy is 50–100 times less efficient than treatment with tumor-specific IL-2-activated TIL, CY and IL-2.

The authors discuss T helper lymphocytes in tumor immunology. Besides a tumor's proliferative and invasive capacity, it is also capable of diminishing host resistance through the induction of both specific and non-specific suppressor mechanisms. The suppressor effect plays a major role in rendering the host incapable of successfully resisting tumor progression. Numerous attempts during past years have been aimed at increasing the immunogenicity of tumor-associated molecules by coupling helper determinants to tumor cells. These have included proteins, nucleic acids, viral or transplantation antigens. Recent efforts have employed virus and trinitrophenyl (TNP) helper activity to augment tumor-specific immunity. Significant regression occurred in mice presensitized against the helper epitope following inoculation of the helper determinant into a tumor site, if suppressor cells had first been eradicated. Helper determinants must be present on the same cell as the antigen to be effective.

Exogenous IL-2 injected into normal mice stimulates NK cell activity and increases cytolytic activity against allogeneic tumor and lymphoid cells. IL-2 aids immune recognition of tumors in vivo.

Clinically, the LATI system represents IL-2-mediated immunotherapy associated with direct reactivity to a tumor. The local injection of IL-2 avoids the problems of toxicity associated with systemic administration of large doses of the lymphokine. The authors administered IL-2 and lymphocytes to patients with tumors of the head and neck, which are associated with an unusually high incidence of immunosuppression mediated by suppressor cells. Head and neck tumors offer the further advantage of ease of manipulation and ready access to draining lymph nodes for the local administration of IL-2 and lymphocyte suspensions. The ineffectiveness of conventional therapy coupled with the suppression of immune competence make these types of tumors ideal for the investigation of local stimulation of immune reactivity through immunotherapy.

Experimental methylcholanthrene-induced murine sarcomas and human bladder tumors may sometimes be induced to regress following intratumor inoculation of IL-2. The authors demonstrated through LATI experiments in animals that initiator lymphocytes potently enhanced the action of exogenous IL-2 inoculated at a tumor growth site. Draining lymph nodes are involved mostly with the activation of multiple reaction processes. In clinical trials, IL-2 was injected around draining lymph nodes rather than into the

lesion or around the tumor to activate mechanisms resembling those stimulated in murine LATI to decrease the tumor mass.

Yigata and Grimm present an elegant survey of current understanding of the LAK cell phenomenon. When peripheral blood lymphocytes (PBL) are cultured with IL-2 without antigen or mitogen effector cells are generated that are cytotoxic for tumor cells resistant to NK cells. These cells are defined by their function and have been used successfully to treat advanced tumor patients with partial success. LAK cell precursors have been described to be negative for T3, T4, T8, M1 and Leu-7 markers, yet LAK effector cells bear identifiable pan-T cell markers, T3 and Leu 1. However, most LAK activity in PBL cultured in the presence of IL-2 for 1–7 days is attributable mainly to IL-2-activated Leu-11 positive cells with some T3 positive cell contribution. B cells have also been implicated in the LAK system. Thus, various cell types may mediate LAK function.

Studies on the development of LAK activity from NK and T lymphocyte-enriched populations reveal the extensive heterogeneity of LAK precursors with the most active LAK precursors residing in the Leu 11 positive and low-density Percoll fraction cells. Current data reveal that LAK cell precursors include $CD16^+$ (NK) cells, T lymphocytes and B lymphocytes which become cytotoxic following culture with IL-2.

In addressing the question of how Tac-negative LAK precursors respond to IL-2, Yagita and Grimm discuss high and low affinity IL-2 receptors. Although resting lymphocytes are described as IL-2 receptor negative, as assayed by the inability to detect expressed Tac antigen, exposure to IL-2 without mitogen or antigen permits the rapid appearance of detectable IL-2 receptors.

LAK effector cells are likewise heterogeneous. However, they have in common the ability to kill all forms of cultured and fresh tumor cells without MHC restriction. LAK is identified by function and the requirement of IL-2 for activation. As outlined previously, adoptive immunotherapy trials in humans bearing tumors involves the in vitro culture of autologous lymphocytes with IL-2 and adoptively reinoculating these cells into the patients from which they were derived. Besides intravenous administration, localized intratumor injections have been carried out. Relatively large numbers of these effector cells are required for tumor regression and systemic administration of IL-2 induces systemic toxicity such as capillary leak syndrome. The authors cite the advantages of locally administering LAK cells in association with IL-2 which has been used successfully in combination with surgical debulking to reduce tumor mass. These localized treatments have not been