Clinical and Biological Evaluation of the Immunomodifiers

Clinical and Biological Evaluation of the Immunomodifiers

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Contents

Cancer Treatment Symposia
Volume 1, 1985

1 Introduction. **P. Reizenstein, M. Chirigos, H. Fudenberg, and P. Pontiggia.** Karolinska Hospital and Institute, Stockholm, Sweden.

3 Mechanism of Melphalan-Mediated Modulation of Antitumor Immune Potential of Tumor-Bearer Lymphoid Cells. **S. Dray, R. C. Bocian, S. Ben-Efraim, and M. B. Mokyr.** University of Illinois at Chicago.

11 Cellular Regulation by Immunomodifiers MVE-2 and Poly ICLC and Their Therapeutic Application. **M. A. Chirigos, T. Saito, E. Schlick, and R. Ruffman.** National Cancer Institute, Frederick Cancer Research Facility, Maryland.

19 Effects of Cytotoxic Chemotherapy on Immune Function in Cancer Patients. **D. P. Braun and J. E. Harris.** Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois.

27 Immunomodulation in Cancer Patients by Synthetic Biological Response Modifiers. **M. Micksche, M. Colot, A. Uchida, E. M. Kokoschka, T. A. Luger, C. Dittrich, K. Moser, H. Rainer, R. Lenzhofer, R. Kolb, R. Jakesz, M. Schemper, O. Kokron, H. Zwick, A. Scheiner, H. Flicker, and P. Sagaster.** Institute for Applied and Experimental Oncology, University of Vienna, Austria.

37 Antimicrobial Chemotherapy in Immunocompromised Host. **G. Gillissen.** Medical Faculty, Aachen, West Germany.

45 Antibodies to Human Interleukin-2 Induced by Synthetic Peptides. **A. Altman, J. M. Cardenas, R. A. Houghten, N. Isakov, F. J. Dixon, and A. N. Theofilopoulos.** Scripps Clinic and Research Foundation, La Jolla, California.

53 Interleukins in Murine Lupus. **A. N. Theofilopoulos, G. J. Prud'homme, and F. J. Dixon.** Scripps Clinic and Research Foundation, La Jolla, California.

61 Purification and Properties of Human Interleukin-1. **L. B. Lachman.** The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

69 Better-Defined B-Cell Assays for T-Cell Factors. **R. H. Zubler.** Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland.

75 T-Cell Factors Regulating Macrophage Functions, Especially Activation for Tumor Cell Killing. **S. W. Russell.** University of Florida, Gainesville.

83 Interleukin-2 Reconstitution of Aged Immune Function. **M. L. Thoman and W. O. Weigle.** Scripps Clinic and Research Foundation, La Jolla, California.

89 Modifiers of Normal Immune Status and Restorers of Immune Function in Immunodepressed Animals. **G. Mathé, J. Martinez, and F. Winternitz.** Institut de Cancérologie et d'Immunogénétique, Hôpital Paul-Brousse, Villejuif, France.

95 Significance and Value of Thymic Preparations Used for Immunotherapy. **J.-F. Bach and D. Kaiserlian.** Immunologie Clinique, Hôpital Necker, Paris, France.

101 Biological Response to Acute Leukemia. I. Tumor-Associated Antigens and Antigen-Independent Tumor Surveillance. **P. Reizenstein, A. Andreasen, P. Biberfeld, C. Canon, R. Hast, B. Lagerlof, L. Olsson, and Å. Öst.** Karolinska Hospital and Institute, Stockholm, Sweden.

107 Generation and Biological Implications of Intratumoral Phenotypic Diversity. **L. Olsson.** Cancer Biology Laboratory, State University Hospital, Copenhagen, Denmark.

113 Immunologic Recognition of Tumor Cells in Humans Measured by Lymphocyte Cytotoxicity. **F. Vánky and E. Klein.** Karolinska Institute, Stockholm, Sweden.

121 Use of Allostimulation To Generate Killer Lymphocytes Against Autologous Human Melanoma. **G. Parmiani, A. Balsari, G. Fossati, D. Taramelli,**

I. Storchi, and N. Cascinelli. Istituto Nazionale Tumori, Milan, Italy.

127 Evaluation of Effectiveness of Intrapleural *Nocardia rubra* Cell Wall Skeleton Against Lung Cancer.

K. Yasumoto and Y. Yamamura. Kyushu University Medical School, Fukuoka, Japan.

135 Announcements

Introduction

The biological response to disease is a complex system of reactions, including the mediators of inflammation, hormones, cytokines, the complements, kinin and coagulation cascades, and reactions mediated by macrophages, leukocytes, and different lymphocyte subpopulations. The metabolism of several proteins and nutrients is also affected, with "nutritional immunity" as one of the results.

The system is characterized by remarkable complexity, which is due in part to interaction between apparently unrelated elements of this defense system. The increased production by granulocytes of lactoferrin, which mediates hyposideremia, an important part of nutritional immunity, also inhibits the macrophage production of colony-stimulating activity, and thus granulopoiesis. Steroid and thymic hormones, prostaglandins, cytokines, and histamine-induced suppressor factor interact with lymphocytes.

There are numerous biological and synthetic products that can influence the biological response. Microbiological products, hormones, peptides, and substances as different as cephalosporins, azimexon, vitamin A, levamisole, and cimetidine are among them. Most of these products, however, have nonlinear dose-response curves and responses that are limited in time. This means that high doses or prolonged administration may lead to responses opposite to those that are found after low doses or short-term administration. In addition, the effects of cimetidine in cell cultures or animal systems are different from those in humans. These factors increase the complexity inherent in the use of biological response modifiers.

To some extent, however, the mechanisms of these substances are becoming known. We know, for instance, that interferon stimulates the activity of natural killer cells, that BCG stimulates the helper function of macrophages, and that low doses of certain cytostatics may be more toxic to suppressor T cells than to helper T cells. Research in this area is progressing rapidly, and it is in the service of investigations of the *biological* evaluation of response modifiers that this symposium was arranged.

Greater difficulties are involved in the *clinical* evaluation of biological response modifiers, because it is frequently unknown which effects on the biological response to disease are clinically desirable. For example, interferon can stimulate natural killer cells and interleukin-2 induces cytotoxic T cells, but the clinical benefit is uncertain. Low doses of certain cytostatics can induce an immunostimulatory effect by inhibiting the suppressor cells, but it has not been demonstrated that this exerts any clinically beneficial effect.

It is important to establish, not only correlations, but also cause-effect relationships between the various elements of the biological response and the clinical effects on tumors. In this area, the present symposium is intended only to be a beginning.

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Mechanism of Melphalan-Mediated Modulation of Antitumor Immune Potential of Tumor-Bearer Lymphoid Cells[1]

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The antitumor immune potential of immunosuppressed spleen cells from a mouse bearing a large MOPC-315 plasmacytoma is augmented when the spleen cells are exposed in vivo or in vitro to a low concentration of melphalan (L-PAM). The mechanism of this L-PAM-mediated enhancement consists of the elimination of the immunosuppressive activity of macrophages and metastatic tumor cells as well as the appearance of immunostimulatory activity. Thus, a low dose of L-PAM, < 1/20th of the maximum tolerated dose, mediates its curative effect in mice bearing very large tumors and extensive metastases by facilitating the generation of antitumor immunity in the host, which, together with the tumoricidal action of the drug, is effective in tumor eradication. The curative effectiveness of a very low dose of L-PAM at an advanced stage of tumor growth is made possible by the drug's immunomodulatory activity, which shifts the balance from immunosuppression to immunopotentiation, resulting in effective, potent antitumor immunity. The possibility that a low dose of drug may have a substantial beneficial immunomodulatory effect in humans, as it does in mice, offers an approach to cancer therapy that might avoid the complications of commonly used high-dose chemotherapy. [Cancer Treat Symp 1:3–10, 1985]

Many drugs currently used in cancer chemotherapy cause severe depression of bone marrow and lymphoid cell functions. However, under defined conditions, some of these otherwise immunosuppressive drugs can potentiate immune responsiveness (1–3). For example, cyclophosphamide (CY), one of the most potent immunosuppressive chemotherapeutic drugs available, has been shown under certain experimental conditions to enhance IgE (4,5), delayed type hypersensitivity (6–8), and antitumor (9,10) responses. From these studies, it became apparent that the timing of CY administration relative to immunization is critical to the drug's immunomodulatory effect. Thus, treatment with CY prior to immunization potentiated IgE production and cell-mediated immune responses, whereas CY treatment after immunization suppressed cell-mediated immune responses. The potentiation of cell-mediated immune responses by CY (≥ 100 mg/kg) was attributed originally to suppression of antibody production as a consequence of depletion of B lymphocytes (11,12). By lowering the dose of CY to 20 mg/kg, Askenase et al (7) achieved enhancement of delayed type hypersensitivity without suppressing antibody production. The authors suggested that this enhancement is due to the effect of CY on precursors of suppressor T cells (7,13).

Recently, CY has been implicated as an immunomodulator of antitumor response in tumor-bearing mice (1,14). This conclusion stems from the observation that administration of a low dose of CY to tumor-bearing mice, whose spleens exhibit suppressed antitumor immune potential, leads to an enhancement of their antitumor immune potential, which is associated with the elimination of suppressor cell activity from the spleen. The absence of suppressor cell activity in the spleen following CY therapy of tumor-bearing mice might not be due to CY-induced selective elimination of suppressor cell activity but might instead be the result of CY-induced alteration of the migratory pattern of suppressor cells out of the spleen.

To establish that CY does mediate the selective elimi-

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nation of splenic suppressor cell activity, it would be necessary to demonstrate that incubation of immunosuppressed tumor-bearer spleen cells with the drug in vitro results in an augmented antitumor immune potential that cannot be augmented further by subjecting the spleen cell suspension to fractionation procedures that selectively deplete suppressor cells. Such an approach is complicated in the case of CY because the drug is inactive in its native form and requires activation by hepatic microsomal enzymes to exert its toxic effects (15,16). Moreover, 4-hydroxycyclophosphamide, the first activation product of CY, and aldophosphamide, which exists in equilibrium with 4-hydroxycyclophosphamide, are quite labile (16,17). To circumvent these obstacles, we have employed another alkylating agent, melphalan (L-PAM). Unlike CY, L-PAM is both active and stable in its native chemical form (18,19) and, like CY, is used extensively in treatment of patients with cancer, particularly those with multiple myeloma (20).

MURINE MOPC-315 PLASMACYTOMA MODEL

Our studies have utilized the weakly immunogenic murine MOPC-315 plasmacytoma of BALB/c origin (21). Progressively growing plasmacytomas in mice initiate many of the pathophysiologic phenomena associated with multiple myeloma in humans, including a specific gammopathy (22), proteinuria (23), kidney lesions (24), and osteolytic bone destruction (22). Routinely, mice were inoculated sc with 300 times the minimum lethal dose of MOPC-315 tumor cells; this inoculum size leads to the appearance of palpable tumor nodules in 8 days, which grow progressively and kill the mice in 18 days. At advanced stages of tumor growth, spleen cells from such mice exhibit suppressed antitumor immune potential, compared to the immune potential of normal mice, as judged by the ability of the spleen cells to mount an antitumor cytotoxic response following in vitro immunization with stimulator tumor cells (25). The suppressed antitumor immune potential of tumor-bearer spleen cells is due to the inhibitory activity of splenic macrophages (26) and metastatic tumor cells (27).

EFFECT OF L-PAM

Spleen Cell-Mediated Antitumor Immunity

Low-dose treatment in vivo

Initially, we had to establish that low-dose L-PAM therapy of mice bearing a large MOPC-315 tumor can enhance the antitumor immune potential of their immunosuppressed spleens. For this purpose, mice bearing a large MOPC-315 tumor were given a single ip injection of 0.75 mg of L-PAM/kg of body weight and, subsequently, on Days 2, 5, or 8 after chemotherapy, their spleen cells were evaluated for antitumor immune potential. The antitumor immune potential of spleen cells from L-PAM-treated tumor-bearing mice was compared to that of spleen cells from untreated tumor-bearing mice (fig 1). L-PAM therapy of tumor-bearing mice enhanced the antitumor immune potential of their immunosuppressed spleen cells such that, following in vitro immunization, they mounted a level of antitumor cytotoxicity similar to that exhibited by in vitro-immunized normal spleen cells. This L-PAM-induced enhancement occurred within 2 days after chemotherapy and lasted for at least 8 days; it was not due to L-PAM-induced alteration in the migratory patterns of suppressor cells and/or cells involved in the generation of antitumor immunity, since in vitro incubation of immunosuppressed tumor-bearer spleen cells with L-PAM also enhanced their antitumor immune potential.

Treatment in vitro

Immunosuppressed tumor-bearer spleen cells were incubated in vitro for 1 hour with graded concentrations of L-PAM ranging from 0.15 to 1.5 nmol of L-PAM/ml and subsequently subjected to in vitro immunization (fig 2). In vitro exposure of immunosuppressed tumor-bearer spleen cells to as little as 0.15 nmol of L-PAM/ml enhanced their antitumor immune potential; exposure to 0.5–1.5 nmol of L-PAM/ml resulted in much greater enhancement of antitumor immune potential.

To ascertain whether L-PAM-induced enhancement of antitumor immune potential of immunosuppressed tumor-bearer spleen cells is due to selective elimination of suppressor cell activity, we determined whether the antitumor immune potential of L-PAM-treated tumor-bearer spleen cells can be further augmented by subjecting them to glass wool fractionation; this procedure selectively depletes the suppressor cells that operate in the spleens of MOPC-315 tumor-bearing mice, namely, macrophages and metastatic tumor cells (fig 3). Subjecting L-PAM-treated immunosuppressed tumor-bearer spleen cell suspensions to glass wool fractionation did not further augment the antitumor immune potential of the spleen cells and, in a few instances, actually decreased it. Although these results suggest that exposure of immunosuppressed tumor-bearer spleen cells to L-PAM leads to selective elimination of the suppressive activity of glass-adherent splenic cells, the possibility exists that, following L-PAM treatment, the suppressor cells no longer adhere to glass and/or that the cells involved in the generation of antitumor immunity become unresponsive to suppression mediated by the glass-adherent suppressor cells. To establish that L-PAM treatment leads to selective elimination of sup-

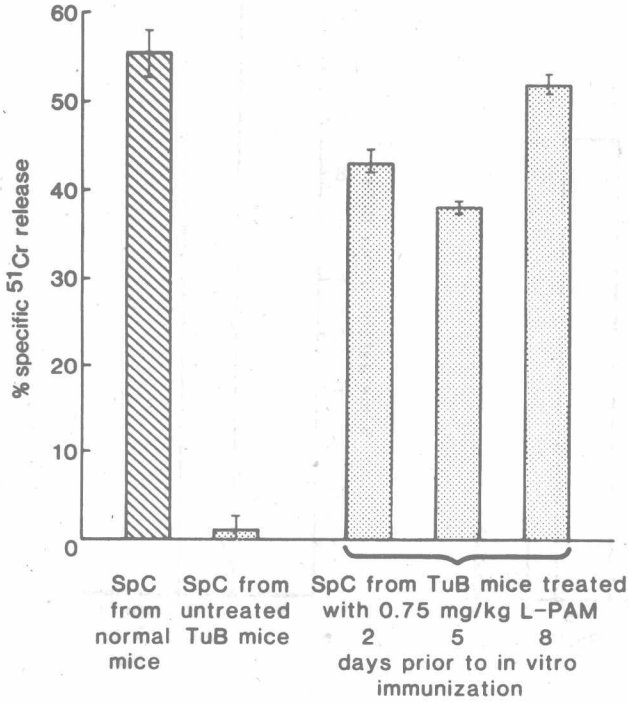

FIGURE 1.—Low-dose L-PAM therapy of mice bearing large MOPC-315 tumor enhances antitumor immune potential of immunosuppressed spleens. Spleen cell suspensions (SpC) obtained from tumor-bearing (TuB) mice treated with 0.75 mg/kg of L-PAM 2, 5, or 8 days earlier were subjected to in vitro immunization by cocultivation with mitomycin-treated stimulator tumor cells. 5 days later, spleen cells were evaluated for ability to lyse target tumor cells by 4-hr ^{51}Cr-release assay. Cytotoxicity exhibited by in vitro immunized spleen cells from normal mice is also included as control.

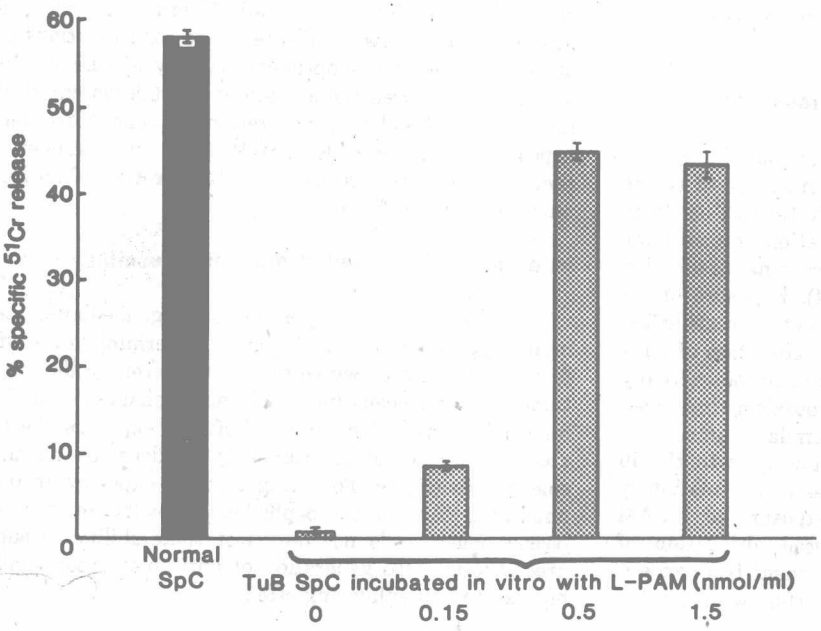

FIGURE 2.—Enhancement of antitumor immune potential of immunosuppressed tumor-bearer spleen cells is shown following in vitro exposure to L-PAM. Tumor-bearer (TuB) spleen cell suspensions (SpC) were incubated in vitro at 37°C for 1 hr with 0.15, 0.5, or 1.5 nmol of L-PAM/ml and subsequently subjected to in vitro immunization. 5 days later, spleen cells were evaluated for ability to lyse target tumor cells by 4-hr ^{51}Cr-release assay.

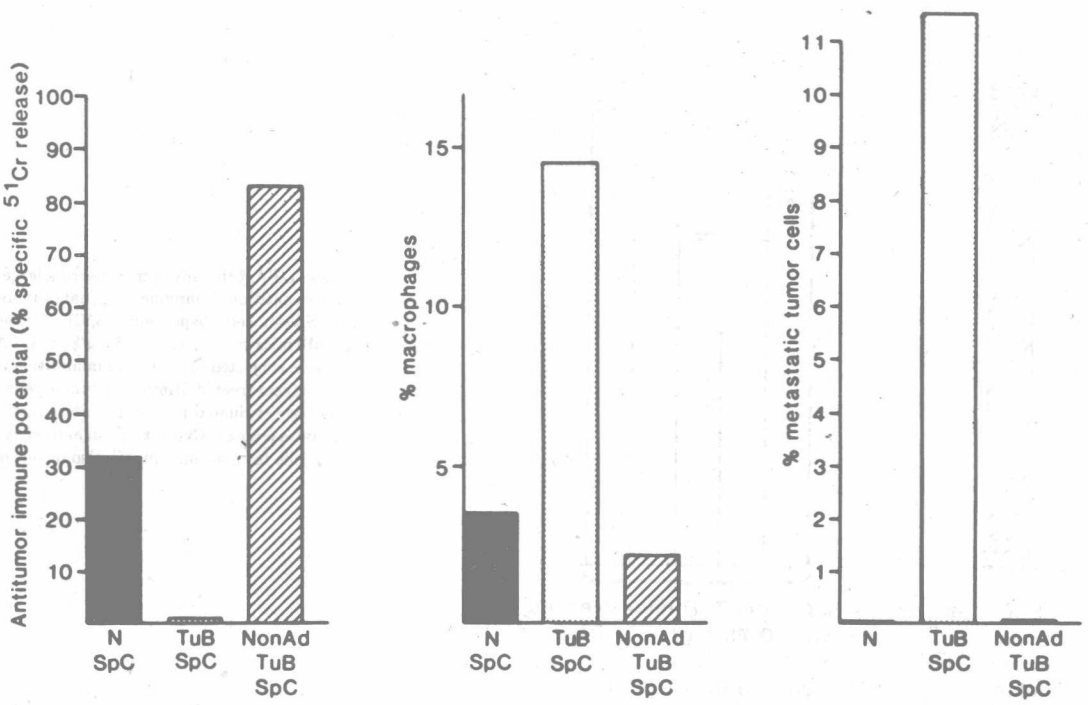

FIGURE 3.—Compared to suspensions of normal spleen cells (N SpC) subjected to in vitro immunization, spleen cell suspensions from BALB/c mice bearing large sc MOPC-315 tumor (TuB SpC) exhibit suppressed antitumor immune potential, as evidenced by reduced ability to mount antitumor cytotoxic response following in vitro immunization with stimulator tumor cells. Suppression of antitumor immune potential of tumor-bearer spleen cells is mediated by influx of macrophages and metastatic tumor cells. Depletion of most macrophages and tumor cells is accomplished by fractionation on glass wool columns, thereby allowing remaining nonadherent (NonAd) TuB SpC to mount secondary antitumor cytotoxic response following in vitro immunization.

pressor cell activity, we evaluated the effect of the drug on the inhibitory activity of metastatic tumor cells and of splenic macrophages.

Tumor Cell-Mediated Immunosuppression

The effect of L-PAM on tumor cell-mediated immunosuppression was assessed by determining the effect of incubating tumor cells in vitro with L-PAM on their ability to suppress the in vitro generation of antitumor cytotoxicity by normal spleen cells immunized with mitomycin-treated tumor cells (fig 4). Exposure of tumor cells to 0.5 nmol of L-PAM/ml not only abolished their ability to suppress the in vitro generation of antitumor cytotoxicity by normal spleen cells but actually resulted in augmented antitumor cytotoxic activity well beyond that achieved by adding similar numbers of supplemental mitomycin-treated tumor cells to the in vitro immunization mixture. The loss of the inhibitory activity of tumor cells following exposure to L-PAM cannot be attributed to drug-induced abrogation of their proliferative capacity. This is evident from experiments in which incubation of tumor cells with 0.5–1.5 nmol of L-PAM/ml did not abolish their ability to incorporate ^{3}H-thymidine or to establish lethal tumors upon inoculation into new recipients; these concentrations of drug abrogated the suppressive activity of tumor cells as well as enhanced the antitumor immune potential of immunosuppressed tumor-bearer spleen cells. Moreover, exposure of tumor cells to L-PAM in vitro rendered them superior stimulators for the generation of antitumor cytotoxicity (fig 5).

Macrophage-Mediated Immunosuppression

The effect of L-PAM on macrophage-mediated immunosuppression was assessed by determining the effect of incubating Sephadex G-10-adherent cells from tumor-bearer spleens (primarily macrophages) with 0.5 nmol of L-PAM/ml on their ability to suppress the in vitro generation of antitumor cytotoxicity by normal spleen cells (fig 6). Following in vitro exposure to 0.5 nmol of L-PAM/ml, the Sephadex G-10-adherent tumor-bearer spleen cells not only lost their ability to suppress the in vitro generation of antitumor cytotoxicity but exerted an enhancing effect.

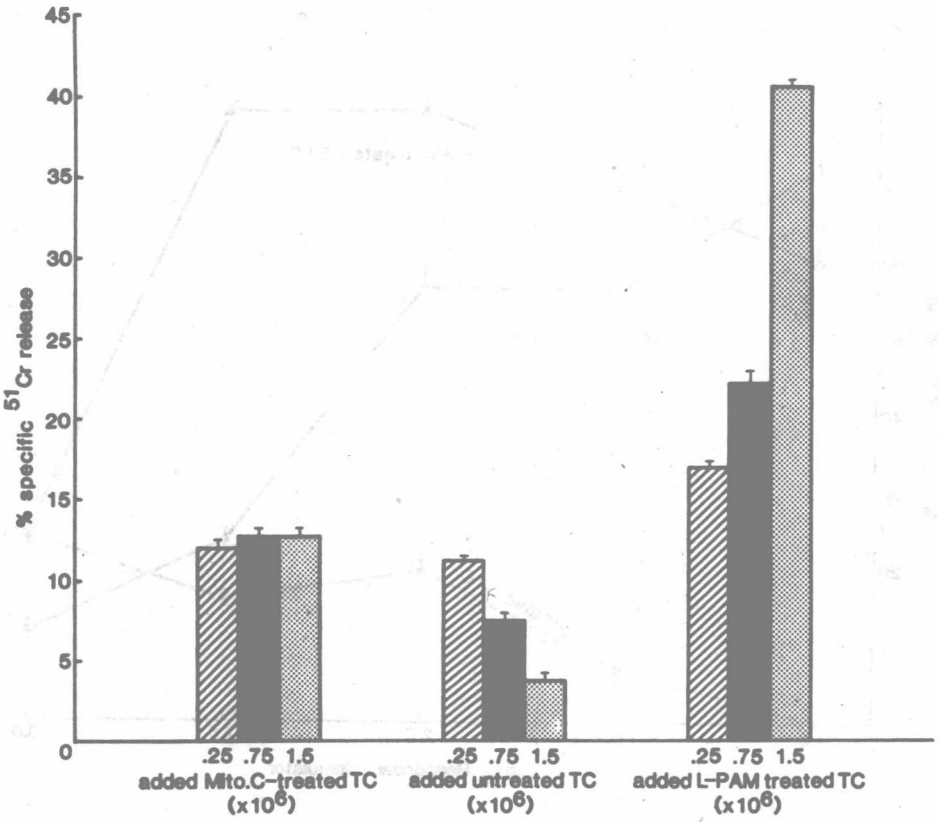

FIGURE 4.—Treatment of tumor cells (TC) with 0.5 nmol of L-PAM/ml abolishes ability to suppress in vitro generation of antitumor cytotoxicity. Untreated, mitomycin-treated (Mito.C-treated), or L-PAM-treated tumor cells (0.25, 0.75, or 1.5 × 10^6) were added to the immunization culture of normal spleen cells (75 × 10^6) and mitomycin-treated tumor cells (2.5 × 10^6). Subsequently, level of antitumor cytotoxicity generated was evaluated by ^{51}Cr-release assay.

CONCLUSIONS AND SIGNIFICANCE

Our results demonstrate that L-PAM acts as an immunomodulator, shifting the balance between immunosuppression and immunopotentiation in favor of antitumor immunity. The resultant strong antitumor immunity, in conjunction with the tumoricidal activity of a low dose of L-PAM, brings about the cure of mice bearing a large tumor (28). The kinetics of tumor eradication following low-dose L-PAM therapy is illustrated in figure 7. Following administration of L-PAM (0.75 mg/kg) to mice bearing a large tumor, a significant decrease in tumor size was observed by Day 3 after chemotherapy, and the tumor regressed completely by Day 10. The curative effectiveness of the low dose of L-PAM for mice bearing a large tumor is not due solely to the drug's tumoricidal effect, since viable, tumorigenic cells are present in the primary tumor nodule long after the drug has been cleared from the circulation. Tumor eradication occurs as a consequence of cooperation between the toxic effects of L-PAM and T-cell-dependent antitumor immunity. This is evident from experiments in which the curative effectiveness of a low dose of L-PAM was abrogated in most mice bearing a MOPC-315 tumor, if they were treated with anti-thymocyte serum (fig 8). Mice cured by the low dose of L-PAM in conjunction with T-cell-dependent antitumor immunity are resistant to a tumor challenge with at least 300 times the minimal lethal tumor dose.

Mice bearing a large MOPC-315 tumor can be cured also by a high dose of L-PAM (15 mg/kg) without the contribution of T-cell-dependent antitumor immunity in tumor eradication (28). The curative effectiveness of the high dose of L-PAM is due primarily to the tumoricidal activity of the drug, since viable, tumorigenic cells could not be detected in the primary tumor nodule even 1 day after chemotherapy. Although mice bearing a large MOPC-315 tumor can be cured by a high dose of L-PAM, these mice are not resistant to a subsequent tumor challenge (28).

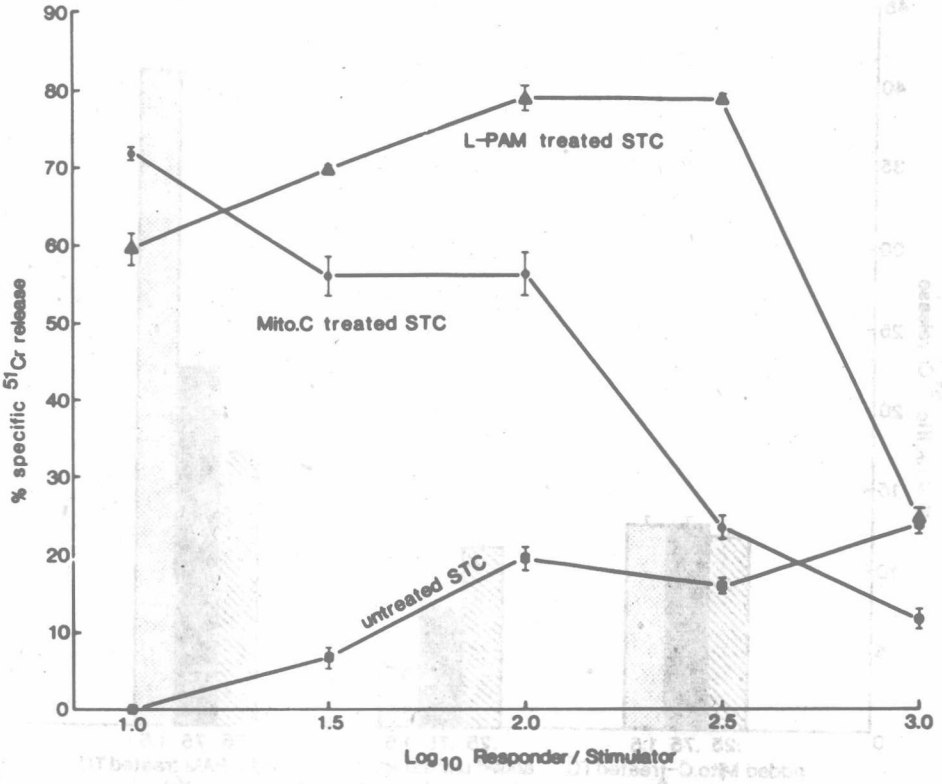

FIGURE 5.—L-PAM (0.5 nmol/ml) enhances ability of tumor cells to stimulate generation of antitumor cytotoxicity. Untreated (■), mitomycin-treated (Mito.C-treated) (●), or L-PAM-treated (▲) tumor cells were used as stimulator cells (STC) for in vitro generation of antitumor cytotoxicity. In vitro immunization was performed at various responder/stimulator cell ratios.

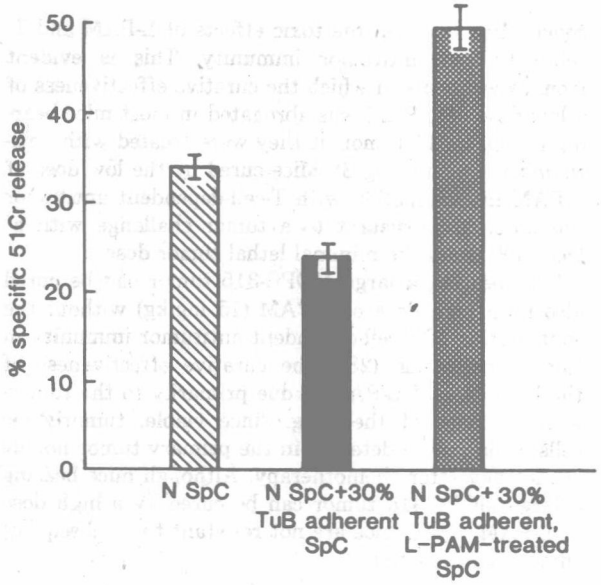

FIGURE 6.—L-PAM (0.5 nmol/ml) abolishes immunosuppressive activity of Sephadex G-10-adherent cells from tumor-bearer (TuB) spleens. Untreated or L-PAM-treated Sephadex G-10-adherent spleen cells (SpC) were added to in vitro immunization culture of normal spleen cells (N SpC), to constitute 30% of spleen cell population.

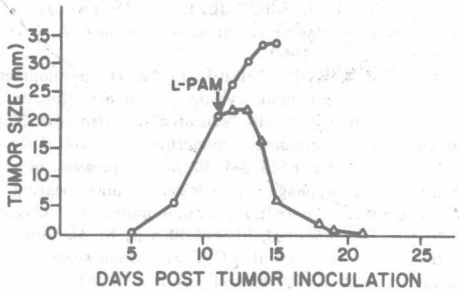

FIGURE 7.—Kinetics of regression of large sc tumor nodule following therapy with single low dose of L-PAM. Mice bearing 20-mm tumor received ip injection of 0.75 mg/kg of L-PAM (Δ). Untreated tumor-bearing mice are included as controls (O). Reprinted with permission from Ben-Efraim S, Bocian RC, Mokyr MB, et al. Increase in the effectiveness of melphalan therapy with progression of MOPC-315 plasmacytoma tumor growth. Cancer Immunol Immunother 15:101-107, 1983.

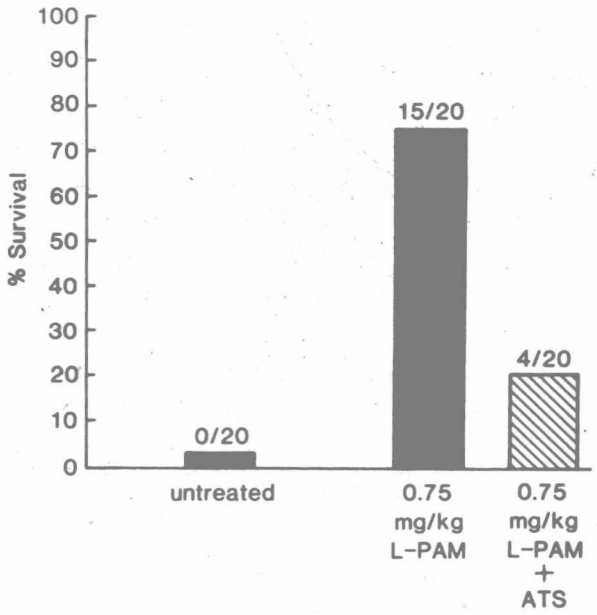

FIGURE 8.—Antithymocyte serum abolishes curative effectiveness of low dose of L-PAM (0.75 mg/kg) for most mice bearing large tumor. Mice were given 0.25 ml of rabbit antimouse thymocyte serum (ATS).

These results demonstrate that mice bearing a large tumor can be cured by a lower dose if T-cell-dependent antitumor immunity also aids in tumor eradication than if tumor eradication occurs solely via the drug's tumoricidal activity. This is important since high-dose chemotherapy is often immunosuppressive, thus increasing the susceptibility of the host to infection and decreasing host ability to prevent the development of dormant tumor foci. Moreover, tumor bearers treated "successfully" with high-dose chemotherapy are at a higher risk of developing a different type of cancer (29,30). The curative effectiveness of a low dose of L-PAM at an advanced stage of tumor growth is made possible in the MOPC-315 system by the drug's immunomodulatory activity. The possibility that a low dose of drug may have a substantial beneficial immunomodulatory effect in humans, as it does in mice, offers an approach to cancer therapy that might avoid the complications of commonly used high-dose chemotherapy.

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