1990

Modulation of the Host Response to a Wealthy Immunogenic Murine Tumor by Interleukin-1

Sylvia M. Kiertscher
Loyola University Chicago

MODULATION OF THE HOST RESPONSE TO A WEAKLY IMMUNOGENIC MURINE TUMOR BY INTERLEUKIN-1

by

Sylvia M. Kiertscher

A Dissertation Submitted to the Faculty of the Graduate School of Loyola University of Chicago in Partial Fulfillment of Requirements for the Degree of Doctor of Philosophy

September

1990

ACKNOWLEDGEMENTS

My gratitude is extended to Dr. Herbert L. Mathews, whose assistance and guidance have been invaluable throughout my graduate career. In addition, I wish to acknowledge the generous contribution of time and assistance by my research committee members; Drs. Thomas Ellis, Charles F. Lange, H. Terry Wepsic, and M. Rita Young.

I wish to thank Mr. David Beno, Ms. Tina Taylor, and the members of the Lange lab for their support and encouragement during my time at Loyola.

Lastly, I acknowledge the love and support of my family throughout my years in higher education.

VITA

The author, Sylvia Marie Kiertscher, is the daughter of Patricia Kiertscher and the late Henry Kiertscher. She was born September 28, 1960, in Hinsdale, Illinois.

Her secondary education was obtained at the Downers Grove Community High School South, Downers Grove, Illinois, where she was graduated in June, 1978.

She received the degree of Bachelor of Science with a major in biology from the University of Illinois at Urbana-Champaign in May, 1982.

From 1983 to 1985, she was employed as a research specialist in the health sciences at the University of Illinois Medical Center in Chicago, Illinois. In July, 1985, she entered the graduate program of the Department of Microbiology of Loyola University Stritch School of Medicine.

She was awarded an honorable mention in the National Science Foundation pre-doctoral fellowship competition in 1986. In 1988, she received the Cancer Federation Scholarship Award, Riverside, California, and the Graduate Dissertation Fellowship Award, Loyola University of Chicago. She has been awarded a National Institutes of Health Training Grant Fellowship in tumor immunology at the University of California, Los Angeles.

Following are the author's present publications:

Friedenson, B., and Kiertscher, S. 1985. Increase in protein -SH groups by 2-mercaptoethanol in mitogen stimulated lymphocytes. Fed. Proc. 44:1311.

Kiertscher, S., and Mathews, H. 1987. Augmentation of the tumor immune response with recombinant interleukin-1. Midwest Autumn Immunology Conference, Chicago, Illinois.

Kiertscher, S., and Mathews, H. 1988. Augmentation of localized cytolytic activity by systemic administration of recombinant interleukin-1. FASEB J. 2:A688.

Kiertscher, S.M., and Mathews, H.L. 1989. The effect of systemic IL-1 administration on local immunity. FASEB J. 3:A782.

Mathews, H.L., and Kiertscher, S.M. 1989. Effect of systemic IL-1 on tumor immunity. Abstract 106-57. 7th International Congress of Immunology: Berlin.

Kiertscher, S.M., and Mathews, H.L. 1989. Cytotoxic precursor augmentation by IL-1. Abstract 335. 2nd International Workshop on Cytokines: Hilton Head Island, South Carolina.

Kiertscher, S.M., and Mathews, H.L. (1990). Augmentation of local immune responsiveness by interleukin 1. Submitted.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS

ACTH	Adrenocorticotropic hormone
ATCC	American Type Culture Collection
CFS	Culture fluid supernatant
cpm	Counts per minute
CTL	Cytotoxic T lymphocyte
dpm	Disintegrations per minute
DTH	Delayed-type hypersensitivity
FACS	Fluorescence-activated cell sorter
FBS	Fetal bovine serum
GM-CSF	Granulocyte-monocyte colony-stimulating factor
h	Hour(s)
HBSS	Hank's balanced salt solution
i.d.	Intradermally
IL-1	Interleukin-1
IL-2	Interleukin-2
IL-4	Interleukin-4
IL-6	Interleukin-6
i.p.	Intraperitoneally
LAK cell	Lymphokine-activated killer cell
LLC	Lewis lung carcinoma
LPS	Lipopolysaccharide
min	Minute(s)
mRNA	Messenger RNA

ND	None detected
NK cell	Natural killer cell
PBS	Phosphate-buffered saline
PGE_2	Prostaglandin E_2
SD	Standard deviation
SE	Standard error
SI	Stimulation index
TNP	Trinitrophenyl

CHAPTER I

INTRODUCTION

The primary goal of this dissertation research has been to investigate the effects of systemic interleukin-1 (IL-1) therapy on local immune function in an experimental murine tumor model. In this model, the administration of systemic IL-1 in conjunction with adjuvant therapy allows for enhanced development of tumor immunity (Hornung and Mathews, 1985; Hornung, dissertation, 1987). Little is known about the ability of IL-1 to augment immune responsiveness in weakly immunogenic tumor systems. Because weakly immunogenic tumors respond less readily to some forms of therapy, the use of cytokines to enhance immune responsiveness may be a valuable addition to current therapeutic protocols. While the ability of IL-1 to affect various immunocompetent cells is well established *in vitro*, information involving its *in vivo* activities in tumor systems is more limited. Although other investigators have demonstrated the efficacy of IL-1 in promoting host survival of tumor inoculation (Nakamura *et al.*, 1986; North *et al.*, 1988; McCune and Marquis, 1990), little is known about the IL-1 effects which may lead to host protection.

The current study examined the effects of systemic IL-1 therapy on regional lymphoid tissue draining the site of tumor challenge. This was accomplished by; *in vitro* assessments of lymph node cell populations from IL-1 treated and non-IL-1 treated animals, cytotoxic cell frequency analysis of the lymph node cells, the assessment of

tumor-specific proliferation, and the identification of tumor-protective cell populations by *in vivo* adoptive transfer experiments. The results from this study provide information about the effects of IL-1 in an *in vivo* tumor model, and aid in the assessment of IL-1 as a potential therapeutic agent.

CHAPTER II

REVIEW OF THE RELATED LITERATURE

A. Interleukin-1: An overview.

Interleukin-1 (IL-1) has been described as a basic mediator of intercellular communication both within the immune system, and between the immune system and virtually all other organ systems (di Giovine and Duff, 1990). IL-1 is produced *in vivo* by a variety of cell types, including monocytes (Gery and Lepe-Zuniga, 1984), tissue macrophages (Simon and Willoughby, 1981; Murphy *et al.*, 1985), and lymphocytes (Scala *et al.*, 1984; Matsushima *et al.*, 1985; Tartakovsky *et al.*, 1986). IL-1 is also produced by cells in the vasculature (Libby *et al.*, 1986), skin (Luger *et al.*, 1982), and brain (Fontana *et al.*, 1984). Two forms of the cytokine have been identified (IL-1 alpha and IL-1 beta) which have 26% amino acid homology and bind to the same receptor (Mosley *et al.*, 1987).

IL-1 has been shown to have a number of effects upon physiological functions when administered to animals. These activities are important factors to consider when evaluating IL-1 as a potential therapeutic agent. The administration of IL-1 to animals or humans may affect other functions than those which are therapeutic. The effects of IL-1 administration on neurological functions include the induction of fever (Dinarello *et al.*, 1986), increased adrenocorticotropic hormone (ACTH) (Besedovsky *et al.*, 1986), and increased slow-wave sleep (Krueger *et al.*, 1984; Tobler *et al.*, 1984). Effects

on metabolic functions include hypozincemia, hypoferremia, and hypercupraemia (Kampschmidt and Pulliam, 1978), increased acute phase protein synthesis (Sipe et al., 1979; Dinarello et al., 1984), and increased insulin and corticosteroid production (Besedovsky and del Rey, 1987). These effects on metabolic functions may be relevant to the host response to infectious agents. IL-1 effects on endothelial and vascular cells result in hypotension (Dinarello, 1987; Okusawa et al., 1988), increased leukocyte adherence (Bevilacqua et al. 1985) and chemoattractant effects (Hunninghake et al., 1986). These effects are important in lymphocyte trafficking, and in the inflammatory response.

B. Effects of interleukin-1 on immunocompetent cells.

IL-1 is known to modulate a number of hematopoietic and immunological processes which may result in the modification of a tumor immune response. IL-1 induces the production of a variety of hemopoietic growth factors (Rennick, et al., 1987; Zucali, et al., 1987) and acts synergistically with certain colony stimulating factors to stimulate murine macrophage progenitors and primitive stem cells (Moore and Warren, 1987; Mochizuki et al., 1987). IL-1 administered in vivo can promote the cycling of hemopoietic stem cells in normal (Neta et al., 1987) and 5-fluorouracil-treated mice (Moore and Warren, 1987), perhaps by inducing the production and/or release of hematopoietic growth factors. IL-1 treatment resulted in bone marrow cell enlargement and increased cycling of the enlarged cells. Bone marrow cells from IL-1 treated animals were better able to proliferate in the presence of granulocyte-monocyte colony stimulating factor (GM-CSF) in in vitro experiments. Other IL-1 effects upon hematologic cells in-

clude radioprotection (Neta et al., 1986), and bone marrow stimulation.

IL-1 is known to affect a number of immunological cell populations. IL-1 has been shown to function as an immunological adjuvant in vivo (Staruch and Wood, 1983; Reed et al. 1989). In vitro, the cytokine can regulate a variety of cell surface receptors and determinants correlated with maturation and functional activation of not only T, but also B lymphocyte differentiation and proliferation (Lipsky et al., 1983; Pike and Nossal, 1985). IL-1 affects both resting and activated B cells, resulting in increased proliferation and plaque-forming activity (Chiplunkar et al., 1986). IL-1 also increases antibody production in B cell lines (Giri et al., 1984). Therefore IL-1 may play a role in B lymphocyte growth and maturation.

The role of IL-1 in T lymphocyte-mediated immune responses is well established. IL-1 functions as an activating signal for T cells (Larsson et al., 1980; Meuer and Meyer zum Buschenfelde, 1986), and can enhance T lymphocyte proliferation (Smith, 1980). IL-1 can augment the generation of helper and cytotoxic T lymphocytes by induction of interleukin-2 (IL-2) (Koopman et al., 1978; Farrar et al.,1980; McMannis and Plate, 1985). In addition, IL-1 has been reported to promote the generation of helper T lymphocytes and to diminish the generation of suppressor T cells (Durum et al., 1984; Durum et al., 1985). IL-1 synergizes with interleukin-6 (IL-6) in the activation of $CD4^+$ T cells (Houssiau et al., 1989; Holsti and Raulet, 1989), and modulates messenger RNA (mRNA) levels of activation-associated lymphokines in T lymphocytes (Hagiwara et al., 1987). IL-1 induces IL-2

production and increases T cell responsiveness to mitogens and antigens in vitro. IL-1 also induces the production of interleukin-4 (IL-4), as well as the increased expression of the IL-2 receptor (Lowenthal et al., 1986; Ho et al., 1987).

IL-1 has been shown to contribute to the generation of cytotoxic T lymphocyte (CTL) and lymphokine activated killer (LAK) cell activities, as well as the enhancement of natural killer (NK) cell activities. IL-1 may act with IL-6 on $CD8^+$ cells to enhance CTL function (Renauld et al., 1989). In addition, the enhanced activity of cytotoxic cells may be generated indirectly due to the stimulatory effects of IL-1 on helper T cells and their subsequent production of IL-2 (Holsti and Raulet, 1989). In vitro experiments have suggested a role for IL-1 in the generation of LAK activity. IL-1 synergizes with IL-2 in the generation of LAK cells (Crump et al., 1989). The addition of IL-1 to LAK cell cultures resulted in enhanced proliferation (Aribia et al., 1987) and augmented lytic activity (Ochoa et al., 1987). In addition, intratumoral injections of IL-1 in vivo resulted in the preferential homing of intravenously injected LAK cells to the tumor site (Migliori et al., 1987).

IL-1 increases the binding of NK cells to tumor targets. Tumor cells can induce the synthesis of IL-1 by NK cells (Herman et al., 1985). The same report has shown that patients with large tumor burdens show significant reduction in IL-1 production with concomitant decreased killing activity of NK cells. The impaired NK function was reconstituted with exogenous IL-1. Davis et al. (1990) have shown an augmentation of in vivo NK activity with IL-1. IL-1 enhances NK func-

tion synergistically with IL-2 and interferon gamma (Ostensen et al., 1989). An NK-like cell line expressed increased binding of IL-2 and increased IL-2 receptor mRNA synthesis in response to IL-1 (Lubinski et al., 1988).

Based on its multiple effects on various cell populations, IL-1 has a number of potential therapeutic uses. The in vivo administration of IL-1 can protect mammalian hosts against lethal challenges with microorganisms (Ozaki et al., 1987; Czuprynski and Brown, 1987; Van't Wout, et al., 1988) and from lethal irradiation (Neta et al., 1986). The radioprotective effects are due to IL-1 stimulation of bone marrow cells, and may be caused by the induction of large numbers of bone marrow cells into the radioresistant late S phase (Neta et al., 1987). The antimicrobial resistance caused by IL-1 treatment may be the result of an accumulation of neutrophils and mononuclear phagocytes. The antimicrobial and radioprotective effects of IL-1 may be useful in the prevention of infection in individuals undergoing various cancer therapies. Morikage et al. (1990) evaluated the preventive capability of IL-1 alpha against bacterial infections in normal and anticancer drug-treated mice. Pretreatment with IL-1 resulted in enhanced survival of both the normal and drug-treated animals.

The ability of IL-1 therapy to restore impaired T cell functions in vivo was demonstrated by Yamashita and Shirakawa (1987). They examined the effect of in vivo administration of recombinant human interleukin 1 on T-cell functions in tumor-bearing mice using an in vitro assay system. In tumor-bearing animals, the in vitro induction of trinitrophenyl (TNP)-specific cytotoxic T cells and proliferative T

cell responses from spleen cells were impaired. The administration of IL-1 alpha or IL-1 beta resulted in a dose dependent restoration of impaired T and NK cell function and the activation of protective immunity against the tumor.

c. Antitumor effects of interleukin-1.

The potential use of IL-1 as an antitumor agent has been examined by several investigators. Nakamura et al. (1986) examined the antitumor effect of IL-1 alpha against various murine syngeneic tumors. By using large doses of IL-1 (1-30 ug per injection) they were able to demonstrate antitumor effects. These effects were generally dose and route dependent. Treatment with IL-1 in vivo inhibited the growth of a number of intradermally transplanted tumors, including the Meth A sarcoma in BALB/c mice, the B16 melanoma in C57Bl/6 mice, and the colon 26 adenocarcinoma in CDF1 mice. Mice cured of the Meth A sarcoma were capable of rejecting the tumor upon rechallenge. In addition, IL-1 therapy was effective against the spontaneous pulmonary metastases of Lewis lung carcinoma in BDF1 mice. The intradermal route was most effective, followed by intramuscular and intravenous injections. Intraperitoneal therapy was not assessed. Treatment regimes ranged from one dose of 10-30 ug to 7 daily doses of 1-10 ug. Several small-dose injections were as efficacious as one larger injection when administered intratumorally. IL-1 was most effective early after tumor inoculation (day 1 tumors responded better to treatment than did day 7 tumors). The authors suggest that the antitumoral activity of IL-1 may be due to direct cytotoxicity for the tumor cells, facilitated cytolysis of the tumor cells, or chemotactic ef-

fects upon tumoricidal monocytes. In addition, the authors suggest that IL-1 may play a role in the establishment of immunity. In these same tumor systems, it was shown that concomitant therapy with indomethacin augmented the antitumor effect, in part by reducing the efficacious dose (Nakata et al., 1988). Prostaglandin E_2 (PGE_2) produced by the host cells in response to IL-1 and/or by the tumor mass may disrupt the antitumor activity of IL-1. PGE_2 has known immunosuppressive properties for NK and macrophage-mediated cytotoxicity (Lala et al., 1986; Kunkel et al., 1986), T cell mitogenic responses (Barker et al., 1981) and cytokine production by lymphocytes (Walker et al., 1983). The investigations by Nakamura et al. (1986) were among the first which indicated a possible role for IL-1 therapy in the treatment of neoplasia. Disadvantages to the methods included the high doses of IL-1 required for tumor regression, and the appearance of tumor immunity in only one tumor model. In addition, the authors did not investigate possible mechanisms for the IL-1 effects.

More recently, investigators have examined the effect of IL-1 therapy on the generation of systemic immunity, in addition to regression of primary tumors. Ebina and Ishikawa (1989) investigated the antimetastatic effects of recombinant human IL-1 beta. They found that intratumoral injections of IL-1 inhibited the growth of Meth A solid tumors. IL-1 treatment lead to the complete regression of established tumors, and resistance to reinoculated tumors. In a double grafted tumor system, the authors found that intratumoral injections of IL-1 in one location had an effect on tumor growth in another region. This effect was dependent upon the presence of tumor cells in

the region of IL-1 injection. IL-1 therapy appeared to induce responses to the non-treated tumor of the double grafted tumor system, and therefore may bring about the regression of metastatic tumors. Spleen cells from immunized animals were capable of adoptively transferring tumor resistance.

The ability of IL-1 to induce systemic immunity was also shown by its use as an adjuvant in specific immunotherapy (McCune and Marquis, 1990). These authors administered IL-1 therapy in conjunction with a vaccine of irradiated line 1 alveolar cell lung carcinoma cells. Animals were rechallenged with viable tumor; those which had received 120-360 ng/dose of IL-1 therapy demonstrated an enhanced ability to survive tumor rechallenge. IL-1 therapy could be administered either locally (at the chest wall inoculation site) or distally (in the leg). Higher doses of IL-1 (720 ng/animal) proved to be less effective therapeutically, and produced signs of illness in the mice. Some suppression of weight gain was seen with 8 daily doses of 360 ng/animal. IL-1 alpha, IL-1 beta, and an IL-1 peptide (163-171) all showed antitumor effects. These experiments suggest that IL-1 can act as a systemic adjuvant in this tumor system. Because IL-1 therapy was administered only at the time of tumor vaccination, the authors suggest that IL-1 acts through its effects on cell populations, rather than directly against the tumor.

Other investigators examined the effects of the IL-1 peptide (163-171) on the tumor immune response. This peptide is proposed to have the immunostimulatory effects of IL-1, without the potentially harmful inflammatory effects (Antoni et al., 1986). Forni et al.

(1989) examined the effects of both IL-1 beta and the IL-1 peptide on the poorly immunogenic CE-2 tumor in BALB/c mice. The peptide was administered at the tumor challenge site for 10 daily injections. While consistent inhibition of tumor growth was noted, it was less than that seen with intact IL-1 beta. The authors also investigated the effect of IL-1 therapy upon the efficacy of adoptive immunotherapy. Tumor cells were admixed in a 1 to 10 ratio with non-reactive lymphocytes from tumor-bearing animals. Strong inhibition of tumor growth was demonstrated by animals which received local IL-1 therapy along with the lymphocytes. This activity was abolished if the recipient animals were sublethally irradiated, treated with cyclosporin A, or if $L3T4^+$ or asialo $GM1^+$ cells were removed. The authors suggest that $L3T4^+$ cells may enhance host tumor reactivity by the release of cytokines.

While investigators have reported direct IL-1 mediated cytostatic and cytotoxic activities against some tumor cell lines (Onozaki et al., 1985b; Lachman et al., 1986), there is evidence to suggest that the antitumor effects of IL-1 are mediated in vivo by the action of various cell populations. As noted previously, IL-1 has multiple effects upon immunocompetent cells such as NK cells, T lymphocytes, and macrophages. A series of investigations utilizing antibody treatment of cell populations, irradiated animals, and nude mice have implicated T lymphocytes as potential mediators of the in vivo antitumor activities of IL-1 (Izumi et al., 1985; North et al., 1988; Belardelli et al., 1989).

In mice transplanted with highly metastatic Friend leukemia

cells, subcutaneous injections of IL-1 beta resulted in a marked increase in survival time, and inhibition of metastatic tumor growth in liver and spleen (Belardelli et al., 1989). Subcutaneous therapy with IL-2 resulted in inhibition of tumor growth, but not prevention of metastases. Combined treatment with IL-1 and IL-2 produced a synergistic antitumor effect resulting in 60% survival. IL-1 and IL-2 therapy had no antitumor effects in animals which had been treated with anti-Thy 1.2 antibody, or in nude mice. These experiments demonstrate the ability of local IL-1 therapy to prevent the formation of metastases. The inclusion of IL-2 injections with the IL-1 therapy enhanced this effect. The antitumor activities of IL-1 and IL-2 appeared to be dependent on host T lymphocytes.

Izumi et al. (1985) evaluated the effect of in vivo administration of IL-1-containing culture fluid supernatants (CFS) on the induction of tumor-specific immunity. Animals were injected with viable syngeneic X5563 cells, followed by 5 consecutive subcutaneous or intraperitoneal injections of CFS. Lymph node cells or spleen cells from the CFS-treated animals exhibited enhanced CTL and delayed-type hypersensitivity (DTH) responses when compared with tumor-bearing animals which did not receive treatment. CTL responses were measured by in vitro sensitization with mitomycin c treated X5563, followed by cytotoxicity assays. DTH responses were measured by adoptive transfer of immune cells and mitomycin c treated X5563 cells, followed by the measurement of footpad swelling 24 hours after transfer. Lymph node and spleen cells from treated animals also exhibited complete tumor-neutralizing effects in Winn-type adoptive transfer assays. Antibody

depletion experiments demonstrated that Lyt 1^+2^- T cells were responsible for both the DTH and tumor-neutralizing activities. Depletion of Thy 1.2^+ or Lyt 1.1^+ cells decreased these immune activities, while depletion of Lyt 2.1^+ cells had no effect. The *in vivo* protective immunity was tumor-specific as demonstrated by the lack of activity against MH134 cells. These experiments demonstrated that IL-1 may increase CTL priming in lymph node and spleen, as well as increasing DTH and tumor-neutralizing activity. As suggested previously (Fujiwara *et al.*, 1984), the adoptively transferred tumor-neutralizing activity appeared to be more closely associated with the DTH response, rather than with CTL generation. Because these authors used lipopolysaccharide (LPS)-stimulated culture supernatants instead of recombinant IL-1, it is unclear which effects are directly attributable to IL-1 activity, and which may be the result of other factors. In addition, the actual doses of IL-1 administered are difficult to determine because the injection protocols list only the volume of supernatant injected. However, these experiments do suggest a possible role for IL-1 in the induction of tumor-specific immune effects, and imply that T lymphocytes may be mediators of these activities.

In an immunogenic murine tumor model, North *et al.* (1988) found that systemic IL-1 therapy could induce T-cell-mediated tumor regression. Intraperitoneal injections of human recombinant IL-1 alpha were shown to cause complete regression of a relatively large, immunogenic murine sarcoma growing subcutaneously. The efficacy of IL-1 therapy was dependent on the number of IL-1 injections and the time at which

they were administered. Intraperitoneal IL-1 therapy was shown to be most effective against subcutaneous SAI sarcoma, and less effective against L51784 and P815. The authors suggest that this difference may be due to immunogenic differences between the tumors, and that less immunogenic tumors may be less responsive to IL-1 therapy. The tumor regression was dependent upon underlying concomitant T-cell mediated immunity, as determined by the timing of most effective therapy and experiments with various phenotypic cell populations. IL-1 therapy was most effective when administered after an immune response to the tumor was underway, and was not therapeutic if given too early during tumor growth. In addition, experiments in T cell deficient mice infused with donor spleen cells showed that removal of either $CD4^+$ or $CD8^+$ cell populations abrogated the antitumor effects. In this system, IL-1 appeared to promote tumor regression by stimulating an increase in SAI-sensitized T cells, thereby converting a subtherapeutic cell number to a therapeutic response. IL-1 augmented the response to tumors of sufficient immunogenicity by expanding host populations responsive to the tumor. These experiments showed that systemic IL-1 therapy was capable of mediating effects against tumors, and that T lymphocytes were involved in this response.

Taken together, the investigations performed thus far suggest that IL-1 may be useful in the treatment of immunogenic tumors. However, few of the groups have examined the immunological basis of the increased tumor protection induced by IL-1 treatment. Investigators have found that the augmenting effect of IL-1 may be mediated by T lymphocytes in immunogenic tumor systems (North et al., 1988; Belar-

delli et al., 1989). These authors were able to demonstrate immunity only with immunogenic tumors, and suggest that weakly immunogenic tumors are less likely to respond to IL-1 therapy. In most of the tumor systems examined, relatively large doses of IL-1 are necessary to promote tumor survival (Nakamura et al., 1986, North et al., 1988). These high doses may cause the disruption of other physiological processes affected by IL-1 administration. Few examinations have been made of low-dose IL-1 therapy in the treatment of weakly immunogenic tumors, or of its use in conjunction with other forms of anticancer treatment.

In the current investigation, the tumor system to be examined is the EL-4 lymphoma of C57Bl/6 mice. EL-4 is an NK-resistant T-cell lymphoma of low immunogenicity, which rapidly progresses to produce a high degree of mortality in its syngeneic host (Cantrell et al., 1979; Gorer, 1950). Non-viable microorganisms have been shown to exert antitumor effects against the EL-4 lymphoma in vivo (Mathews, 1981; McDaniel and Cozad, 1982). Animals which received the EL-4 lymphoma without local adjuvant therapy with Corynebacterium parvum succumbed to the tumor (Hornung and Mathews, 1985; Hornung, dissertation, 1987). In contrast, animals which received C. parvum showed an increased survival rate with a high percentage of the animals remaining tumor-free. However, when these animals were rechallenged with EL-4 tumor cells, none of the animals survived. These results suggested that no systemic immunity was generated as a consequence of C. parvum treatment of the EL-4 lymphoma. These results are consistent with previous reports which demonstrated that adjuvant therapy of less immunogenic

tumors resulted in survival of the animals, but no subsequent systemic immunity to the tumor (Dye et al., 1981; McDaniel and Cozad, 1982).

An experimental protocol has been developed, whereby systemic IL-1 therapy is administered in conjunction with local adjuvant therapy (Hornung and Mathews, 1985; Hornung, dissertation, 1987). Animals treated with both IL-1 and C. parvum at the time of primary tumor challenge demonstrate significantly enhanced survival upon rechallenge. The survival rate was related in a dose dependent manner to the amount of IL-1 administered. Maximal survival was seen with the administration of 500 U (60 ng) of IL-1 per injection (2500 U total dose). The effective IL-1 doses were much lower than those used by other investigators. These results suggest that administration of IL-1 during the primary tumor challenge modifies the host environment, resulting in an enhancement of tumor immunity.

In summary, it is apparent that IL-1 can function to augment a variety of compartments of the immune system which may play a role in host immunity. As described earlier, other investigators have examined the use of IL-1 as a therapeutic agent in various tumor systems. However, the ability of IL-1 to augment other forms of immunotherapy has not been well characterized. In particular, little research has been conducted utilizing tumors of weak immunogenicity, which may require multiple modalities of treatment. In addition, most studies used relatively large doses of IL-1, which may have deleterious effects in vivo.

The objective of this study was to investigate the nature of the local lymphoid responsiveness generated by IL-1 treatment in an ex-

perimental murine tumor model, and to evaluate low-dose IL-1 as an immune-augmenting agent in animals undergoing adjuvant therapy of a weakly immunogenic tumor.

CHAPTER III

MATERIALS AND METHODS

A. Animals.

Female C57Bl/6 mice, 7 to 8 weeks of age were obtained from Jackson Laboratories (Bar Harbor, ME) and acclimated for one to two weeks prior to use. Cell number, phenotypic, proliferative, and cytotoxic experiments were performed concurrently using three groups of five animals each, per treatment protocol, per day post tumor challenge (225 total). Adoptive transfer assays were performed using a total of 154 animals as cell donors, and 160 animals as cell recipients. Limiting dilution analysis experiments were performed using groups of 10 animals per treatment group, per day post tumor challenge (210 total). Antigenic responsiveness assays were performed on 5 animals per group, per day (40 total).

B. Cytokines.

Recombinant human IL-1 alpha (specific activity 8.6 x 10^6 U/mg) was generously provided by Dr. Peter Lomedico (Hoffmann-LaRoche Inc., Nutley, N.J.). One unit of activity produced half maximal proliferation in the thymocyte co-stimulation assay. Prior to use in therapeutic injections, the IL-1 was diluted in Hank's balanced salt solution (HBSS, GIBCO Laboratories, Grand Island, NY) adjusted to pH 7.4 with sodium bicarbonate and supplemented with 0.01% human serum albumin. Human recombinant IL-2 (activity 2.5 x 10^6 U/ml) was provided by Cetus Corporation (Emeryville, CA).

C. Cell lines.

The EL-4 lymphoma, a 7,12 dimethylbenz(a)-anthracene induced tumor syngeneic in the C57Bl/6 mouse, was obtained from Dr. R. Herberman. The YAC-1 lymphoma is a Moloney murine virus-induced lymphoma (Sjogren and Hellstrom, 1965) which was obtained from Dr. J. Clancy, Loyola University of Chicago, Maywood, IL. The P815 mastocytoma (ATCC-TIB-64)(Plaut et al., 1973) was obtained from the American Type Culture Collection (Rockville, MD). The Lewis lung carcinoma (LLC) was obtained from Dr. M.R. Young, Hines Veterans Administration Hospital, Hines, IL.

The cell lines were maintained in vitro in complete medium [RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS, Hazleton Biologics Inc., Lenexa, KS), 2 mM L-glutamine, 0.1 mM non-essential amino acids, 100 U/ml penicillin, 100 ug/ml streptomycin, 0.25 ug/ml fungizone (GIBCO Laboratories, Grand Island, NY), and containing 5×10^{-5} 2-mercaptoethanol (Sigma Chemical Co., St. Louis, Mo.)].

D. In vivo tumor model.

1. Tumor inoculation.

Mice were inoculated intradermally in the right flank with 0.2 ml of an admixture of 5×10^{9} heat-inactivated Corynebacterium parvum and 5×10^{3} EL-4 lymphoma cells in HBSS. Animals receiving IL-1 therapy were treated with five equal dosages of 0.1 ml each, administered intraperitoneally on alternate days beginning on the day of tumor challenge. Except where noted, animals received 500 U of IL-1 per injection. The C. parvum strain 4182 was obtained from the Pasteur

Institute (Paris, France) and cultured anaerobically. The organisms were heat-killed at 80°C for 30 min prior to use. EL-4 cells for tumor injections were maintained in vivo by weekly passage, and were removed from the peritoneal cavity and washed once in HBSS immediately before use.

2. Lymph node cell preparation.

Axillary and brachial lymph nodes draining the tumor site were excised. Single cell suspensions were prepared in HBSS by passage through 60-gauge wire mesh and the cells were washed and counted by hemocytometer. Cell viability was determined by trypan blue exclusion.

E. Phenotypic analysis of lymph node cell populations.

Antibodies used to analyze the phenotype of various lymph node cell populations included; monoclonal anti-mouse CD8 (anti-Lyt 2.2, clone 53-6.7, biotin-conjugated), monoclonal anti-mouse Thy 1.2 (clone 30H12, biotin-conjugated), and monoclonal anti-mouse CD4 (anti-L3T4, clone GK 1.5, phycoerythrin-conjugated), all from Becton-Dickinson (Mountain View, CA). Streptavidin-phycoerythrin was obtained from Vector Laboratories (Burlingame, CA).

Lymph node cells (1×10^6 per marker assessed) were prepared for phenotyping by washing once with cold phosphate buffered saline (PBS, 0.145 M sodium chloride, 0.02 M sodium phosphate, pH 7.4). The cells were incubated with the primary antibody for one half h in an ice bath. Following washes in cold PBS, cells were incubated with the secondary antibody for an additional half h on ice, protected from light. The cells were washed in cold PBS, and then fixed with 0.1 ml

of 1% paraformaldehyde in 0.15 M NaCl solution and stored at 4°C. The samples were washed and resuspended in 0.1 PBS prior to flow cytometric analysis (FACS Analyzer IFA, Becton-Dickinson, Mountain View, CA). The number of cells expressing each surface marker was determined by multiplying the lymph node cell number by the percent positive cells.

F. Adoptive transfer assay.

Lymph node cells, prepared as in D.2., were admixed with 5 x 10^3 EL-4 and injected intradermally into naive animals. Lymph node cell to EL-4 ratios of 500:1 in a total volume of 0.5 ml were used. Other animals were injected with the EL-4 tumor alone. In some experiments certain cell populations were removed by complement-mediated depletion prior to use in the adoptive transfer assay. The rate and size of tumor formation were assessed by measurement of perpendicular diameters on various days following tumor inoculation.

G. Complement-mediated cell depletion.

Antibodies used to deplete certain lymph node cell populations included monoclonal anti-CD4 (anti-Lyt 1.2, clone CG16, 1:10 dilution) and monoclonal anti-CD8 [anti-Lyt 2.2, clone AD4(15), 1:20 dilution] both from Accurate Chemical and Scientific Corp. (Westbury, NY). Rabbit anti-asialo GM1 was obtained from Wako Chemicals USA, Inc. (Dallas, TX), and used at a 1:33 dilution. Antibodies were diluted in HBSS with 1% FBS immediately prior to use.

Lymph node cell preparations were depleted of cells bearing specific cell markers by incubating 1 x 10^7 lymph node cells with 0.5 ml of diluted antibody for 1 h in an ice bath. The cells were washed 3

times with HBSS with 1% FBS to remove excess antibody and then 100 ul of Low Tox M rabbit complement (Accurate Chemical and Scientific Corp., Westbury, NY) was added to the cell pellet. After 1 h of incubation in a 37°C water bath, the cells were washed and resuspended and cell viability determined by trypan blue exclusion. These cells were then used in adoptive transfer assays as described in section F.

H. IL-2 responsiveness.

1. In vitro proliferation in response to IL-2.

Lymph node cells prepared as in D.2. were cultured in 96-well flat-bottomed plates (Corning Glass Works, Corning, NY) at 5 x 10^5 cells/ml in complete medium containing 1500 U/ml of IL-2 (total well volume equaled 0.2 ml). The lymph node cells were incubated at 37°C and 5% CO_2 for 5, 6 or 7 days, after which 1.0 uCi of [^{3}H]thymidine (ICN Radiochemicals, Irvine, CA) was added to each well. After 6 h of incubation, the cells were harvested with a Cambridge PHD automated cell harvester (Cambridge Technology, Cambridge, MA) and the amount of radiolabel incorporation for each sample determined using a Beckman LS 5801 liquid scintillation counter (Beckman Instruments, Irvine, CA). Data are expressed as stimulation indices (SI):

$$SI = \frac{\text{dpm experimental}}{\text{dpm control}} .$$

2. In vitro stimulation with IL-2.

Lymph node cells, prepared as in D.2., were cultured in 24-well plates (Becton-Dickinson & Co., Lincoln Park, NJ) at 2.5 x 10^6/ml in complete medium containing 1500 U/ml of IL-2 (total well volume

equaled 1 ml). After 7 days of culture at 37°C and 5% CO_2, the cells were removed from the plates and collected by centrifugation. The cell pellet was resuspended in 5 ml of HBSS and layered onto 5 ml of lymphocyte separation medium (Organon Teknika Corp., West Chester, PA). After centrifugation for 20 m, the viable cells were collected from the interface, washed once in HBSS, resuspended in complete medium, and quantified as in section D.2. Some of these cells were used in chromium release assays as described in section I. below.

I. Chromium release assay.

Tumor cells were radiolabeled by incubation of 2 x 10^6 cells with 100 uCi sodium [^{51}Cr]chromate (DuPont NEN, Wilmington, DE) for 1 h at 37°C with frequent agitation. After three washes in HBSS, the cells were resuspended in complete medium at 5 x 10^4/ml and 0.1 ml volumes placed in 96-well round-bottomed plates (Corning Glass Works, Corning, NY) with varying effector cell numbers. Effector to target ratios of 50:1, 25:1, 12:1 and 6:1 were utilized. All assessments were performed in triplicate. Following 4 h of incubation the supernatants were removed using a Skatron harvesting press (Skatron Inc., Sterling, VA) and associated radioactivity determined with a 4/600 ME PLUS automatic gamma counter (ICN Micromedic Systems, Horsham, PA). Percent specific release was calculated by the following formula:

$$\% \text{ specific release} = \frac{(\text{cpm experimental}) - (\text{cpm spontaneous})}{(\text{cpm maximum}) - (\text{cpm spontaneous})} \times 100.$$

Spontaneous release was determined by incubation of labeled target cells with medium alone. Maximum release was determined by 0.5% Non-

idet P-40 (Sigma Chemical Co., St. Louis, MO) lysis of labeled cells. Linear regression analysis was used to determine the number of lytic units/10^7 cells. One lytic unit was defined as the number of cells required to specifically lyse 30% of the target cells.

J. Limiting dilution assay.

Lymph node cells were harvested from animals injected with C. parvum and EL-4, or C. parvum and EL-4 with IL-1 therapy. Axillary and brachial lymph nodes were removed on various days post-tumor challenge, and single cell suspensions prepared. The cells were cultured in 96-well plates at concentrations of 1 x 10^4, 5 x 10^4 and 1 x 10^5/well with 12 replicates of each dilution. Following a 7 day incubation with 120 U/well (800 U/ml) of IL-2, 5 x 10^3 chromium-labeled target cells were added to each well. On a per cell basis, the concentration of IL-2 used in the limiting dilution assay corresponds to at least twice that used in the IL-2 stimulation protocol above. EL-4, YAC-1 and P815 were used as tumor targets. After 4 h of incubation at 37^{o}C, the amount of chromium released in each well was quantified as in Section I. above. The wells were scored as positive for cytotoxic activity when the chromium release exceeded 3 SD over the minimum release of chromium. Estimates of precursor frequencies were made by the minimal chi-square method (Taswell, 1981).

Preliminary experiments with lower IL-2 doses in culture resulted in a non-linear relationship between the fraction of non-responding wells and the input cell number, when plotted semi-logrithmically. This phenomenon was particularly apparent at low lymph node cell numbers and may indicate the loss of a cell necessary

for cytotoxic development. In order for accurate assessments of precursor frequency to be made, the precursor must be the only limiting factor (Miller, 1982). The use of supraoptimal IL-2 concentrations overcame this deficiency and permitted assessment of precursor frequency.

K. Antigenic responsiveness assay.

1. Secondary tumor challenge.

IL-1 treated and untreated animals which survived the primary tumor challenge were rechallenged on the opposite (left) flank with either 5 x 10^4 EL-4 or LLC in 0.1 ml of HBSS. Axillary and brachial lymph nodes draining the site of rechallenge were removed on various days post secondary challenge and were assessed for antigenic responsiveness to EL-4 or LLC in vitro as described below.

2. Stimulation in vitro.

EL-4 or LLC cells (4.5 x 10^7) were incubated with 100 ug of sterile-filtered mitomycin c (Sigma Chemical Co., St. Louis, MO) in a 1 ml volume of complete medium with 1% FBS in a 37^{o}C water bath with agitation at frequent intervals. After 1 h of incubation the cells were washed 3 times with HBSS and resuspended in complete medium with 1% FBS.

Lymph node cells from animals undergoing secondary tumor challenge (see above) were used as responder cell populations. The responders were plated at 5 x 10^5 cells/well with either 2 x 10^5 or 4 x 10^5 of EL-4 or LLC. Control wells consisted of responders alone or stimulators alone. All assessments were performed in replicates of three or five. The cells were incubated for 7 days, after which 1 uCi

of [^{3}H]thymidine (ICN Radiochemicals, Irvine, CA) was added to each well. After 6 h of incubation the cells were harvested and analyzed as described above.

L. Statistical analysis.

Statistical analyses of differences in cell number, proliferation, and cytotoxicity between IL-1 treated and untreated mice were performed by the two-tailed Students t-test. The 95% confidence levels obtained from the minimal chi-square analysis (Taswell, 1981) were used to analyze the limiting dilution data. The two-tailed Students t-test was used to assess differences in day of tumor appearance and animal survival in the adoptive transfer assays, while chi-square analysis was used to assess total survival data.

CHAPTER IV

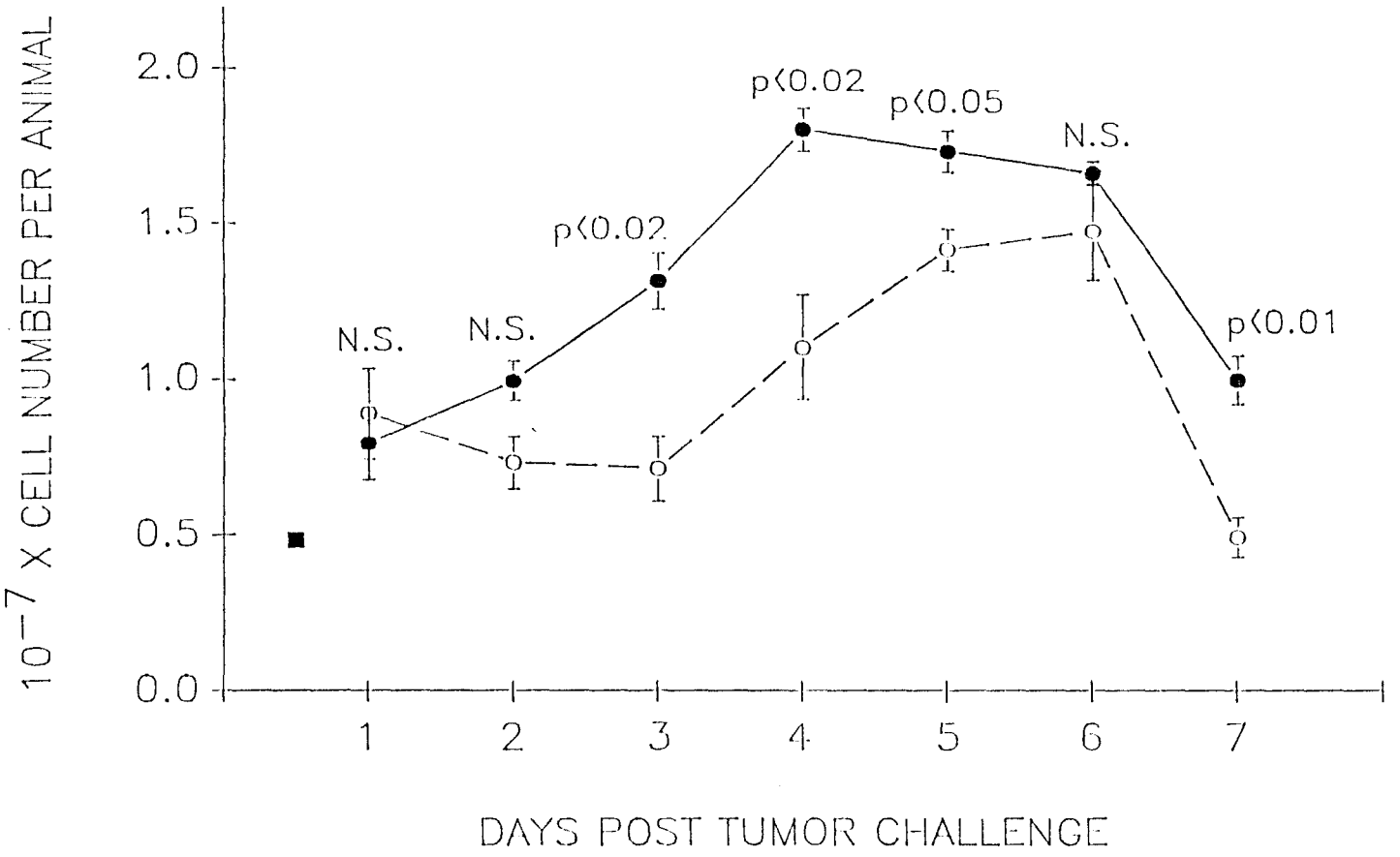
RESULTS

Systemic administration of interleukin-1, in conjunction with adjuvant therapy, provides for animal survival and the generation of tumor immunity in the tumor model utilized (Hornung and Mathews, 1985). IL-1 may augment local lymphoid responsiveness to the tumor, and therefore allow for enhanced host survival. An examination was made of the lymph nodes draining the site of tumor challenge in order to assess the ability of IL-1 to modulate local immune responsiveness.

A. Effect of IL-1 treatment on lymph node cell number.

The ability of IL-1 to increase the number of lymph node cells draining the site of antigenic challenge was evaluated. Lymph node cells were derived sequentially from animals that had been challenged with adjuvant and tumor and either administered IL-1 (500 U/injection) or no further treatment (225 total animals; 3 groups of 5 each, per treatment group, per day post tumor challenge). An earlier increase in lymph node cell number was observed in animals which received IL-1, when compared to those animals which did not (Fig. 1.). No difference in lymph node cell number was observed between the two groups of animals on days 0 through 2 post tumor and adjuvant administration. When compared to the day 1 values, both groups showed a cell number increase beginning on day 3 for the IL-1 treated ($p<0.01$) and on day 5 for the non-IL-1 treated animals ($p<0.05$). This cell number increase continued until day 6. The cell numbers for the IL-1 treated animals

Fig. 1. Effect of IL-1 treatment on lymph node cell number. Brachial and axillary lymph nodes draining the site of tumor challenge were sequentially derived from IL-1 treated (●) and non-IL-1 treated (O) animals. Single cell suspensions were prepared and the total number of lymph node cells/animal determined by trypan blue exclusion. Cell number values represent the mean of 3 groups of 5 animals each ± SE. Average lymph node cell numbers for naive animals ($4.8 \pm 0.091 \times 10^6$) are represented by the filled square (■). In this experimental model, tumor cells do not appear in the regional lymph nodes prior to 17 days after intradermal injection.

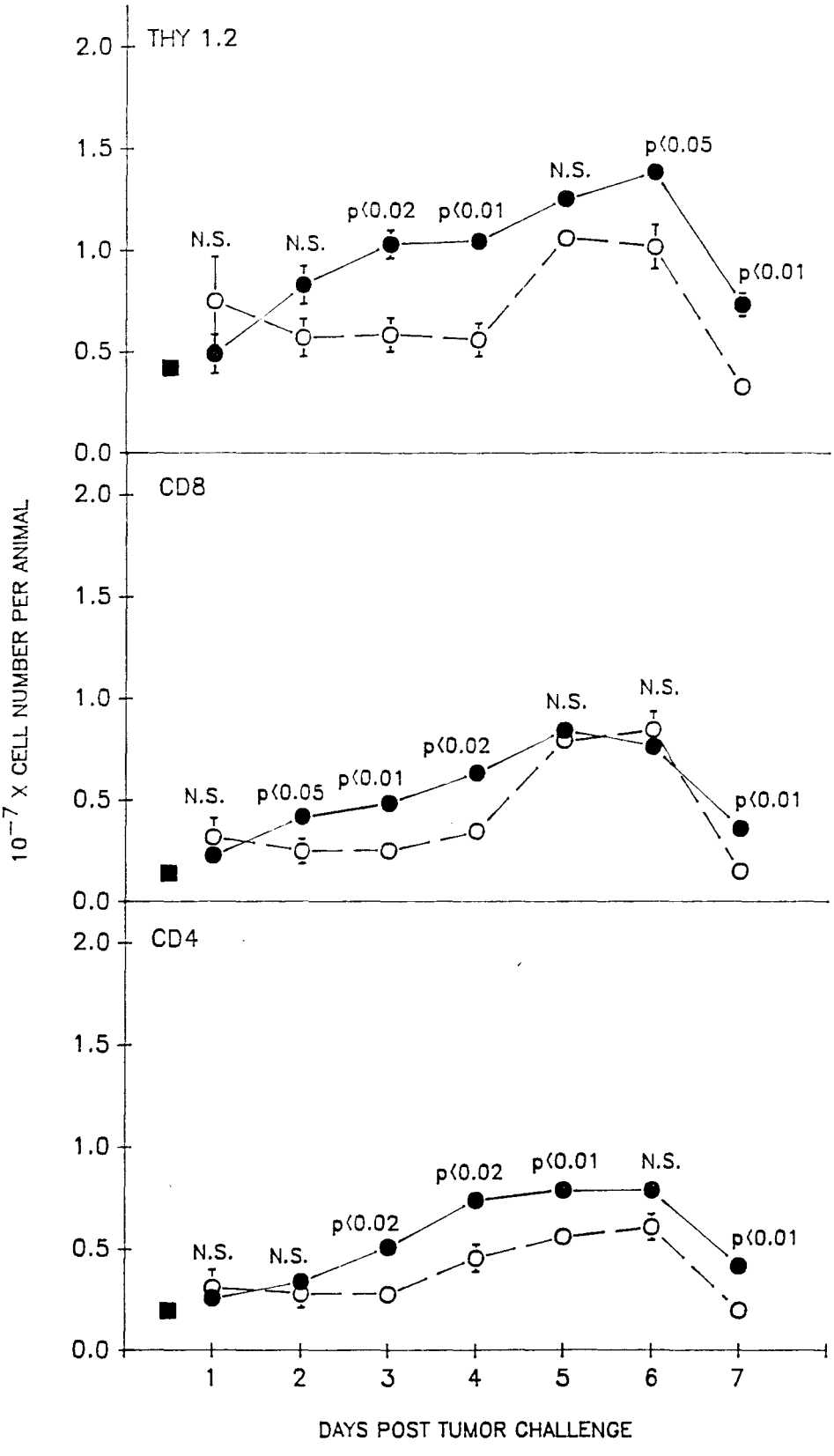

10^{-7} X CELL NUMBER PER ANIMAL
2.0
1.5
1.0
0.5
0.0
1
2
3
4
5
6
7
DAYS POST TUMOR CHALLENGE
N.S.
N.S.
p<0.02
p<0.02
p<0.05
N.S.
p<0.01

were significantly higher when compared to the non-IL-1 treated group on days 3 ($p<0.02$), 4 ($p<0.02$), and 5 ($p<0.05$) post tumor challenge. Maximal cell numbers were recovered on day 4 for the IL-1 treated group and day 6 for the non-IL-1 treated animals. Lymph node cell numbers for both of the treatment groups declined by day 7. These data indicate that systemic IL-1 therapy significantly increased the number of lymph node cells draining the site of antigenic challenge in animals bearing tumors. The increase in lymph node cell number was both earlier and greater than that seen in non-IL-1 treated animals.

B. Phenotypic analysis of draining lymph node cells.

The observed increase in lymph node cell number may represent an overall expansion of cells in the lymph nodes, or alternatively may represent an increase in only a single cell population. Using the same animals as above, phenotypic analysis of draining lymph node cells was performed in order to determine the cell types responsible for the cell number increase. Lymph node cells from IL-1 treated and non-IL-1 treated animals were assessed for their expression of Thy 1.2, CD8, and CD4 cell surface markers. The actual number of cells bearing these surface markers increased in the IL-1 treated animals, when compared to the non-IL-1 treated group (Fig. 2). The cell number increase was due predominately to an increase in Thy 1.2^+ cells. Enhanced numbers of Thy 1.2^+ cells were present in the IL-1 treated animals on days 3 ($p<0.02$) and 4 ($p<0.01$) post tumor challenge, with maximal numbers on day 6. The cell number increase was further characterized by assessing the expression of CD4 and CD8 cell surface markers. Phenotypic analysis determined that the cell number increase

Fig. 2. Phenotypic analysis of lymph node cells. Lymph node cells from IL-1 treated (●) and non-IL-1 treated (○) animals were assessed for their expression of T cell markers. The positive cell number/animal was determined by multiplying the percent positive cells by the lymph node cell number/animal and is expressed as the mean value ± SE. The filled squares (■) represent the number of cells expressing these surface markers in naive animals: $4.2 \pm 0.079 \times 10^6$ for Thy 1^+, $1.42 \pm 0.027 \times 10^6$ for $CD8^+$, and $1.96 \pm 0.037 \times 10^6$ for $CD4^+$. Data points represent the mean of three groups of 5 animals each.


THY 1.2
CD8
CD4
2.0
1.5
1.0
0.5
0.0
N.S.
N.S.
p<0.02
p<0.01
N.S.
p<0.05
p<0.01
N.S.
p<0.05
p<0.01
p<0.02
N.S.
N.S.
p<0.01
N.S.
N.S.
p<0.02
p<0.01
N.S.
p<0.01
1
2
3
4
5
6
7
10^{-7} X CELL NUMBER PER ANIMAL
DAYS POST TUMOR CHALLENGE

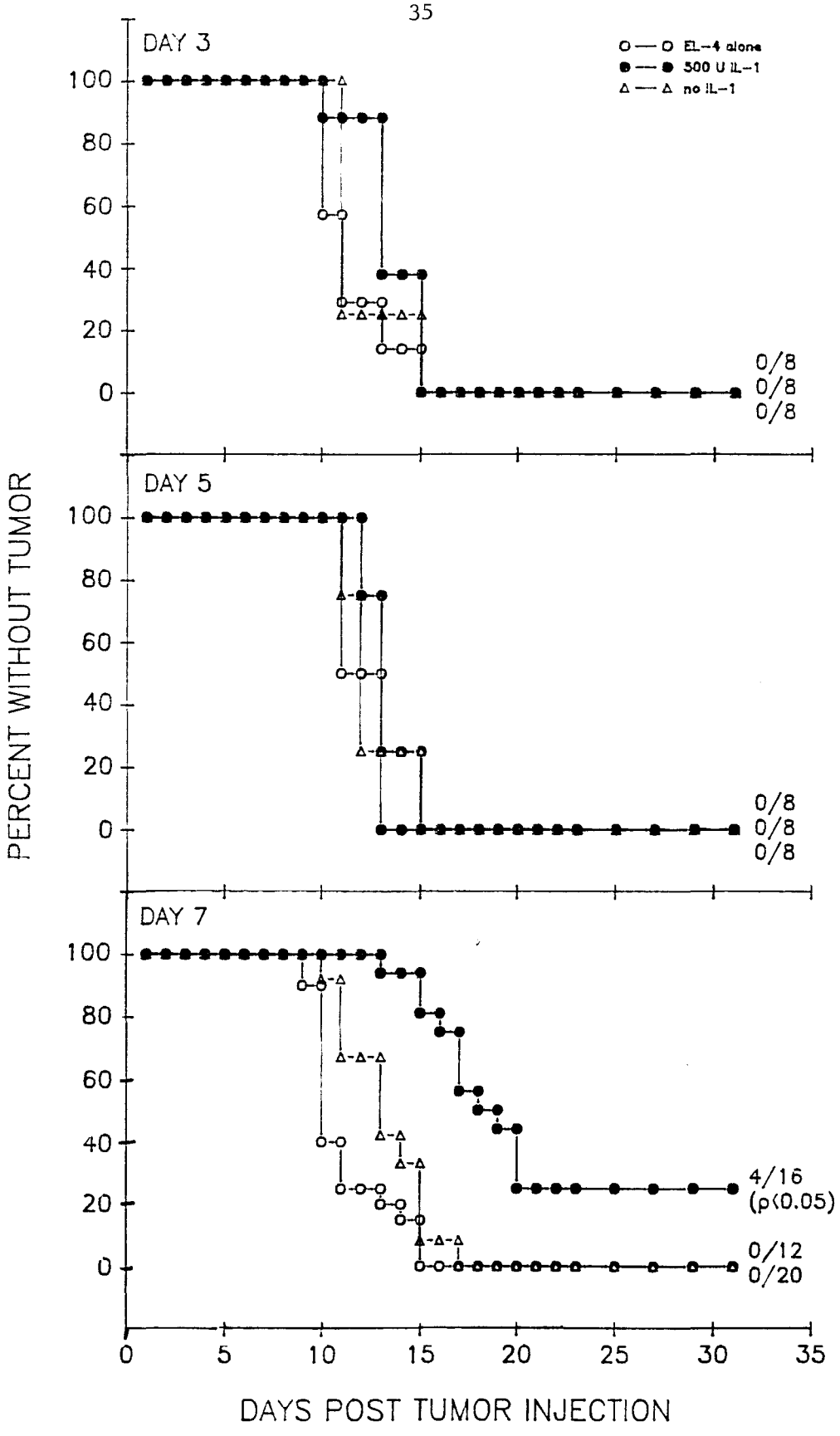
in IL-1 treated animals was due to an increase in both CD4 and CD8 cells. The $CD8^+$ cell numbers were higher in the IL-1 treated animals on days 2 ($p<0.05$), 3 ($p<0.02$) and 4 ($p<0.02$) post tumor challenge, while an increased number of $CD4^+$ cells were present on days 3, 4, and 5 ($p<0.02$, $p<0.02$, and $p<0.01$, respectively). The $CD8^+/CD4^+$ ratio remained constant (~1.0) regardless of the treatment group. These data indicate that IL-1 treatment increased numbers of T lymphocytes in the lymph nodes draining the site of tumor challenge. The increase in T lymphocytes was a consequence of increases in either $CD4^+$ or $CD8^+$ lymphocytes.

C. Adoptive transfer assays.

1. Effect of lymph node cells on tumor development and animal survival.

Increased numbers of T lymphocytes in the draining lymph nodes of IL-1 treated animals may enhance the ability of these animals to respond to the tumor. Adoptive transfer assays were utilized to assess the ability of lymph node cells from IL-1 treated and non-IL-1 treated animals to mediate tumor protective effects against EL-4 *in vivo*. Lymph node cells were derived from IL-1 treated and non-IL-1 treated animals, admixed with EL-4, and adoptively transferred into naive mice (day 3, n = 8 per group; day 5, n = 8 per group; day 7, n = 20 for IL-1 treated group, n = 12 for non-IL-1 treated group, and n = 20 for EL-4 alone group). Lymph node cells which were derived 7 days post tumor challenge from animals which received systemic IL-1 therapy demonstrated an ability to delay tumor development, while lymph node cells derived 3 or 5 days after tumor challenge did not (Fig. 3). An

Fig. 3. Effect of day 3, 5 and 7 lymph node cells from IL-1 treated and non-IL-1 treated animals on tumor development. Lymph node cells from IL-1 treated (●) and non-IL-1 treated (Δ) animals were removed on days 3, 5, and 7 post primary tumor challenge. The cells were admixed with a 5 x 10^3 EL-4 in a 500:1 lymph node cell to EL-4 ratio and injected intradermally into naive animals (day 3, n = 8 per group; day 5, n = 8 per group; day 7, n = 20 for EL-4 alone group, n = 20 for IL-1 group, n = 12 for non-IL-1 group). The animals were examined periodically for the appearance of tumors. Animals injected with EL-4 alone (○) were assessed also. These data are the summary of 3 experiments.


DAY 3
O — O EL-4 alone
● — ● 500 U IL-1
Δ — Δ no IL-1
100
80
60
40
20
0
0/8
0/8
0/8
DAY 5
100
80
60
40
20
0
0/8
0/8
0/8
DAY 7
100
80
60
40
20
0
4/16
(p<0.05)
0/12
0/20
0
5
10
15
20
25
30
35
PERCENT WITHOUT TUMOR
DAYS POST TUMOR INJECTION

equal number of day 7 lymph node cells from non-IL-1 treated donors also had no effect. Twenty-five percent (4/16) of the animals which received day 7 lymph node cells from IL-1 treated animals did not develop tumors. This percentage was significantly ($p<0.05$) higher than for animals which received either day 7 lymph node cells from non-IL-1 treated animals (0/12 without tumor), or EL-4 alone (0/20 without tumor). In addition, the animals which received lymph node cells from IL-1 treated animals and did develop tumors (75% of the total) did so later than animals in the other treatment groups. As shown in Table 1, the mean day of tumor appearance was significantly later in these animals when compared to animals which received lymph node cells from non-IL-1 treated animals ($p<0.001$) or EL-4 alone ($p<0.001$).

Adoptive transfer of day 7 lymph node cells from IL-1 treated animals also allowed for enhanced animal survival, while cells from 3 or 5 days post tumor challenge did not (Fig. 4). Animals which received cells from IL-1 treated animals exhibited significantly ($p<0.05$) higher total survival when compared to animals which received cells from non-IL-1 treated animals or EL-4 alone. The surviving animals were immune to a subsequent rechallenge with EL-4. In addition, the animals which received lymph node cells from IL-1 treated animals, but did not survive, lived longer than the animals in the other treatment groups. As shown in Table 2, the mean survival in days was significantly longer in these animals when compared to animals which received lymph node cells from non-IL-1 treated animals ($p<0.005$) or EL-4 alone ($p<0.001$). These data indicate that lymph

Table 1. Effect of day 3, 5, and 7 lymph node cells from IL-1 treated and non-IL-1 treated animals on tumor development.[a]

	Mean Day of Tumor Appearance		
Lymph node cell source (Day)	EL-4 alone	Lymph node cells from IL-1 treated animals	Lymph node cells from non-IL-1 treated animals
3	11.4 ± 1.9 (n = 8)	13.4 ± 1.7 (n = 8)	12.0 ± 2.0 (n = 8)
5	12.5 ± 2.0 (n = 8)	12.8 ± 0.5 (n = 8)	12.5 ± 1.7 (n = 8)
7	11.2 ± 2.0 (n = 20)	17.3 ± 2.3[b] (n = 20)	13.1 ± 2.1[c] (n = 12)

[a] Lymph node cells were derived from IL-1 treated and non-IL-1 treated animals on days 3, 5, and 7 post tumor challenge, and adoptively transferred with 5 x 10^3 EL-4 into naive animals. Animals were observed for tumor appearance. Data are expressed as mean values ± SD. Twenty-five percent (4/16) of the animals which received day 7 lymph node cells from the IL-1 treated animals did not develop tumors, while all of the animals in the other treatment groups did. The surviving animals were immune to subsequent rechallenge with EL-4.

[b] $p<0.001$, when compared to EL-4 alone.
$p<0.001$, when compared to the non-IL-1 treated group.

[c] $p<0.02$, when compared to EL-4 alone.

Fig. 4. Effect of day 3, 5, and 7 lymph node cells from IL-1 treated and non-IL-1 treated animals on survival. Lymph node cells from IL-1 treated (●) and non-IL-1 treated (Δ) animals were removed on days 3, 5, and 7 post primary tumor challenge. The cells were admixed with 5 x 10^3 EL-4 in a 500:1 lymph node cell to EL-4 ratio and were injected intradermally into naive animals (day 3, n = 8 per group; day 5, n = 8 per group; day 7, n = 20 for EL-4 alone group, n = 20 for IL-1 group, n = 12 for non-IL-1 group). Tumor growth was assessed by the measurement of perpendicular diameters. Animals with an average tumor diameter of greater than 15mm were considered non-survivors. Animals injected with EL-4 alone (O) were assessed also. These data represent the same animals shown in Fig. 3., and are the summary of 3 experiments.

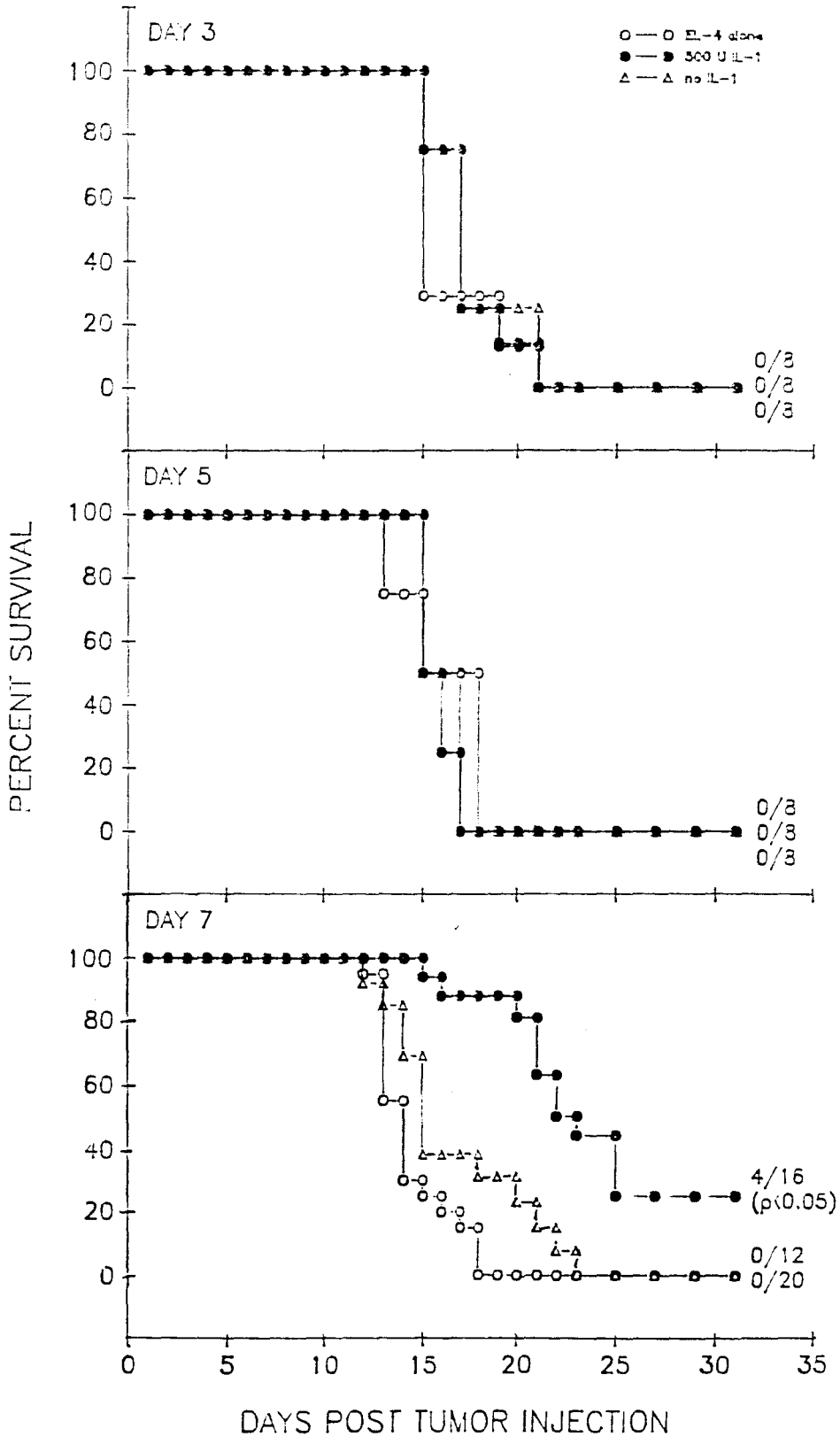

DAY 3
DAY 5
DAY 7
PERCENT SURVIVAL
DAYS POST TUMOR INJECTION
0/8
0/8
0/8
0/8
0/8
0/8
4/16
(p<0.05)
0/12
0/20

Table 2. Effect of day 3, 5, and 7 lymph node cells from IL-1 treated and non-IL-1 treated animals on survival.[a]

Lymph node cell source (Day)	Mean Animal Survival in Days[b]		
	EL-4 alone	Lymph node cells from IL-1 treated animals	Lymph node cells from non-IL-1 treated animals
3	16.1 ± 2.8 (n = 8)	17.3 ± 2.0 (n = 8)	17.5 ± 2.5 (n = 8)
5	16.0 ± 2.4 (n = 8)	15.8 ± 1.0 (n = 8)	16.0 ± 1.2 (n = 8)
7	14.9 ± 2.4 (n = 20)	20.6 ± 3.8[c] (n = 20)	16.8 ± 3.8 (n = 12)

[a] Lymph node cells were derived from IL-1 treated and non-IL-1 treated animals on days 3, 5 and 7 post tumor challenge, and adoptively transferred with 5 x 10^3 EL-4 into naive animals. Animals were observed for survival. Data are expressed as mean values ± SD. These data represent the same animals shown in Table 1.

[b] Animals with an average tumor diameter of greater than 15mm were considered non-survivors.

[c] $p<0.001$, when compared to EL-4 alone.
$p<0.005$, when compared to the non-IL-1 treated group.

node cells from IL-1 treated animals were protective against the EL-4 *in vivo*, while cells derived from animals which did not receive IL-1 had no such protective effect. This was evidenced by delayed tumor development and enhanced animal survival in animals receiving cells from IL-1 treated animals. In addition, protective tumor immunity was generated in the surviving animals, in that all of the animals which remained free of tumor were also resistant to subsequent tumor challenge (4/4 animals).

2. Effect of lymph node cells on average tumor size.

The tumors which developed in animals receiving adoptively transferred cells were measured periodically. As shown in Figure 5, there was no difference in average tumor size between IL-1 treatment groups for animals receiving lymph node cells from days 3 or 5 post tumor challenge. However, the average tumor size of animals receiving day 7 lymph node cells from IL-1 treated animals was signifi-cantly smaller when compared to animals which received cells from non-IL-1 treated animals.

3. Effect of day 7 lymph node and spleen cells on tumor development.

In order to establish whether the capacity to promote *in vivo* effects against EL-4 was a local or systemic immune phenomenon, a comparison was made between the ability of adoptively transferred lymph node and spleen cells to affect tumor development and animal survival. Spleen cells derived 7 days after tumor challenge from either the IL-1 treated or the non-IL-1 treated animals were unable to affect tumor development or animal survival (Fig. 6). As shown in Table 3, animals

Fig. 5. Effect of day 3, 5, and 7 lymph node cells from IL-1 treated and non-IL-1 treated animals on average tumor size. Lymph node cells from IL-1 treated (●) and non-IL-1 treated (o) animals were removed on days 3, 5, and 7 post primary tumor challenge. The cells were admixed with 5 x 10^3 EL-4 in a 500:1 lymph node cell to EL-4 ratio and were injected intradermally into naive animals (n = 5 per group per day assessed). Tumor growth was assessed by the measurement of perpendicular diameters. Data are expressed as mean values $\pm$ SE.

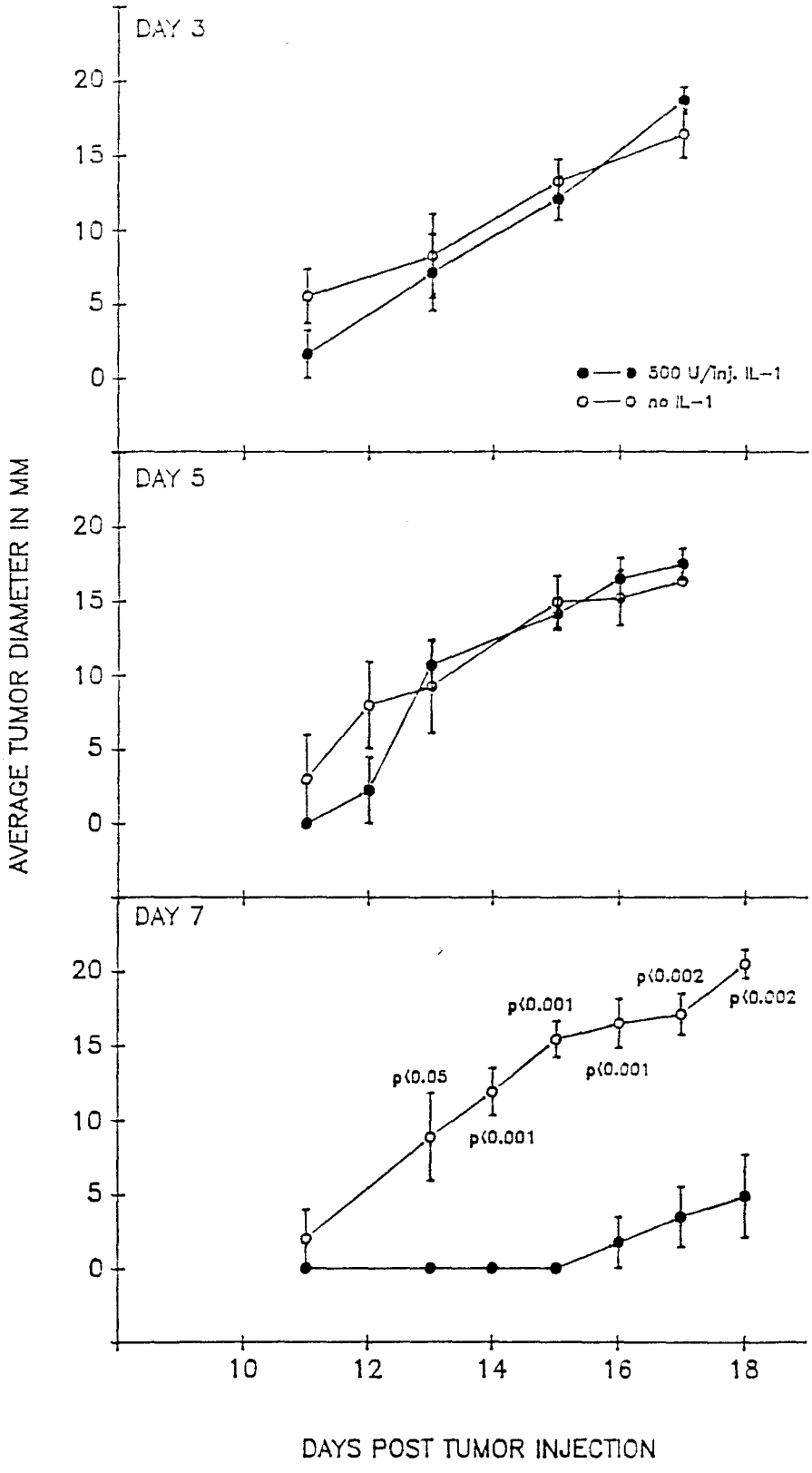

DAY 3
DAY 5
DAY 7
20
15
10
5
0
500 U/inj. IL-1
no IL-1
p<0.05
p<0.001
p<0.001
p<0.001
p<0.002
p<0.002
10
12
14
16
18
AVERAGE TUMOR DIAMETER IN MM
DAYS POST TUMOR INJECTION

Fig. 6. Effect of day 7 lymph node and spleen cells on tumor development and animal survival. Lymph node and spleen cells from IL-1 treated (○, △) and non-IL-1 treated (●, □) animals were removed on day 7 post primary tumor challenge. The cells were admixed with 5 x 10^3 EL-4 in a 500:1 lymph node cell to EL-4 ratio and were injected intradermally into naive animals (n = 4 per group for lymph node cell assessments, n = 5 per group for spleen cell and EL-4 alone assessments). Animals were examined periodically for the development of tumor (panel A.). Tumor growth was assessed by the measurement of perpendicular diameters. Animals with an average tumor diameter of greater than 15mm were considered non-survivors (panel B.). Animals injected with EL-4 alone (■) were assessed as well.

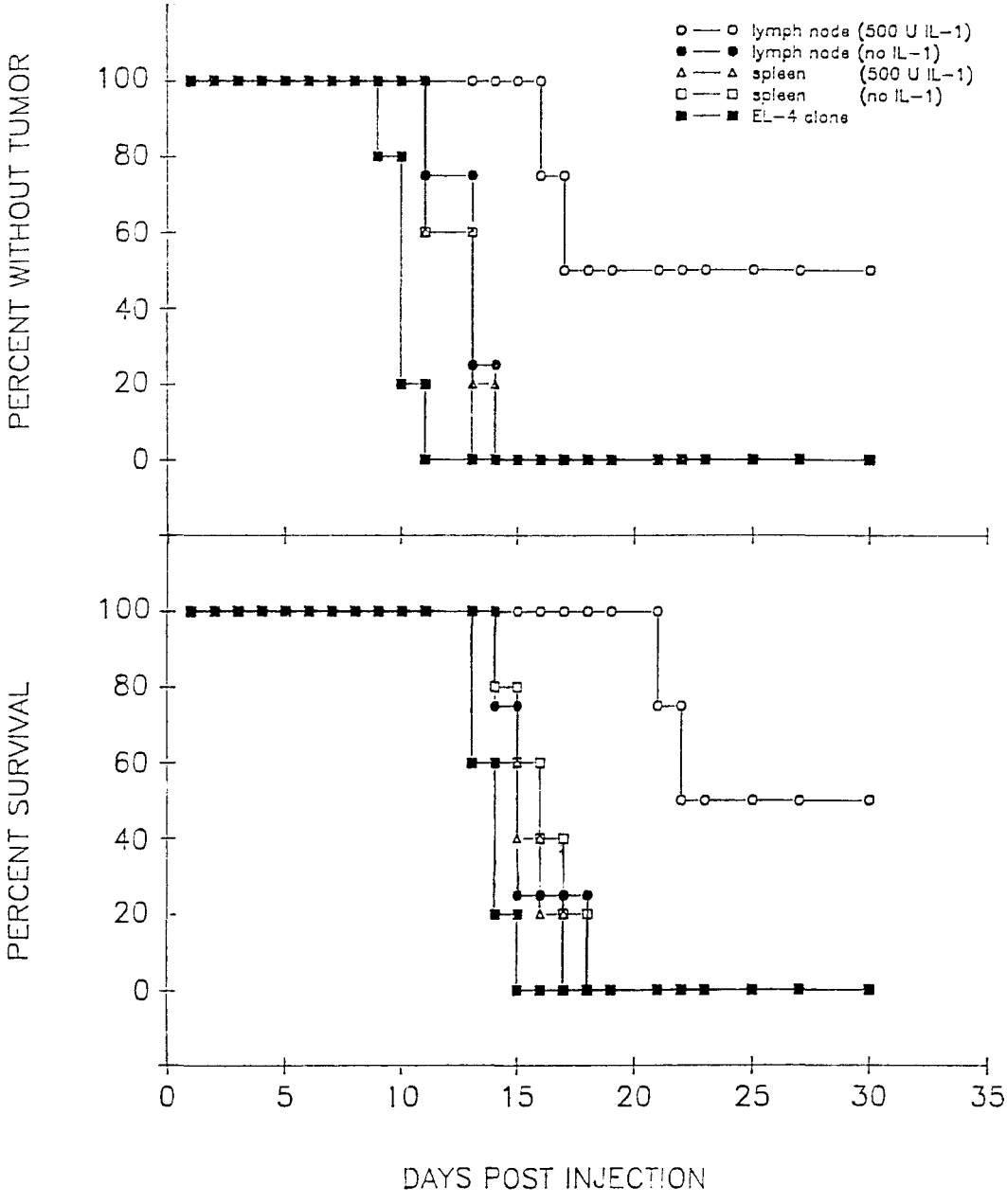

PERCENT WITHOUT TUMOR
PERCENT SURVIVAL
DAYS POST INJECTION
lymph node (500 U IL-1)
lymph node (no IL-1)
spleen (500 U IL-1)
spleen (no IL-1)
EL-4 clone
0
20
40
60
80
100
0
5
10
15
20
25
30
35

Table 3. Tumor protection against EL-4 by adoptive transfer of lymph node and spleen cells.[a]

Treatment group		Mean day of tumor appearance	Mean survival in days[b]
None	(n = 5)	10.0 ± 0.7	13.8 ± 0.8
Spleen (no IL-1)	(n = 5)	11.8 ± 1.6	15.6 ± 1.1
Spleen (IL-1)	(n = 5)	12.0 ± 1.9	14.4 ± 1.5
Lymph node (no IL-1)	(n = 4)	12.8 ± 1.3[c]	15.5 ± 1.7
Lymph node (IL-1)	(n = 4)	16.5 ± 0.7[d]	21.5 ± 0.7[e]

[a] Lymph node and spleen cells derived from IL-1 treated and non-IL-1 treated animals 7 days after tumor challenge were adoptively transferred with 5 x 10^3 EL-4 into naive animals. Animals were observed for tumor appearance and survival. Data are expressed as mean values ± SD.

[b] Animals with an average tumor diameter of greater than 15mm were considered non-survivors.

[c] $p<0.005$, when compared to EL-4 alone.

[d] $p<0.001$, when compared to EL-4 alone.
$p<0.05$, when compared to spleen (IL-1) group.
$p<0.02$, when compared to lymph node (no IL-1) group.

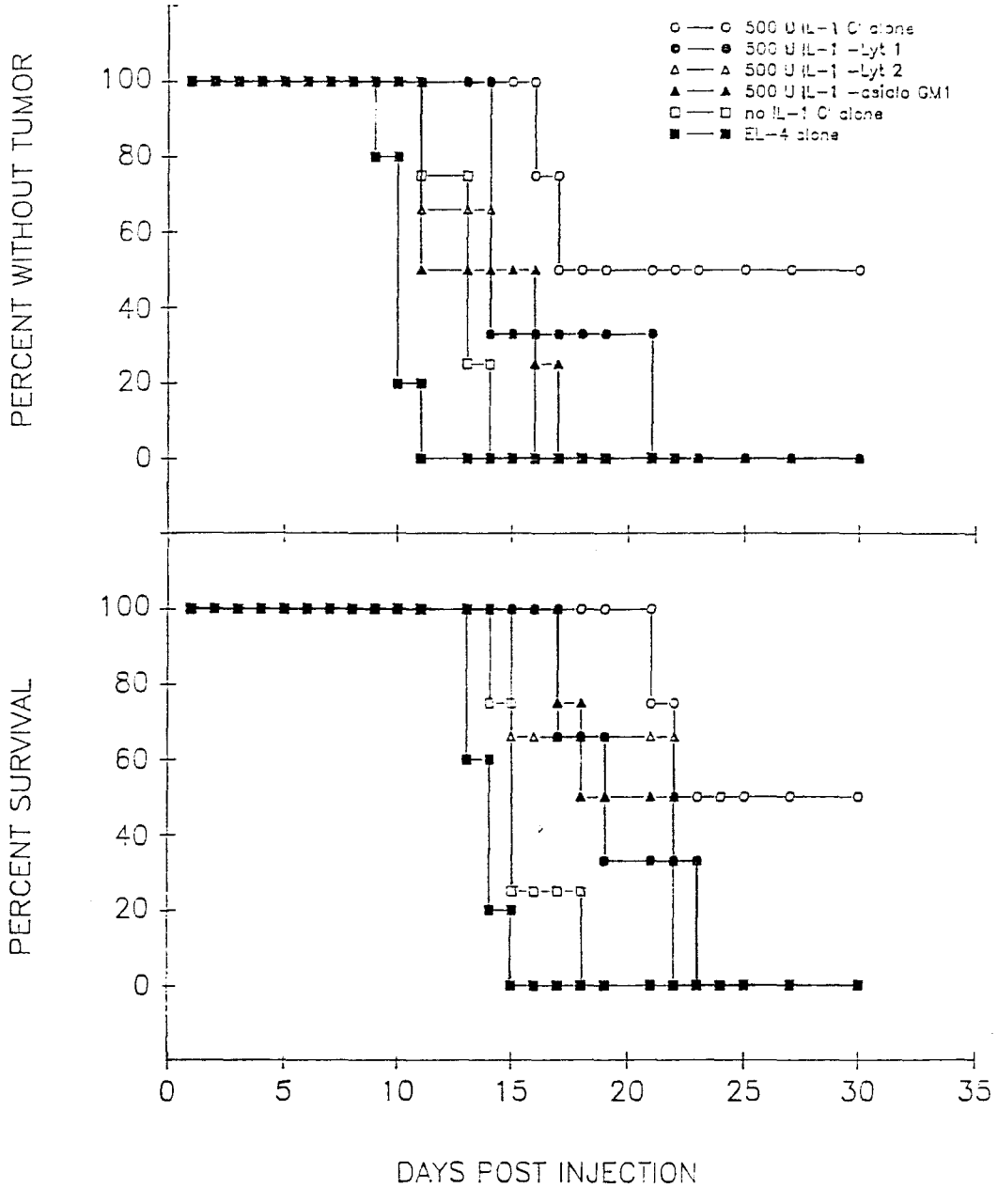
[e] $p<0.001$, when compared to EL-4 alone.
$p<0.002$, when compared to spleen (IL-1) group.
$p<0.02$, when compared to lymph node (no IL-1) group.

which received lymph node cells from the IL-1 treated animals (n = 4) developed tumors significantly later ($p<0.001$) and lived significantly longer ($p<0.001$) than animals which received tumor alone (n = 5). In addition, the mean day of tumor appearance was later than that observed with animals receiving spleen cells from IL-1 treated animals (n = 5, $p<0.05$). Lymph node cells from the IL-1 treated animals were also able to significantly enhance the length of animal survival when compared to spleen cells from IL-1 treated animals ($p<0.002$). Animals which received spleen cells from the IL-1 treated animals did not exhibit significantly later tumor development or enhanced survival when compared to animals from the other treatment groups. These data indicate that, within the time period examined, the ability of lymphoid cells from IL-1 treated animals to delay tumor development and enhance animal survival was present in the draining lymph nodes and was not a systemic phenomenon involving the spleen.

4. Effect of various cell populations on tumor development and animal survival.

Depletion of $CD4^+$, $CD8^+$, or asialo $GM1^+$ cells affected the ability of lymph node cells from IL-1 treated animals to mediate antitumor effects. Antitumor effects were assessed by measuring tumor development and animal survival in adoptive transfer assays ($-CD4^+$, n = 3; $-CD8^+$, n = 3; -asialo GM1, n = 4). Lymph node cells depleted of any of the cell populations were unable to prevent tumor development, although the time of tumor appearance was later than in the animals which received tumor alone (Fig. 7). In contrast, lymph node cells which were treated with complement alone were able to delay tumor

Fig. 7. Effect of lymph node cell population depletion on tumor development and animal survival. Lymph nodes from IL-1 treated and non-IL-1 treated animals were removed on day 7 post primary tumor challenge. Lymph node cells from the IL-1 treated animals were depleted of $CD4^+$ (●), $CD8^+$ (▲), or asialo $GM1^+$ (◀) cells by treatment with specific antibody and complement. In addition, lymph node cells from IL-1 treated (○) and non-IL-1 treated (□) animals were treated with complement alone. The cells were admixed with 5×10^3 EL-4 in a 500:1 lymph node cell to EL-4 ratio and were injected intradermally into naive animals (-$CD4^+$, n = 3; -$CD8^+$, n = 3; -asialo $GM1^+$, n = 4; IL-1 complement-treated, n = 4; non-IL-1 complement treated, n = 4; EL-4 alone, n = 5). Animals were examined periodically for the development of tumors (panel A.). Tumor growth was assessed by the measurement of perpendicular diameters. Animals with an average tumor diameter of greater than 15mm were considered non-survivors (panel B.). Animals injected with EL-4 alone (■) were assessed as well.


PERCENT WITHOUT TUMOR
100
80
60
40
20
0
500 U IL-1 Cl alone
500 U IL-1 -Lyt 1
500 U IL-1 -Lyt 2
500 U IL-1 -asialo GM1
no IL-1 Cl alone
EL-4 alone
PERCENT SURVIVAL
100
80
60
40
20
0
0
5
10
15
20
25
30
35
DAYS POST INJECTION

development, and to prevent tumors in 50% of the animals.

An assessment was made of the relative ability of $CD4^+$ and $CD8^+$ lymph node cell populations from IL-1 treated and non-IL-1 treated animals to mediate effects against EL-4 in naive animals. $CD4^+$ populations were isolated from lymph nodes of IL-1 treated and non-IL-1 treated animals. These cells were combined with $CD8^+$ lymph node cells (as described in Fig. 8), and adoptively transferred with EL-4 into naive animals [$CD4^+$ (IL-1) + $CD8^+$ (IL-1), n = 5; $CD4^+$ (IL-1) + $CD8^+$ (no IL-1), n = 4; $CD4^+$ (no IL-1) + $CD8^+$ (no IL-1), n = 5). Delayed tumor development was observed when both the $CD4^+$ cells and the $CD8^+$ cells were from the IL-1 treated animals. As shown in Table 4, animals which received recombined cells from IL-1 treated animals developed tumors significantly ($p<0.02$) later than animals which received EL-4 alone. In addition, these animals showed enhanced survival ($p<0.02$) when compared to the EL-4 alone animals. However, no significant differences were observed when a direct comparison was made between animals which received $CD4^+$ and $CD8^+$ cells from IL-1 treated animals and animals which received $CD4^+$ and $CD8^+$ cells from non-IL-1 treated animals. Adoptive transfer of $CD4^+$ and $CD8^+$ cell populations was not as effective as whole lymph node cell populations in promoting animal survival (no animals survived beyond 25 days). When both the $CD4^+$ cells and the $CD8^+$ cells were from non-IL-1 treated animals, no significant delay in tumor development or increase in animal survival was observed when compared to animals injected with EL-4 alone. These data suggest that $CD4^+$ and/or $CD8^+$ cells may be involved in the tumor protective effects observed in the adoptive trans-

Fig. 8. Effect of recombined lymph node cell populations on tumor development and animal survival. Lymph nodes from IL-1 treated and non-IL-1 treated animals were removed on day 7 post primary tumor challenge. $CD4^+$ and $CD8^+$ cell populations were obtained by negative selection with specific antibody and complement treatment. These populations were recombined in the following manner: $CD4^+$ (IL-1) + $CD8^+$ (IL-1); $CD4^+$ (IL-1) + $CD8^+$ (no IL-1); and $CD4^+$ (no IL-1) + $CD8^+$ (no IL-1). The cells were admixed with 5×10^3 EL-4 in a 500:1 lymph node cell to EL-4 ratio and were injected intradermally into naive animals [$CD4^+$ (IL-1) + $CD8^+$ (IL-1), n = 5; $CD4^+$ (IL-1) + $CD8^+$ (no IL-1), n = 4; $CD4^+$ (no IL-1) + $CD8^+$ (no IL-1), n = 5]. Animals were examined periodically for the development of tumor (panel A.). Tumor growth was assessed by the measurement of perpendicular diameters. Animals with an average tumor diameter of greater than 15mm were considered non-survivors (panel B.). Animals injected with EL-4 alone (o , n = 4) were assessed also.

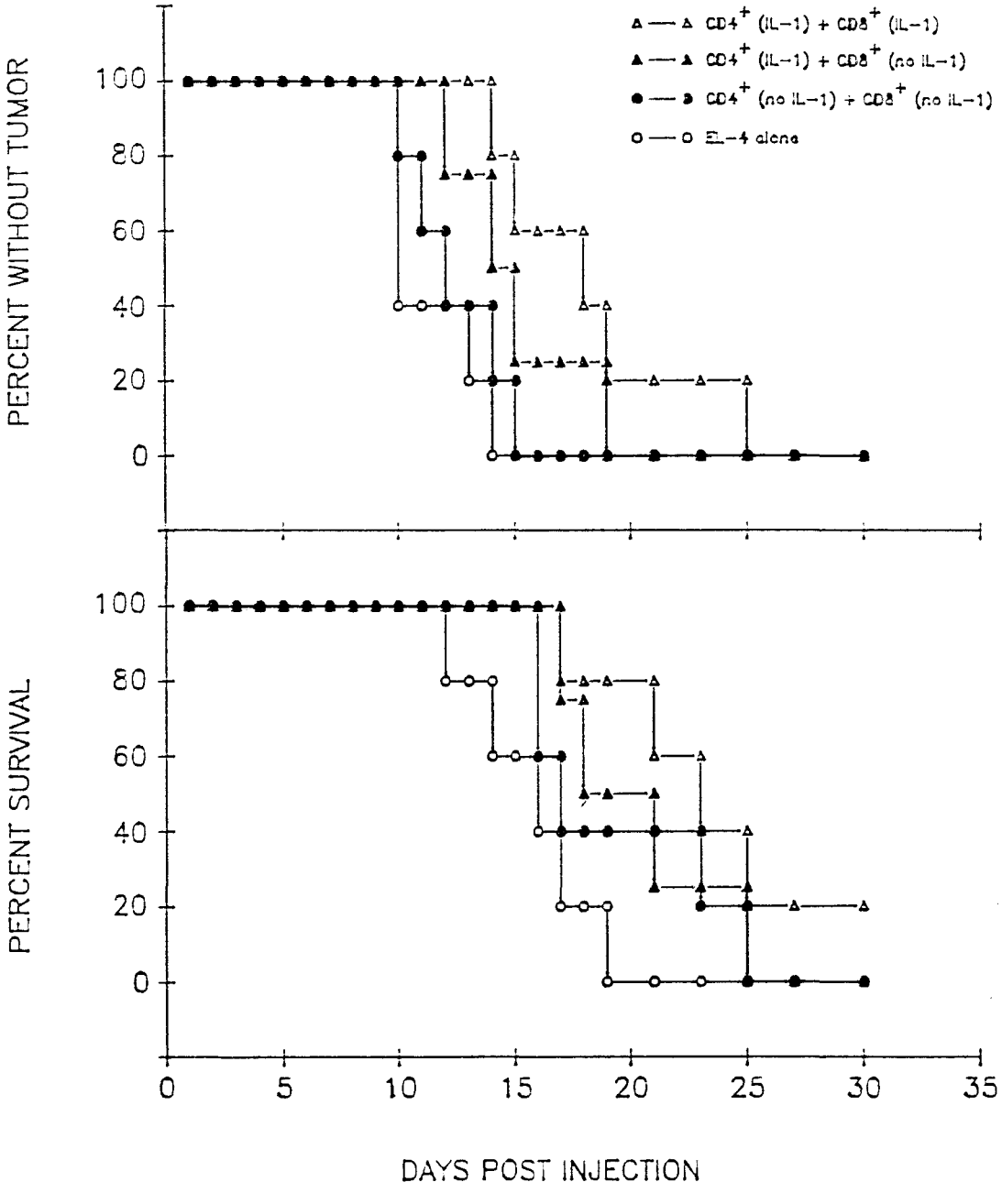

PERCENT WITHOUT TUMOR
PERCENT SURVIVAL
DAYS POST INJECTION
CD4+ (IL-1) + CD8+ (IL-1)
CD4+ (IL-1) + CD8+ (no IL-1)
CD4+ (no IL-1) + CD8+ (no IL-1)
EL-4 alone
0
20
40
60
80
100
0
5
10
15
20
25
30
35

Table 4. Tumor protection against EL-4 by adoptive transfer of recombined lymph node cell populations to naive animals.[a]

Treatment group		Mean day of tumor appearance	Mean survival in days[b]
None	(n = 5)	11.4 ± 1.9	15.0 ± 1.8
$CD4^+$ (IL-1) $CD8^+$ (IL-1)	(n = 5)	18.2 ± 4.3[c]	23.4 ± 5.2[c]
$CD4^+$ (IL-1) $CD8^+$ (no IL-1)	(n = 4)	15.0 ± 2.9	20.3 ± 3.6[d]
$CD4^+$ (no IL-1) $CD8^+$ (no IL-1)	(n = 5)	12.4 ± 2.0	19.4 ± 4.3

[a] Negatively selected lymph node cell populations from IL-1 treated and non-IL-1 treated animals were recombined and adoptively transferred with 5 x 10^3 EL-4 into naive animals. Animals were observed for tumor appearance and survival. Data are expressed as mean values ± SD.

[b] Animals with an average tumor diameter of greater than 15mm were considered non-survivors.

[c] $p<0.02$, when compared to EL-4 alone.

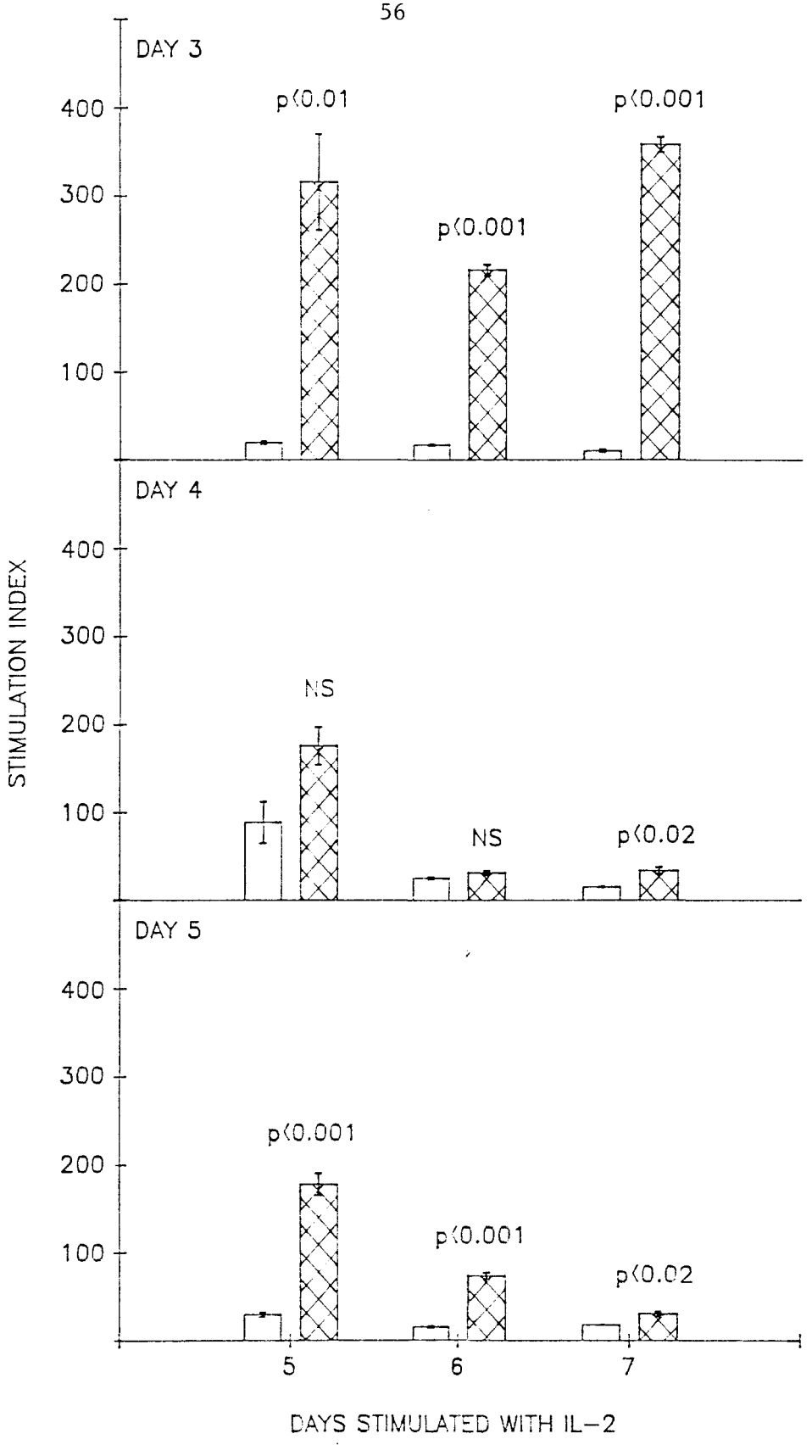
[d] $p<0.05$, when compared to EL-4 alone.

fer assays. In summary, the adoptive transfer assay data demonstrate the ability of lymph node cells from IL-1 treated animals to mediate tumor protective effects *in vivo* as evidenced by delayed tumor development, enhanced animal survival, and generation of protective immunity in surviving animals.

D. Effect of IL-1 treatment on IL-2 responsiveness of lymph node cells.

The preceding experiments established the ability of lymph node cells from IL-1 treated animals to mediate responsiveness to EL-4. A series of experiments was performed to assess the effects of systemic IL-1 therapy on the local immune parameters of lympho-proliferation and cytotoxic potential. Enhanced responsiveness to IL-2 could lead to the expansion of cell populations important in tumor survival and the generation of immunity (Izumi *et al.*, 1985; North *et al.*, 1988). In the IL-1 treatment groups, lymph node cell numbers were increased on days 3, 4 and 5 post primary tumor challenge (Fig. 1). Therefore these lymph node cells were assessed for their capacity to respond to IL-2. As shown in Figure 9, lymph node cells from the IL-1 treated animals had greater proliferative capacity in response to IL-2. The day 3 lymph node cells from the IL-1 treated animals showed a greater proliferative ability after 5, 6, and 7 days in IL-2 ($p<0.01$, $p<0.001$, $p<0.001$) as compared to the non-IL-1 treated animals. The day 4 cells showed a lower degree of proliferation than the day 3 cells. The day 5 lymph node cells also showed a lower proliferative ability when compared to day 3 cells, but the differences between IL-1 and non-IL-1 treated groups were significant after all three culture periods in IL-

Fig. 9. Effect of IL-1 treatment on IL-2 responsiveness of lymph node cells. Lymph node cells from IL-1 treated (■) and non-IL-1 treated (□) animals were stimulated with 1500 U IL-2/ml for 5, 6 or 7 days. Proliferation was assessed by incorporation of [^{3}H]thymidine, and the stimulation indices determined. The stimulation index is equal to the radioactivity incorporation of cells stimulated with IL-2, divided by the radioactivity incorporation of cells without IL-2. Assessments were performed in triplicate on lymph node cells pooled from 15 animals per group. Data are expressed as the mean value ± SE.


DAY 3
p<0.01
p<0.001
p<0.001
DAY 4
NS
NS
p<0.02
DAY 5
p<0.001
p<0.001
p<0.02
400
300
200
100
STIMULATION INDEX
5
6
7
DAYS STIMULATED WITH IL-2

2 ($p<0.001$, $p<0.001$, $p<0.02$). Peak proliferative responses were observed after 5 days of stimulation with IL-2.

In addition, lymph node cells from IL-1 treated and non-IL-1 treated animals were evaluated to determine if the enhanced IL-2 responsiveness in the IL-1 treated animals could lead to increased cell numbers following IL-2 stimulation *in vitro*. Although the direct number of recoverable cells was similar in both treatment groups (Table 5), the total number of recoverable cells in the IL-1 treated animals was significantly higher on four of the five days tested (Fig. 10). These data indicate that systemic IL-1 treatment *in vivo* enhanced lymph node cell responsiveness to IL-2 *in vitro*.

E. IL-1 effects on cytotoxic ability of lymph node cells.

1. Effect of IL-1 treatment on cytotoxic potential of lymph node cells.

The next series of experiments was performed to determine if systemic IL-1 treatment enhances the cytotoxic ability or potential of draining lymph node cells. When first removed from the animal, lymph node cells from both IL-1 treated and non-IL-1 treated animals showed little or no cytotoxic activity against EL-4, YAC-1, or P815. Lytic activities were less than 10 lytic units/10^7 cells at 30% lysis for all targets tested, regardless of the treatment group (data not shown). Following stimulation with IL-2, however, cytotoxic activity was observed against both natural killer cell (NK)-sensitive (YAC-1) and NK-resistant (EL-4, P815) tumor cells.

Lymph node cells from IL-1 treated and non-IL-1 treated animals were stimulated with IL-2 for 7 days, and assessed for cytotoxic ac-

Fig. 10. Effect of IL-1 treatment on the number of recoverable cells following IL-2 stimulation. Triplicate cultures of lymph node cells from IL-1 treated (▩) and non-IL-1 treated (□) animals were stimulated with 1500 U IL-2/ml for 7 days. Viable cells were isolated and the percent recovery determined. The percent recovery equals the number of viable cells, divided by the number of cells placed in culture, multiplied by 100. These percentages were multiplied by the lymph node cell number to obtain the total number of recoverable cells/animal. Data points represent the mean of 3 groups of 5 animals each and are expressed as the mean value $\pm$ SE.

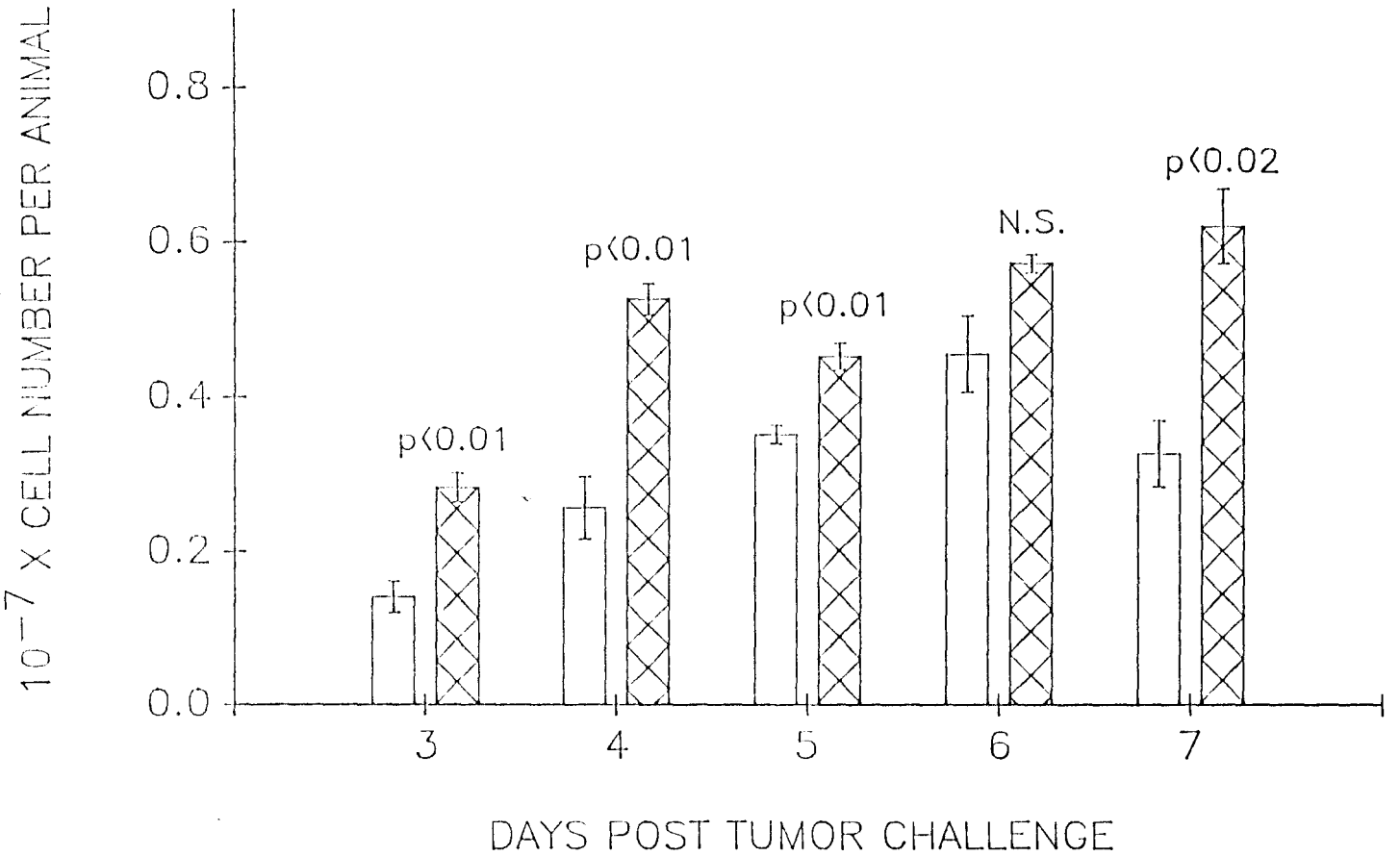

10^{-7} X CELL NUMBER PER ANIMAL
0.0
0.2
0.4
0.6
0.8
p<0.01
p<0.01
p<0.01
N.S.
p<0.02
3
4
5
6
7
DAYS POST TUMOR CHALLENGE

Table 5. Effect of IL-1 treatment on lymph node cell number and the number of recoverable cells following IL-2 stimulation.

	Lymph node cell number[a] (cell number x 10^{-6})		Recoverable cells per 5 x 10^6 cells stimulated with IL-2[b] (cell number x 10^{-6})	
	Treatment group		Treatment group	
Day	IL-1	no IL-1	IL-1	no IL-1
3	13.1 ± 0.9	7.1 ± 1.0	1.08 ± 0.20	0.99 ± 0.47
4	18.0 ± 0.7	10.1 ± 1.7	1.46 ± 0.17	1.16 ± 0.16
5	17.3 ± 0.7	14.1 ± 0.7	1.31 ± 0.21	1.22 ± 0.23
6	16.6 ± 0.4	14.7 ± 1.6	1.73 ± 0.45	1.54 ± 0.14
7	9.9 ± 0.8	4.9 ± 0.6	3.13 ± 0.45	3.31 ± 0.30

[a] Lymph node cell numbers represent values depicted in Fig. 1. Data are expressed as mean values ± SE and represent the mean of 3 groups of 5 animals each, per treatment group, per day post tumor challenge.

[b] Lymph node cells were stimulated with IL-2 as described in Fig. 10. Data are expressed as the number of cells recovered per 5 x 10^6 lymph node cells stimulated.

tivity against EL-4, YAC-1, and P815 (a representative experiment is shown in Table 6). While reactivity was generated in both the IL-1 treated and non-IL-1 treated groups, the IL-1 treated group showed the greatest enhancement in terms of lytic units of activity per axillary and brachial lymph node (Fig. 11). This activity varied depending on the time at which lymph node cells were obtained post tumor challenge and appeared to be a function of the increase in lymph node cell number seen in the IL-1 treated animals. Significantly higher cytotoxicity was observed in the IL-1 treated animals on days 4 through 6 post tumor challenge for EL-4 and YAC-1, and days 3 through 7 for P815. Total activity was highest against the NK-sensitive target for both treatment groups. These data indicate that IL-1 treated animals had a greater capacity to develop cytotoxic activity against a variety of tumor targets, and suggest that a heterogenous population of effectors may be generated by IL-2 stimulation.

2. Effect of IL-1 treatment on cytotoxic cell frequency.

The enhanced in vitro cytotoxic capability of the IL-2 stimulated lymph node cells (from the IL-1 treated animals) may be due to a greater number of IL-2 inducible cytotoxic cells in the lymph nodes of IL-1 treated animals. In order to assess this possibility, limiting dilution analysis was performed. Lymph node cells were harvested on sequential days following primary tumor challenge (10 animals per treatment group, per day. Figure 12 shows the cytotoxic cell precursor frequencies for bra-chial and axillary lymph node cells derived days 1 through 4 post tumor challenge. Each panel depicts the cytotoxic cell frequency for an individual day post tumor challenge. Lit-

Table 6. Effect of IL-1 treatment on lytic activity of IL-2 stimulated lymph node cells.[a]

	IL-1 treated animals			non-IL-1 treated animals		
	Tumor target			Tumor target		
Day	EL-4	YAC-1	P815	EL-4	YAC-1	P815
3	10[b]	74	64	17	135	64
4	10	84	24	10	22	10
5	10	204	88	10	100	47
6	48	247	124	28	161	121
7	10	165	108	16	177	90

[a] Lymph node cells were stimulated with IL-2 as described in Fig. 10. After 7 days, the cells were assessed for their cytotoxic activity against EL-4, YAC-1, and P815 in a 4 hour chromium release assay with effector to target ratios ranging from 50:1 to 6:1. Linear regression analysis was used to determine the lytic unit activity.

[b] Data are expressed as lytic units/10^7 cells at 30% lysis, and are from a representative experiment.

Fig. 11. Effect of IL-1 treatment on cytotoxic potential of lymph node cells. Lymph node cells from IL-1 treated (▣) and non-IL-1 treated (□) animals were stimulated with IL-2 as in Figure 10. After 7 days, the cells were assessed for their cytotoxic activity against EL-4, YAC-1, and P815 in a 4 hour chromium release assay with effector to target ratios ranging from 50:1 to 6:1. Linear regression analysis was used to determine the number of lytic units/10^7 cells at 30% lysis. These values were multiplied by the recoverable cell numbers from Figure 10 to obtain the number of lytic units per animal. Data points represent the mean of three groups of five animals each and are expressed as the mean value $\pm$ SE.

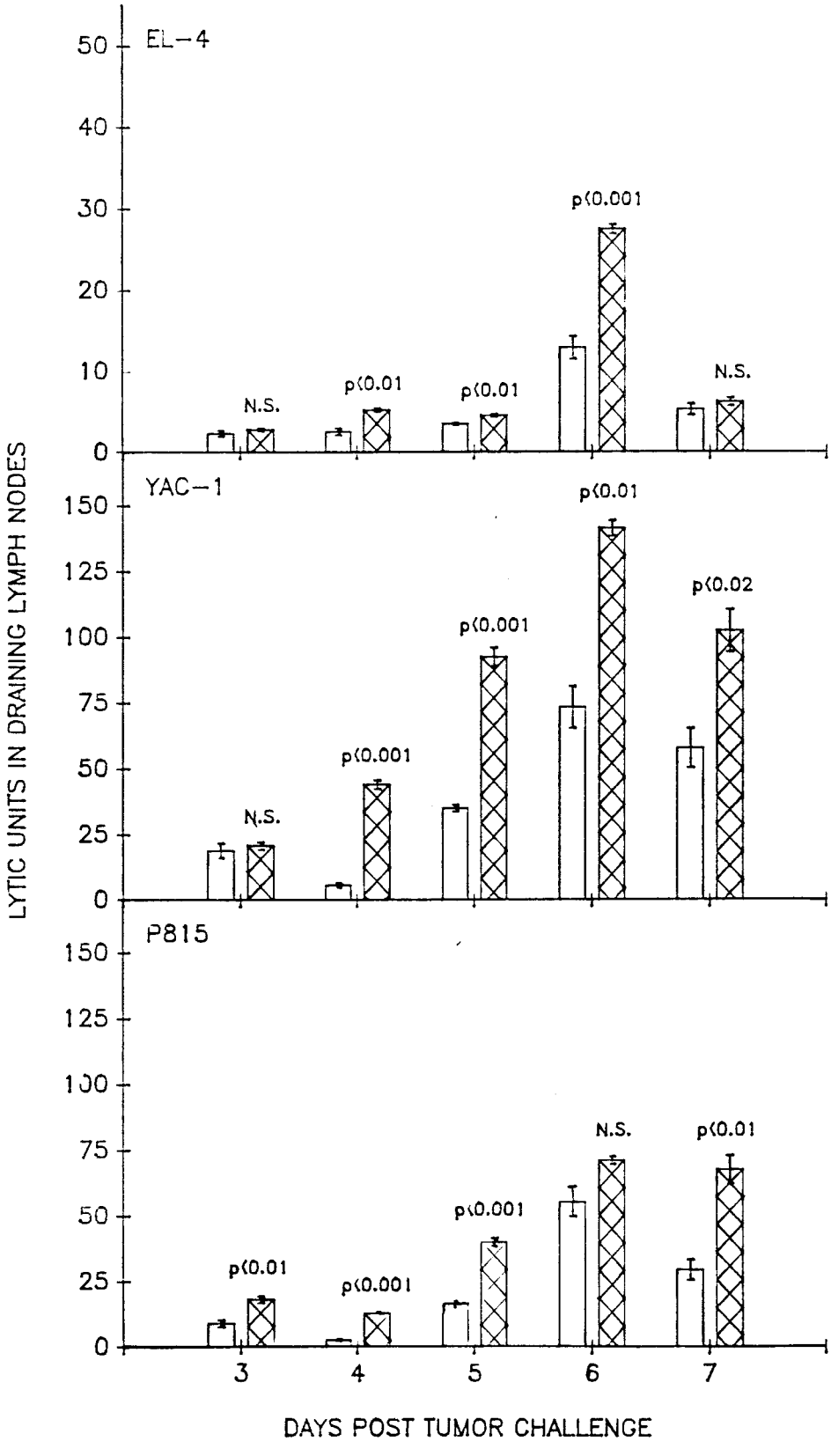
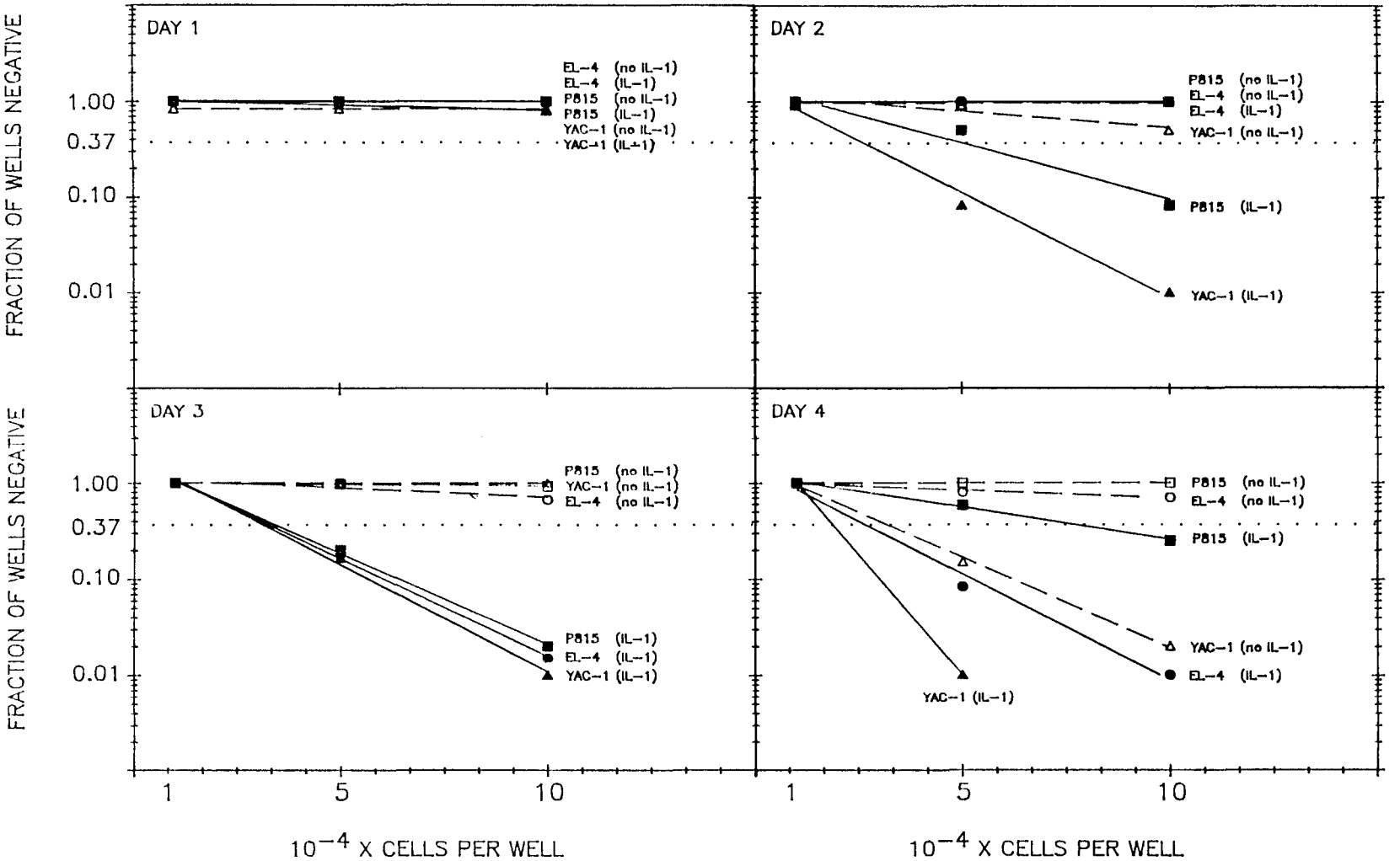

EL-4
YAC-1
P815
LYTIC UNITS IN DRAINING LYMPH NODES
DAYS POST TUMOR CHALLENGE
N.S.
p<0.01
p<0.001
p<0.02
0
25
50
75
100
125
150
3
4
5
6
7

Fig. 12. Cytotoxic cell frequency analysis of day 1-4 lymph node cells. Limiting dilution analysis was performed on lymph node cells from IL-1 treated (●, ▲, ■; n = 10 animals per day) and non-IL-1 treated (○, △, □; n = 10 animals per day) animals. Various numbers of lymph node cells were cultured with 120 U/well (800 U/ml) of IL-2. After 7 days, the cells were assessed for cytotoxic activity by the addition of chromium-labeled tumor targets. Both NK sensitive (YAC-1) and NK resistant (EL-4, P815) tumor targets were used. Positive wells were those with chromium release greater than 3 SD above spontaneous release. The data is presented as the fraction of wells which failed to develop lytic activity versus the number of lymph node cells/well.


DAY 1
EL-4 (no IL-1)
EL-4 (IL-1)
P815 (no IL-1)
P815 (IL-1)
YAC-1 (no IL-1)
YAC-1 (IL-1)
DAY 2
P815 (no IL-1)
EL-4 (no IL-1)
EL-4 (IL-1)
YAC-1 (no IL-1)
P815 (IL-1)
YAC-1 (IL-1)
DAY 3
P815 (no IL-1)
YAC-1 (no IL-1)
EL-4 (no IL-1)
P815 (IL-1)
EL-4 (IL-1)
YAC-1 (IL-1)
DAY 4
P815 (no IL-1)
EL-4 (no IL-1)
P815 (IL-1)
YAC-1 (no IL-1)
EL-4 (IL-1)
YAC-1 (IL-1)
FRACTION OF WELLS NEGATIVE
1.00
0.37
0.10
0.01
1
5
10
10^-4 X CELLS PER WELL

tle cytotoxic activity was noted on day 1 for any of the tumor targets, regardless of the therapeutic protocol. By day 2, the potential for activity against YAC-1 developed in the IL-1 treated group. Day 3 lymph node cells from the IL-1 treated group demonstrated the ability to develop activity against all three tumor targets, while cells from the non-IL-1 treated group had no detectable cytolytic cell activity. By day 4 the potential for activity against YAC-1 was present in the non-IL-1 treated group. In Figure 13, the cell frequencies for days 5 through 7 post tumor challenge are depicted. Lymphoid cytotoxic activity for days 5 through 7 showed a decrease in frequency for the IL-1 treated group and an increase for the non-IL-1 treated group. Day 5 and 6 lymph node cells from the non-IL-1 treated group showed the potential to develop activity against YAC-1, and to a lesser extent against EL-4. By day 7 post tumor challenge neither group displayed the potential to develop cytotoxic activity. No difference in activity between groups was noted after day 6.

The cytolytic cell frequencies derived from the frequency graphs are summarized in Table 7. The 95% confidence levels obtained from the minimum chi-square analysis (Taswell, 1981) revealed a significant increase in cytolytic cell frequency in the lymph node cells of IL-1 treated animals on days 2 and 3, for all tumor targets tested, when compared to cells from the non-IL-1 treated group. On day 4, the frequency of cytolytic cells with activity against EL-4 and P815 was significantly higher in the IL-1 treated group. However, the frequency of cytolytic cells with activity against YAC-1 was similar in the IL-1 treated and non-IL-1 treated animals. By 5 days after tumor

Fig. 13. Cytotoxic cell frequency analysis of day 5-7 lymph node cells. Limiting dilution analysis was performed on lymph node cells from IL-1 treated (● , ▲ , ■ ; n = 10 animals per day) and non-IL-1 treated (○ , △ , □ ; n = 10 animals per day) animals as in Figure 12. Positive wells were those with chromium release greater than 3 SD above the spontaneous release.

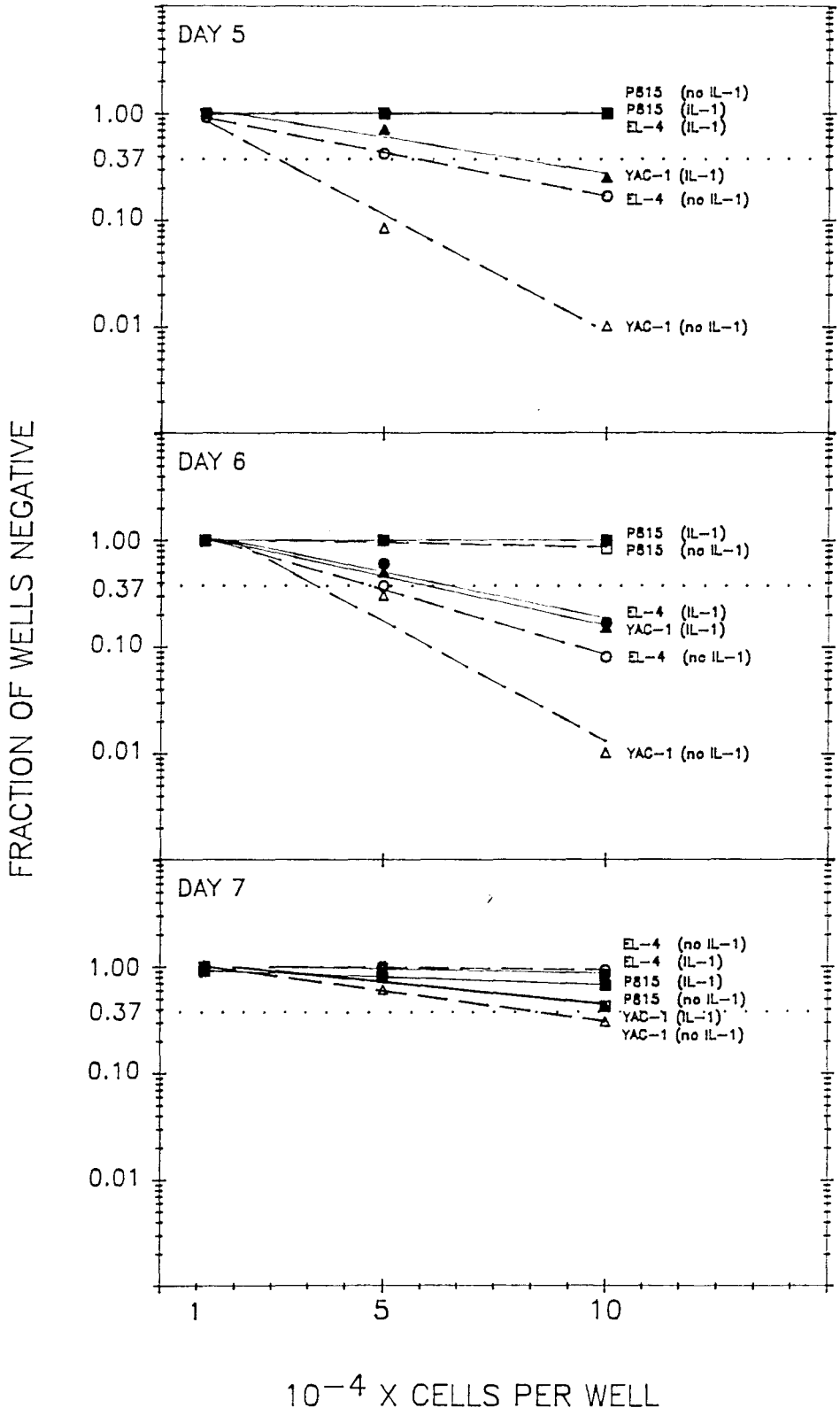

DAY 5
1.00
0.37
0.10
0.01
P815 (no IL-1)
P815 (IL-1)
EL-4 (IL-1)
YAC-1 (IL-1)
EL-4 (no IL-1)
YAC-1 (no IL-1)
DAY 6
1.00
0.37
0.10
0.01
P815 (IL-1)
P815 (no IL-1)
EL-4 (IL-1)
YAC-1 (IL-1)
EL-4 (no IL-1)
YAC-1 (no IL-1)
DAY 7
1.00
0.37
0.10
0.01
EL-4 (no IL-1)
EL-4 (IL-1)
P815 (IL-1)
P815 (no IL-1)
YAC-1 (IL-1)
YAC-1 (no IL-1)
1
5
10
FRACTION OF WELLS NEGATIVE
10⁻⁴ X CELLS PER WELL

Table 7. IL-2 inducible lymph node cytolytic cell frequencies for various tumor targets.[a]

	IL-1 treated animals			non-IL-1 treated animals		
	Tumor target			Tumor target		
Day	EL-4	YAC-1	P815	EL-4	YAC-1	P815
1	n.d.[b]	n.d.	n.d.	n.d.	n.d.	n.d.
2	n.d.	1/34000[c]	1/83000[c]	n.d.	n.d.	n.d.
3	1/47000[c]	1/35000[c]	1/52000[c]	n.d.	n.d.	n.d.
4	1/34000[c]	1/25000	1/86000[c]	n.d.	1/65000	n.d.
5	n.d.	1/103000	n.d.	1/60000[c]	1/34000[c]	n.d.
6	1/155000	1/164000	n.d.	1/103000	1/150000	n.d.
7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

[a] Limiting dilution analysis was performed as described in Figure 12. Frequencies are expressed as cytolytic cell frequency/number of lymph node cells. Statistical significance was determined by 95% confidence limits of the minimal chi-square analysis. Data represent pooled lymph node cell populations from 10 animals per treatment group, per day post tumor challenge.

[b] none detected.

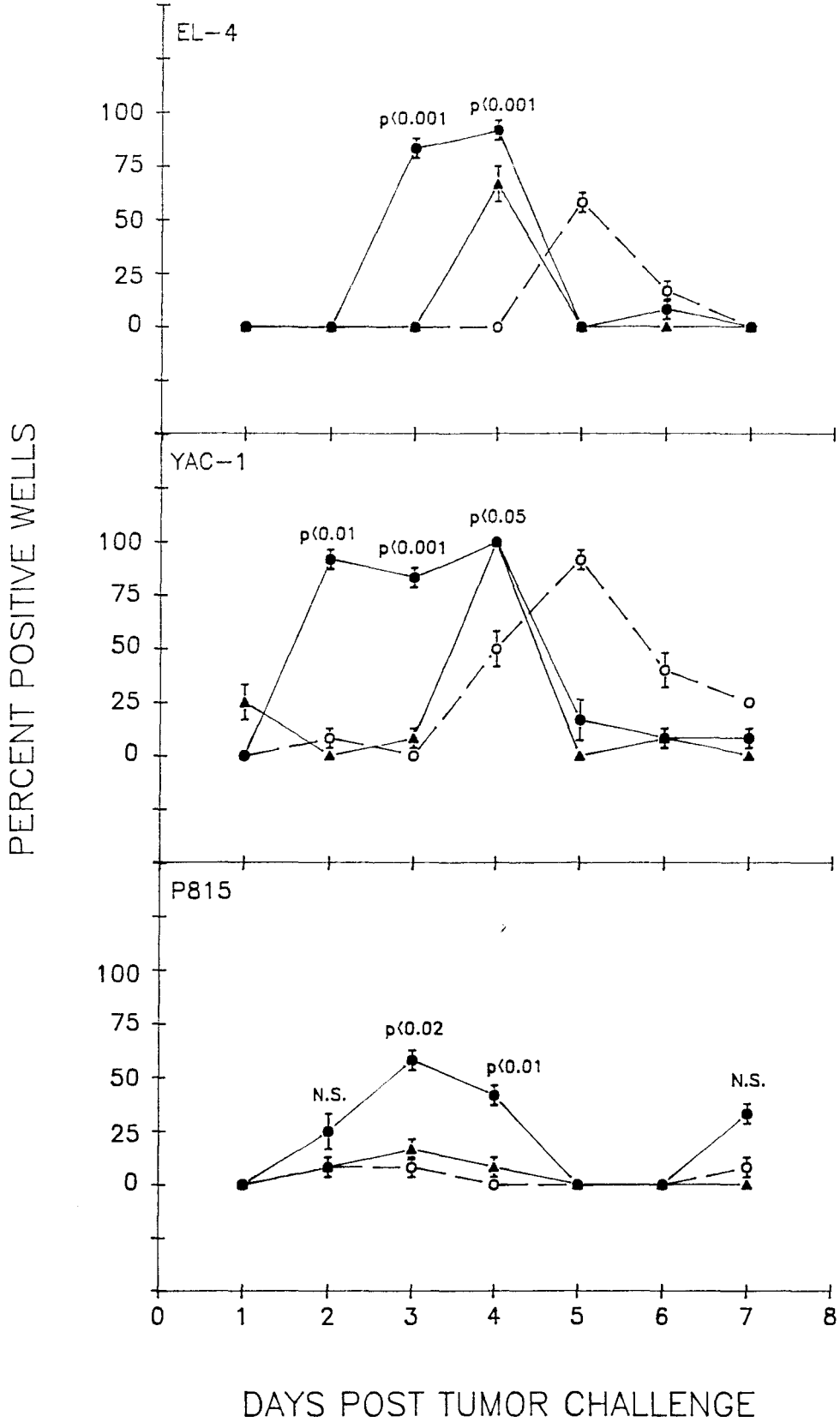
[c] $p<0.05$, when compared to frequency against same target cell in the other treatment group.

challenge, the non-IL-1 treated animals had a significantly higher frequency of cytolytic cells with activity against YAC-1 and EL-4 when compared to the IL-1 treated animals.

In order to demonstrate the temporal difference in cytotoxic cell development between IL-1 treated and non-IL-1 treated animals, the cell frequency data were alternatively analyzed and the percentage of positive scoring wells for one particular cell dilution (5×10^4 lymph node cells) depicted (Fig. 14). When compared to cells from the non-IL-1 treated animals, lymph node cells from the IL-1 treated animals demonstrated an earlier, augmented development of cytotoxic activity. A more modest response was observed in animals which received 200 U per injection of IL-1. For example, activity against EL-4 developed on day 3 for the 500 U IL-1 group, on day 4 for the 200 U IL-1 group, and on day 5 for the non-IL-1 treated group. Similarly, the potential for maximal activity against YAC-1 was observed on days 2 through 4 for the 500 U IL-1 group, day 4 for the 200 U IL-1 group, and day 5 for the non-IL-1 treated group. These data indicate that *in vivo* systemic IL-1 treatment enhanced the capacity to induce total lymph node cytotoxic activity *in vitro* as assessed by enhanced cytotoxic activity and increased cytotoxic cell frequency.

Maximal cytotoxic cell frequencies in the limiting dilution assay occurred with lymph node cells derived before day 6, with variable cytotoxic activity depending upon the treatment group from which the cells were derived. In contrast, maximal cytolytic activity in the bulk-cultured cells was observed with lymph node cells derived 6 days post tumor challenge (Table 6), and cytotoxicity against all

Fig. 14. Relationship of IL-1 dose to cytotoxic cell frequency. The percentage of positive wells for a single cell concentration (5×10^4 lymph node cells/well) was determined using limiting dilution analysis. Data shown are for animals treated with 0 (○), 200 (▲), and 500 (●) U IL-1/injection and represent the same animals as Fig. 12 and 13. Individual panels represent the data for EL-4, YAC-1 and P815 tumor targets.

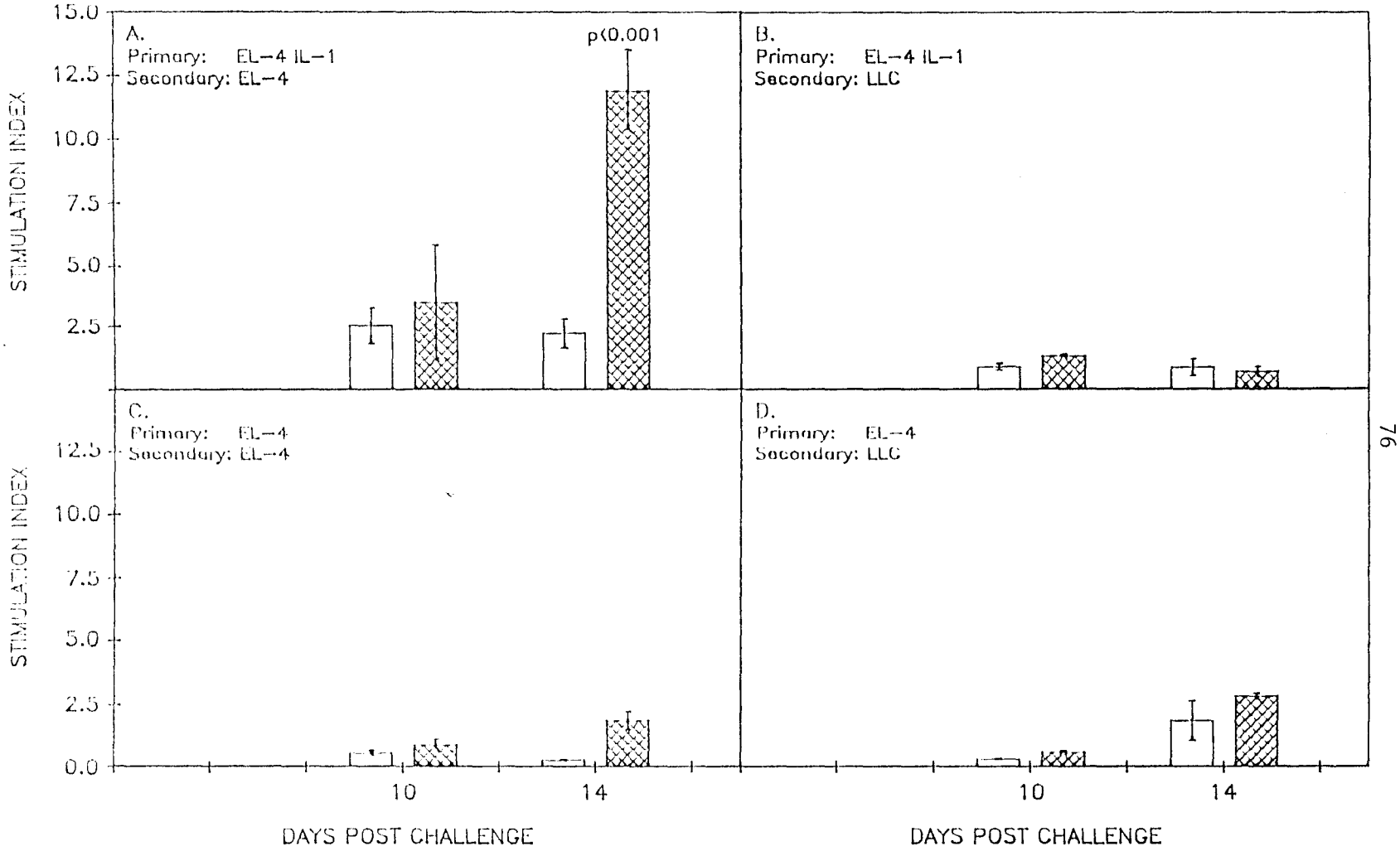

EL-4
p<0.001
p<0.001
YAC-1
p<0.01
p<0.001
p<0.05
P815
p<0.02
p<0.01
N.S.
N.S.
100
75
50
25
0
0
1
2
3
4
5
6
7
8
PERCENT POSITIVE WELLS
DAYS POST TUMOR CHALLENGE

three tumor targets was generated regardless of the treatment group.

F. Antigenic responsiveness.

Because the protective immunity generated in this system is tumor-specific (Hornung and Mathews, unpublished observation), it may be hypothesized that animals receiving IL-1 therapy develop reactivity to EL-4. In accordance with the enhanced local responsiveness demonstrated above, it could be suggested that this reactivity may be found in the local lymph nodes. Therefore, the ability of lymph node cells from IL-1 treated animals to proliferate in vitro in response to stimulation with EL-4 was assessed in an antigenic responsiveness assay. Briefly, animals which survived primary tumor challenge (with or without IL-1 therapy) were rechallenged in vivo with either EL-4 or the unrelated tumor LLC (n = 5 animals per group, per day; 40 total). The rechallenge served to increase the number of cells in the lymph nodes and therefore to enable multiple assessments of activity. Lymph node cells were removed from these animals at various times post rechallenge and stimulated in vitro with EL-4 or LLC. As shown in Figure 15, only the animals which received systemic IL-1 therapy at the time of primary tumor challenge were able to respond to EL-4 stimulation in vitro (panel A.). This responsiveness was observed 14 days after secondary tumor challenge, and was significantly higher ($p<0.001$) than that elicited by LLC in vitro. Animals which did not receive IL-1 therapy at the time of primary tumor challenge did not demonstrate enhanced responsiveness to EL-4 (panel C.) Lymph node cells from animals which received adjuvant and IL-1 therapy, but were rechallenged with LLC in vivo (panel B.), also did not show an en-

Fig. 15. Effect of IL-1 treatment on tumor-specific lymph node cell proliferation in secondary challenge animals. Animals which survived primary tumor challenge with either IL-1 and adjuvant therapy, or adjuvant therapy alone, were rechallenged on the opposite flank with either EL-4 or the unrelated tumor LLC (n = 5 animals per treatment group, per day). On various days post secondary challenge, the lymph nodes draining the site of tumor rechallenge were removed. Cells were stimulated in culture for 7 days with either mitomycin c treated EL-4 (⊠) or LLC (□). Tumor-specific stimulation was assessed by the incorporation of [^{3}H]thymidine in a 6 hr assay. Data are expressed as mean stimulation indices ± SE.


A.
Primary: EL-4 IL-1
Secondary: EL-4
p<0.001
B.
Primary: EL-4 IL-1
Secondary: LLC
C.
Primary: EL-4
Secondary: EL-4
D.
Primary: EL-4
Secondary: LLC
STIMULATION INDEX
STIMULATION INDEX
15.0
12.5
10.0
7.5
5.0
2.5
0.0
10
14
DAYS POST CHALLENGE
DAYS POST CHALLENGE

hanced proliferative response. Animals which did not receive IL-1 therapy (panel D.) showed little responsiveness to EL-4 or LLC *in vitro*, following *in vivo* rechallenge with LLC. These data demonstrate that lymph node cells from IL-1 treated animals were able to proliferate in response to EL-4 stimulation. This responsiveness was predominantly tumor-specific as shown by the low level of proliferation in response to LLC *in vitro*. No such lymphoproliferation was observed in animals undergoing primary tumor challenge (data not shown).

CHAPTER V

DISCUSSION

In recent years, the use of biological response modifiers in the treatment of cancer has been evaluated in both animal tumor systems and clinical trials. The use of immunotherapy has been proposed as an adjunct to more traditional forms of therapy such as surgery, radiotherapy, and chemotherapy. In this way, the immune-enhancing effects of various cytokines may work in concert with the direct anti-cancer activities of the other treatments. IL-1 may be useful as a immunotherapeutic mediator because of its multiple effects upon immune cells. IL-1 has a number of effects upon T and B lymphocyte function, as well as on macrophages and NK cells. These effects include enhancement of antibody secretion (Wood, 1979), T lymphocyte proliferation (Smith, 1980), and macrophage cytotoxicity (Onozaki *et al.*, 1985a). As an activating factor for T cells (Larsson *et al.*, 1980), IL-1 induces IL-2 production and therefore augments the generation of helper and cytolytic T-lymphocytes (Koopman *et al.*, 1978; Farrar *et al.*, 1980; McMannis and Plate, 1985). IL-1 synergizes with IL-2 in the generation of lymphokine activated killer (LAK) cells *in vitro* (Crump *et al.*, 1989). IL-1 is directly cytocidal for certain tumor cell lines (Onozaki *et al.*, 1985b) and in high doses can promote tumor regression in some tumor model systems (Nakamura *et al.*, 1986). *In vivo* administration of IL-1 can restore impaired T cell and NK cell functions in tumor-bearing mice (Yamashita and Shirakawa, 1987).

The general enhancement of immune function seen with IL-1 treatment may make it valuable as a therapeutic modality in tumor therapy in conjunction with other therapies. Although other investigators have demonstrated the efficacy of IL-1 in promoting host survival of tumor inoculation (Nakamura et al., 1986; North et al., 1988; McCune and Marquis, 1990), little is known about the IL-1 effects which may lead to host protection. One of the drawbacks to the use of cytokines in immunotherapy is the lack of knowledge about their in vivo effects. Little is known about the immunoregulatory effects of IL-1 in vivo. In addition, many of the in vivo studies of IL-1 effects in experimental tumor models require high doses of IL-1 to produce an antitumor effect (Nakamura et al., 1986; North et al., 1988). In the experimental model under investigation, low dose intraperitoneal IL-1 therapy results in a protective tumor immunity (Hornung and Mathews, 1985). The amount of IL-1, although low, was sufficient to affect immune parameters resulting in subsequent tumor protection. The apparent tumor immunity was specific to the initial tumor and not to another syngeneic tumor (Hornung and Mathews, unpublished observations). This experimental model was selected for further investigation because low doses of IL-1 promoted immunity when administered in conjunction with adjuvant therapy.

Using this experimental model, a series of in vitro experiments were performed to assess EL-4 reactive antibody titers, natural killer cell activity, and EL-4 cytotoxic T cell activity in animals undergoing secondary tumor challenge (Hornung, dissertation, 1987). These animals had received EL-4 and local adjuvant (C. parvum) therapy or

EL-4 and adjuvant plus IL-1 at the time of primary tumor challenge. Little or no cytotoxic T cell activity against the EL-4 was detected in the spleens of either the IL-1 treated or non-IL-1 treated animals. The spleen cell populations from the two treatment groups showed no apparent difference in natural killer cell activity. In addition, no increase in antibody titer was detected in the sera of animals from either treatment group. The IL-1 treated animals showed no augmentation in the immune parameters measured.

Although not demonstrated directly in the previous study, it is possible that IL-1 plays a role in the modulation of the immune response to the EL-4 lymphoma and that this modulation affects the generation of tumor immunity. The objective of this study was to investigate the nature of the local lymphoid responsiveness generated by IL-1 treatment and evaluate the relationship between this lymphoid responsiveness and the induction of tumor immunity in this experimental model. This model was selected because the tumor is responsive to adjuvant therapy, and IL-1 treatment allows for the generation of apparent tumor-specific immunity to a weakly immunogenic tumor.

Because IL-1 therapy is administered only at the time of initial tumor challenge, an evaluation was made of IL-1 treated and non-IL-1 treated animals undergoing primary tumor challenge. A careful assessment of the immune status of animals undergoing primary tumor challenge has not been made previously. These experiments were performed to determine whether systemic IL-1 could modify local immune responses in animals undergoing adjuvant therapy for a primary tumor.

These investigations revealed that systemic administration of IL-1 results in enhanced immune responsiveness in the lymph nodes draining the site of tumor challenge. The systemic administration of IL-1 augmented local lymphoid responsiveness as demonstrated by increased lymph node cell number, enhanced numbers of T lymphocytes, and increased total cytotoxic potential (Fig. 1, 2, 11-14).

The reasons for examining local responses in this system were two-fold: first, little systemic enhancement of immune function has been noted previously (Hornung, dissertation, 1987), secondly, it has been shown that regional lymph nodes play an important role in the host response to disease and tumors. Stephenson et al. (1989) examined the role of regional lymph nodes in the tumor immune response. They inoculated mice with a mixture of viable tumor cells and C. parvum, and excised the regional lymph nodes on various days post tumor challenge. The development of systemic immunity was severely impaired if regional lymph nodes were excised on day 6 post tumor challenge, but excision of the lymph nodes on day 14 had no impact on the development of systemic immunity. Therefore, essential lymph node events in the development of systemic immunity occur early after tumor challenge. In the current investigation, enhancement of immune parameters in the IL-1 treated animals occurred soon after primary tumor challenge. By 3 days post tumor challenge, increases in total cell number and T lymphocytes, as well as enhanced proliferative abilities and cytotoxic potential were observed. By day 7, lymph node cells showed enhanced abilities to modify responses to EL-4 in naive animals.

Other investigators have found a correlation between the degree of lymph node swelling and the amount of antigen administered (Zatz and Lance, 1971). Increased lymph node size due to the accumulation of lymphocytes has been associated with enhanced responsiveness to antigen, and may be the mechanism of action of some adjuvants (Dresser et al., 1970; Frost and Lance, 1978). Increases in lymph node cell number could lead to increases in the numbers of cells available to react to the tumor. EL-4 alone produces no increase in the number of lymph node cells (Hornung and Mathews, 1985). Administration of IL-1 produced an early increase in the number of lymph node cells draining the site of tumor challenge (Fig. 1). This increase was statistically significant when compared with animals which received adjuvant therapy without IL-1. The expansion in cell number in the IL-1 treated animals may represent an expansion of cell populations relevant to the immune response to the tumor. The cell number increase may represent a generalized immune augmentation which allows for sufficient increases in specific populations. IL-1 treatment may convert a subtherapeutic number of sensitized T cells to a therapeutic number by stimulating an increase in the production of sensitized cells (North et al., 1988). In animals which received adjuvant therapy without IL-1, the cell number increase was smaller and was temporally delayed when compared to IL-1 treated animals.

Phenotypic analysis of the lymph node cells in the EL-4 tumor system revealed an overall increase in T lymphocytes, and T lymphocyte subsets in the IL-1 treated animals. Enhanced numbers of Thy 1.2^+ lymphocytes were present in the IL-1 treated animals on days 3 and 4

post tumor challenge. Approximately 80 to 85% of the cell number increase could be accounted for by the increase in Thy 1.2^+ lymphocytes. The cell number increase may reflect an increased localization of antigen-reactive cells. The increase in Thy 1.2^+ lymphocytes was composed of increases in either $CD4^+$ or $CD8^+$ cells, and therefore indicates an overall augmentation of cell number by IL-1 treatment. In an immunogenic tumor model, the efficacy of IL-1 therapy has been shown to be dependent upon an underlying host immune response (North et al., 1988). These authors suggest that IL-1 acts in vivo by augmenting an already ongoing immune response, and therefore can cause the regression of tumors of sufficient immunogenicity. In several tumor models, concomitant immunity develops as the tumor grows; however, this response is insufficient to prevent the progressive growth of the tumor and subsequent animal mortality. North et al. (1988) have demonstrated that systemic IL-1 treatment can augment this immune response to a level sufficient to cause tumor regression and host survival. In the current study, IL-1 non-specifically augments the number of lymph node cells. This may increase a low level of responsiveness to the EL-4 to an amount above the threshold necessary for the development of tumor immunity. This effect has not been demonstrated previously in a weakly immunogenic tumor system.

The cell number increase generated by IL-1 therapy was predominantly T cell in nature. In contrast, other in vivo studies have shown an IL-1 induced effect on B lymphocytes. Killar et al. (1989) administered 2000 to 5000 U of IL-1 systemically on a daily basis in a non-antigen driven system. They found that IL-1 therapy

increased the size of lymph nodes draining the IL-1 injection site, as well as the yield of mononuclear cells. A five-fold increase in cell number was seen in the IL-1 treated animals. The IL-1 treated lymph node cells showed a reduction in the proportion of Thy 1^+ cells, with a concomitant increase in surface immunoglobulin expressing cells. The percentage of Thy 1^+ cells fell from 81% in the control animals to 38% in the IL-1 treated, while the percentage of surface immunoglobulin expressing cells rose from 18% to 46%. Decreases in CD4 and CD8 expression were also noted. Those authors concluded that the increase in B cells was due to IL-1 induced production of IL-4, IL-2, and interferon gamma by $CD4^+$ cells in the lymph nodes.

In contrast, IL-1 adminstration in the EL-4 tumor system did not reduce the percentage of T cell marker expressing cells, and actually increased total Thy 1^+ cell numbers (Fig. 2). In the system used by Killar et al. (1989), no antigen or adjuvant was administered with the IL-1. The presence of antigen and the adjuvant C. parvum in the EL-4 system may affect lymphocyte trafficking and therefore increase T lymphocyte cell numbers. T cell numbers could also be increased by the development of a T cell response to antigenic determinants of the tumor. In addition, C. parvum has been shown to enhance T cell responsiveness (Tuttle and North, 1976), and may function to stimulate T cell-mediated immunity. Dye et al. (1981) determined that the therapeutic effect of C. parvum in an immunogenic tumor system was based on the capacity to potentiate the generation of T cell-mediated immunity. The C. parvum induced antitumor resistance resulted in the generation of T cells sensitized to tumor-specific transplantation antigens.

Mills et al. (1981) proposed that C. parvum acts by augmenting the host response to the tumor to a level sufficient to cause tumor regression. In the weakly immunogenic EL-4 tumor model, C. parvum therapy is sufficient to allow for primary tumor survival, but not the development of subsequent tumor immunity (Hornung and Mathews, 1985; Hornung, dissertation, 1987). IL-1 may promote tumor immunity by further potentiating the T cell immunity generated by adjuvant therapy with C. parvum.

Utilizing adoptive transfer of tumor resistance as an assay of in vivo activity, experiments were performed to assess the ability of IL-1 treatment to modify the tumor-protective properties of regional lymphoid tissue. These experiments showed that lymph node cells from IL-1 treated animals have the potential to mediate effects against EL-4 in vivo as demonstrated by delayed tumor development and enhanced animal survival. Lymph node cells from IL-1 treated animals were unique in their ability to adoptively modify responses to EL-4 in naive animals. Cells derived 7 (but not 3 or 5) days post tumor challenge were capable of mediating this effect (Fig. 3, 4). Animals which received lymph node cells from the IL-1 treated animals demonstrated delayed tumor development and enhanced survival (Table 1, 2). In addition, the animals which survived tumor challenge were immune to EL-4 upon subsequent rechallenge. When these animals were subsequently challenged with the unrelated tumor LLC, they did not survive. Therefore, lymph node cells from IL-1 treated animals not only modified responsiveness to EL-4 in naive animals, but also allowed for the development of tumor-specific immunity in some animals.

As shown in Fig. 7, depletion of $CD4^+$, $CD8^+$, or asialo $GM1^+$ populations reduced the effectiveness of the adoptively transferred cells. Reductions in the ability of the cells to delay tumor development and to promote animal survival were seen. In recombination experiments, $CD4^+$ and $CD8^+$ cells from IL-1 treated animals performed better than either $CD4^+$ and $CD8^+$ cells from non-IL-1 treated animals, or $CD4^+$ from IL-1 treated and $CD8^+$ from non-IL-1 treated animals (Fig. 8, Table 4), although statistically significant differences were not seen. These results are consistent with the suggestion that both helper ($CD4^+$) and cytotoxic ($CD8^+$) populations may be relevant to the antitumor effect of adoptively transferred cells (Crum, 1982). Spleen cells from the IL-1 treated animals were unable to affect responses to the tumor (Fig. 6, Table 3). The inability of spleen cells from day 7 post challenge animals to mediate effects against EL-4 indicates that IL-1 acts upon local lymphoid populations in the time frame examined. These data support the hypothesis that local immune responsiveness is modified by IL-1 induced effects in this experimental tumor model.

In the current investigation, lymphocytes were effective in adoptive transfer assays when used directly after removal from the tumor-bearing animals. Other investigators have used cyclophosphamide or irradiation to reduce suppressor T cell populations in the recipient animals (Fernandez-Cruz *et al.*, 1980; Greenberg *et al.*, 1981; Lee *et al.*, 1984). Greenberg *et al.* (1981) found that immune cell therapy without accompanying chemotherapy treatment had no apparent antitumor effect *in vivo*. Cyclophosphamide had a direct antitumor effect (Greenberg *et al.*, 1980) as well as potentially facilitating

host immune responses. In the current investigation, effects against EL-4 were observed even without treatment of host T cell populations. IL-1 therapy in this tumor system allows for the generation of lymph node cells which are therapeutically effective in naive animals. The therapeutic efficacy of these cells requires neither in vitro manipulation of the cells, nor additional treatment of the host immune system.

Lymph node cells derived from IL-1 treated animals 7 days post tumor challenge did not exhibit direct cytotoxicity to the tumor in in vitro assays. However, these cells were effective in host protection against the EL-4 in the adoptive transfer assays. This apparent discrepancy between in vivo and in vitro activities has been observed by other investigators in a number of adoptive transfer systems (Greenberg et al., 1981; Fujiwara et al., 1984; Lee et al., 1984; Shu and Rosenberg, 1985). These observations imply that tumor resistance can be transferred with cells which are non-cytotoxic in vitro, and that the lymphocytes circulate in a state lacking direct cytotoxic ability. The adoptively transferred cells may function to recruit host effectors, or may require stimulation by the tumor to become active themselves.

In several adoptive immunotherapy models, $CD4^+CD8^-$ cells have been shown to play a major role in the transfer of immunity (Greenberg et al., 1981; Fujiwara et al., 1984; Lee et al., 1984). Greenberg et al. (1981) demonstrated that depletion of non-cytotoxic (non-$CD8^+$) cell populations did not affect animal survival, while the depletion of $CD4^+$ or Thy 1^+ cells abrogated the therapeutic effect. These lym-

phocytes may interact locally with tumor cells at the tumor site and may generate cytotoxic effectors. The implication in many of these systems is that helper T lymphocytes, adoptively transferred into tumor-bearing animals, recruit and activate cytotoxic T lymphocyte (CTL) precursors in the host animal. In immunogenic tumor models, the $CD4^+$ lymphocytes may activate host CTL precursors *in vivo* (Greenberg *et al.*, 1981). While the adoptively transferred cells stimulate tumor-specific immunity in these systems, it appears that the cytotoxicity generated may have some non-specific components (Crum, 1987). In the weakly immunogenic EL-4 system, the presence of some donor effectors may also be required, as suggested by the depletion experiments. The lymphocytes are potentially cytotoxic, but require stimulation to become activated. For example, the therapeutically effective day 7 lymph node cells, when stimulated with IL-2, showed cytotoxic activity against several tumor targets (Fig. 11).

Lymph node cells from day 3 and 5 post tumor challenge have no effect in the adoptive transfer assays; however, in subsequent *in vitro* experiments, these cells showed the potential for cytotoxic activity (Fig. 11). In contrast, the lymph node cells from day 7 animals did promote delayed tumor development and enhanced animal survival in the adoptive transfer assays. These observations suggest that day 3 and 5 cells have the potential to affect tumor immune responses. By 7 days after tumor challenge further development in the lymph nodes has occurred, and the cells are capable therefore of mediating tumor protective effects against EL-4. In the limiting dilution analysis and bulk culture systems, the addition of exogenous

IL-2 may allow for the maturation of day 3 and 5 cells, and this maturation is reflected in the in vitro assays (Fig. 11-14).

While IL-1 therapy has been shown to enhance tumor survival in a number of tumor models (Nakamura et al., 1986; North et al., 1988; McCune and Marquis, 1990), the ability of IL-1 to affect local immune responsiveness has not been examined previously. This investigation assessed IL-1 effects on cytotoxic and proliferative responses in the lymph nodes draining the site of tumor challenge. Investigations were performed to determine the effect of IL-1 therapy on the cytotoxic ability of local lymphocyte populations. These experiments demonstrated that lymph node cells from IL-1 treated animals have enhanced cytotoxic potential when compared to lymph node cells of animals that did not receive IL-1. Lymph node cells obtained directly from tumor-bearing animals did not exhibit significant cytotoxicity against EL-4, YAC-1, or P815 tumor targets. However, after stimulation with IL-2 in vitro, activity was generated against all three tumors (Fig. 11). The lack of cytotoxic activity in freshly isolated lymph node cells has also been observed in antigenic systems, and may be due to premature arrest of the differentiation pathway (Vanhaesebroeck et al., 1989).

Importantly, lymph node cells from IL-1 treated animals demonstrated enhanced cytotoxic potential when compared to cells from non-IL-1 treated animals. The enhanced cytotoxic potential was shown by increased cytotoxic cell frequency and significantly higher total lytic activity following IL-2 stimulation (Fig. 11-14). The differences in total lytic activity were predominantly due to the increased lymph node cell number observed in the IL-1 treated animals. The

addition of exogenous IL-2 *in vitro* was necessary to reveal the differences between the IL-1 treated and non-IL-1 treated groups. The *in vivo* effects of IL-1 treatment may be difficult to measure *in vitro*, and IL-2 may function to amplify these effects. The increased frequency of cytotoxic cells occurred within 2 to 4 days of tumor challenge, and was associated with the enhancement in lymph node cell number.

IL-1 treated animals demonstrated enhanced total cytotoxic potential and increased cytotoxic cell frequency against all tumor targets assessed. Because of the method by which these cells were generated, a number of factors could lead to enhanced cytotoxicity. Increased cytotoxicity following stimulation with IL-2 *in vitro* may be due to increased cytotoxic ability of individual cells, increased ability of cells to survive in IL-2, or increased frequency of cytotoxic cells. In the bulk culture system, numerous interactions may occur between various cell populations, making it difficult to assess the reasons for enhanced cytotoxicity.

Limiting dilution analysis has been used to analyze the frequency of CTL precursors for a variety of antigens (Miller, 1982). In the current study, the ability of IL-1 to modulate the frequency of cytotoxic cells with multiple target specificities was examined. IL-1 treated animals demonstrated increased cytotoxic cell frequency when compared to animals which received adjuvant therapy alone (Fig. 12, 13). Statistically significant increases in frequency occurred early after tumor challenge in the IL-1 treated group (Table 7). A greater number of effector cells may develop in the IL-1 treated animals due

to lymphokine stimuli received *in vivo*. Vanhaesebroeck *et al*. (1989) suggest that an ongoing immune response may activate LAK precursor cells to reach a state of maturation that lies closer to the LAK effector stage. Lymph node cells from animals undergoing an immune response (such as the IL-1 treated animals) may therefore be further on the way to mature effectors. This may facilitate immune responses *in vivo* because these cells would theoretically require less subsequent stimulation to become cytotoxic.

In addition, the cells from the IL-1 treated animals may be better able to respond to IL-2. Increased cytotoxic cell frequency correlates with an enhanced lymphocyte responsiveness to IL-2 (Fig. 9, 12, 13). IL-1 may act directly on the cytotoxic cells by enhancing their responsiveness to IL-2, allowing for greater expansion in cell number following activation. Alternatively, IL-1 may act on another cell population, which in turn affects cytotoxic cell development or maturation, perhaps through the production of cytokines such as IL-2 or IL-4 (Widmer *et al*., 1987; Trenn *et al*., 1988).

Increased cytotoxic cell frequency was observed in the IL-1 treated animals on the same days in which IL-1 induced enhanced lymph node cell numbers (Table 5, 7). This correlation of increased cell number and increased frequency suggests that the accumulation of cytotoxic cells in the IL-1 treated animals may be related to the increase in lymph node cell number. The IL-1 induced increase in cytolytic cell frequency appeared to be dose dependent (Fig. 14). Animals which received a lower dose of IL-1 showed a more modest increase in frequency as compared to animals which received the higher dose. Similar

dose dependence is observed in the increase in lymph node cell number, and also in the IL-1 mediated tumor immunity in this system (Hornung, dissertation, 1987). The cell number increase may also represent an expansion of helper and/or memory cells.

Limiting dilution analysis gives the lower limit to the true frequency (Miller, 1982). The cytotoxic cell frequencies generated by the limiting dilution analysis experiments are modest in comparison to the frequencies of CTL precursors found in more antigenic systems. The weakly immunogenic nature of the EL-4 lymphoma (Cantrell et al., 1979) is the most probable reason for this difference. Another factor may be reduced immune responsiveness due to the presence of the tumor. It could be suggested that the low frequencies and the differences between bulk and limiting dilution analysis cytotoxicity could be due to an insufficient amount of IL-2 in culture. The possibility that insufficient IL-2 was present is unlikely, however, because the amount of IL-2 used in the limiting dilution experiments was at least twice that used in the bulk culture system. The amount of IL-2 is in the range found to generate maximal cytolytic activity in murine lymph node cells (Gao et al., 1987). In addition, linear plots were obtained when the input cell number was graphed against the fraction of negative wells. This demonstrates that only one necessary component was limiting. Linear graphs were obtained for lymph node cells from both IL-1 treated and non-IL-1 treated animals, allowing for an accurate comparison of cytolytic cell frequency between the treatment groups.

When properly performed, limiting dilution analysis measures

only one limiting factor, which is the number of precursor cells. A straight line graph indicates a single-hit curve which is evidence for a single limiting cell type (Lefkovits and Waldmann, 1984). Non-linear curves indicate inadequate culture conditions and/or complex cellular interactions. The two types of non-linear curves are multitarget and multihit. A multitarget curve reflects the need for two or more different cell types to generate effector activities. These curves have a definite linear portion which may be extrapolated to the y-axis to determine the number of cells involved. A multihit curve is characterized by no definite slope. No extrapolation to the axis is possible. Such curves reflect the need for two or more cells of the same kind to interact. A third type of non-linear curve is one in which "leveling off" occurs. At higher cell inputs, where the precursor cells are saturating, another cell type becomes limiting. The curve asymptotically approaches a particular value for y (the fraction of negative cultures per total number of cultures). The graphs generated in this investigation corresponded to the single-hit straight line type (Fig. 12, 13). Preliminary experiments with lower amounts of IL-2 produced non-linear graphs which resembled multitarget curves. In these graphs, linear portions were present between 5×10^4 and 1×10^5 input cells, with a curved portion approaching a y value of 1.0 at 1×10^4 cells.

The limiting dilution analysis results can be used to suggest possible mechanisms for cytotoxic effector development. The use of suboptimal IL-2 concentrations with low lymph node cell numbers generated non-linear precursor plots. Addition of sufficient ex-

ogenous IL-2 allowed for a linear response. Other investigators have noted similar culture requirements in the limiting dilution analysis of CTL precursors (Horohov et al., 1988; Fischer-Lindahl and Wilson, 1977; Teh et al., 1977a; Teh et al., 1977b; Skinner and Marbrook, 1976). One or more of the cells required for the production of IL-2 can be diluted out by limiting dilution before the culture becomes limiting for CTL precursors (Miller, 1982). This effect suggests the involvement of an additional cell-type in the development of cytotoxic activity; this cell may be diluted out at the low responder cell numbers. Individual wells in the limiting dilution assay had much fewer cells than those in the bulk culture system (1/25th to 1/250th of the number). Therefore it is not surprising that a necessary additional factor may be limiting at the lower cell numbers.

Missing cell populations may produce cytokines necessary for the expansion and maturation of cytotoxic cells. Because the cytotoxic activity generated is against several tumor targets, but the immunity to the tumor is specific, it could be postulated that the cell which is diluted out may be the immunologically specific component. In this model, IL-1 could affect the generation of a specific T helper-type cell, which in turn produces cytokines to stimulate the production of non-specific cytotoxic effectors. These cell populations may also develop in the non-IL-1 treated animals, but at a number insufficient to allow for immunity upon secondary tumor challenge. Generally, there is a greater difference between the cytotoxicity generated in the bulk and limiting dilution assays when lymph node cells from non-IL-1 treated animals are used (e.g. no cytotoxicity against P815 under

limiting dilution conditions). A lower number of these helper-type cells in the non-IL-1 treated animals could contribute to this discrepancy.

The limiting dilution analysis data suggest possible mechanisms for IL-1 action in this experimental model. Little specific CTL response to EL-4 has been found in this system. This observation is consistent with the weakly immunogenic nature of the tumor (Gorer, 1950; Cantrell et al., 1979). The limiting dilution analysis performed in this study further reinforces this observation. Reactivity against EL-4 develops concomitantly with reactivity against P815, and slightly after YAC-1 reactivity. These data suggest the possibility of more than one effector population, one with NK-like activity and one with a broader range of reactivity. Local induction of non-specific effectors has been implicated in the development of in vivo tumor resistance (Crum, 1987). Lymph node cells from IL-1 treated animals should be better able to respond to in vivo stimulus to become cytotoxic effectors.

The general enhancement of potential cytotoxic function seen with lymph node cells from IL-1 treated animals may be related to the increase in $CD8^+$ cells observed in the regional lymph nodes. Significantly enhanced numbers of $CD8^+$ cells were present in the IL-1 treated animals on days 2, 3 and 4 post tumor challenge. This cell number increase could represent an expansion of cytotoxic precursor and/or effector cells which have a role in the immune response to the tumor.

The ability of lymph node cells from IL-1 treated animals to

adoptively transfer immunity to the tumor suggests the development of memory populations reactive to the tumor. The results of the antigenic stimulation experiments provide evidence for the presence of cells reactive to EL-4 in the lymph nodes following secondary challenge with the tumor. Experiments showed that lymph node cells from IL-1 treated animals demonstrate enhanced proliferation in response to EL-4 stimulation *in vitro* following secondary challenge *in vivo*. This responsiveness was predominantly tumor-specific as indicated by the low level of proliferation in response to LLC stimulation *in vitro* (Fig. 15). The presence of EL-4-reactive cells in the lymph nodes of IL-1 treated animals may be important in the immune response to the tumor.

Previously, it has been shown that a low level of reactivity to EL-4 is present in the spleens of animals undergoing secondary challenge (Hornung, dissertation, 1987). In those experiments, the stimulation indices obtained ranged from 3.0 to 3.75 for the IL-1 treated and 1.5 to 2.0 for the non-IL-1 treated. The ability of cells to proliferate in response to EL-4 suggests that the tumor expresses some antigenic determinants to which an immune response can be produced. In the current investigation, lymph node cells from the IL-1 treated animals show enhanced reactivity as compared to cells from non-IL-1 treated animals. Some reactivity was present on day 10 post tumor challenge, but day 14 cells showed the greatest responsiveness of the days measured. The stimulation indices for the IL-1 treated animals were 11.9 $\pm$ 1.6 for the EL-4 and 2.2 $\pm$ 0.6 for LLC. The responsiveness in the lymph nodes was substantially higher than that seen pre-

viously in the spleen. In contrast, lymph node cells from non-IL-1 treated animals did not exhibit enhanced responsiveness to EL-4 by day 14 post challenge. Lymph node cells derived more than 14 days post tumor challenge were not examined to determine if a shift in the kinetics of the response could be responsible for this difference. Lymph node cells derived at time points later than 14 days may contain EL-4 cells, and therefore accurate assessments can not be performed using these cells. However, even if reactivity were to develop after day 14 in the non-IL-1 treated animals, it would be too late to prevent tumor development and subsequent animal mortality.

Although lymph node cells from the IL-1 treated animals showed enhanced proliferation in response to EL-4, a low level of proliferation occurred in response to LLC stimulation in vitro (Fig. 15, panel A.). This indicates a non-specific component in the response generated by IL-1 and EL-4 in vivo. The stimulation of tumor-specific cells would lead to the elaboration of cytokines, and an overall increase in responsiveness. Animals which did not receive IL-1 at the time of primary tumor challenge did not show proliferation in response to LLC. Lymph node cells from animals undergoing primary tumor challenge did not proliferate in response to EL-4 in vitro. When examining animals undergoing primary tumor challenge, there is no way to determine which animals will survive and subsequently develop tumor immunity. Therefore, the lack of responsiveness to EL-4 in the primary challenge animals may be due to the presence of cells from non-protected animals. By looking at animals which have already survived the tumor challenge, the percentage of immune cells was increased.

Animals were rechallenged *in vivo* with either EL-4 or LLC. Memory cells may develop at the time of primary tumor challenge, but require secondary stimulation *in vivo* for a sufficient expansion in number to occur to allow for measurement *in vitro*. A secondary challenge *in vivo* also allows for increased numbers of antigen-reactive cells in the draining lymph nodes by attracting circulating lymphocytes to the region (Cohen and Livnat, 1976).

Experiments were performed to determine the effect of IL-1 therapy on the proliferative responses of lymph node cells from primary and secondary challenge animals. These investigations revealed that IL-1 treatment enhanced the ability of lymph node cells from animals undergoing primary tumor challenge to respond to IL-2 as judged by enhanced proliferation and increased recoverable cell numbers (Fig. 9, 10). IL-1 augmentation of lymph node cell responsiveness was associated temporally with the increases in lymph node cell number and cytotoxic potential. Lymph node cells from IL-1 treated animals undergoing primary tumor challenge showed an enhanced proliferative response to IL-2, which resulted in an increased cell number following stimulation with IL-2 (Fig. 9, 10). This effect was noted on days 3 and 5 post tumor challenge, but not on day 4. IL-1 therapy was administered on alternating days begin-ning on the day of tumor challenge (day 0). Animals which received IL-1 18 to 24 hours before their lymph nodes were removed, showed enhanced lymphoproliferation. In contrast, animals which received IL-1 42 to 48 hours before lymph node removal did not. It appears that IL-2 responsiveness was enhanced in correspondence to the administration of IL-1 therapy. The

delay between IL-1 administration and the increase in IL-2 responsiveness implies that the IL-1 effect is indirect. However, the increased IL-2 inducible cell number in the IL-1 treated animals represents an increased potential for tumor reactivity in the draining lymph nodes. In addition, the increase demonstrates that the enhanced proliferative ability of the lymph node cells from the IL-1 treated animals leads to increased viability and cell number in vitro. The enhanced ability to respond to IL-2 may result in the expansion of host protective cell subsets in the IL-1 treated animals.

Increases in cell populations could be important in the generation of cytotoxic responses, as well as in subsequent immunity to the tumor. The increased responsiveness to IL-2 in vitro exhibited by the lymph node cells from the IL-1 treated animals has implications for enhanced immune responsiveness in vivo. An enhanced proliferative ability may lead to the expansion of cell populations important in cytotoxic and helper responses. A more responsive cytotoxic population would result in greater cytotoxic ability due to a quicker response. Quicker responsiveness could be important in the early response to tumors allowing for survival and immunity. Increased responsiveness in helper populations could result in increased cytokine production and may allow for enhanced development of memory populations.

Based on the observations of this study, IL-1 may be valuable as an immunoaugmenting agent in conjunction with other forms of tumor therapy. This study demonstrates that systemic IL-1 treatment affects immune parameters in the regional lymph nodes of animals undergoing

adjuvant therapy. The regional lymph nodes of IL-1 treated animals demonstrated increased lymph node cell number, enhanced numbers of T lymphocytes, and increased cytotoxic cell frequency. Some of these cells were capable of mediating effects against EL-4 in naive animals. This is the first investigation which has identified immune enhancing effects of IL-1 in a weakly immunogenic tumor model in which IL-1 treatment provides for tumor immunity. These effects may be related to the immune response to the tumor and subsequent development of protective immunity. Because some of these effects were enhanced by exogenous IL-2, the in vivo administration of IL-2 in conjunction with IL-1 may increase the therapeutic effects.

The results of this study suggest that the immune-enhancing activities of IL-1 would make it a useful adjunct to other therapeutic modalities in the treatment of neoplasia. These results are consistent with the hypothesis that IL-1 mediates an immunological response which is tumor-protective in this experimental model. This study suggests several areas of investigation to further elucidate the role of IL-1 in the generation of tumor immunity. Among these are an examination of cellular interactions at the tumor site, and an evaluation of the role of $CD4^+$ lymphocytes in the generation and maintenance of tumor immunity. Information about which lymphocyte populations reach the tumor site and what interactions occur could be used to evaluate the relevance of the immune enhancing effects of IL-1 to the tumor immune response. $CD4^+$ lymphocytes have been suggested as mediators of tumor immune effects in various experimental tumor models (Greenberg et al., 1981; Fujiwara et al., 1984). A more careful eval-

uation of these cells in the primary and secondary responses to the tumor may reveal their role in the IL-1 induced immunity in this tumor model.

SUMMARY

The objective of this study was to investigate the local lymphoid responsiveness generated by IL-1 treatment in an experimental murine tumor model, and to evaluate the relationship between this lymphoid responsiveness and the induction of apparent tumor immunity. This model was selected because IL-1 treatment, in conjunction with local adjuvant therapy, allows for the generation of apparent tumor-specific immunity to the weakly immunogenic EL-4 lymphoma.

This study demonstrated that systemic administration of IL-1 modifies local immune responses in animals undergoing adjuvant therapy for a primary tumor. IL-1 treatment enhanced immune responsiveness in the lymph nodes draining the site of tumor challenge as demonstrated by increased lymph node cell number, enhanced numbers of T lymphocytes, and increased cytotoxic potential. Animals which received IL-1 and adjuvant therapy showed a significantly higher increase in draining lymph node cell number following tumor challenge. The cell number increase correlated with an overall increase in $CD4^+$, $CD8^+$, and Thy 1.2^+ cells. Approximately 80 to 85% of the cell number increase could be accounted for by the increase in Thy 1.2^+ lymphocytes. The increase in Thy 1.2^+ cells represented an overall increase in cell number, and not a specific or selective increase in either $CD4^+$ or $CD8^+$ lymphocytes.

Experiments which evaluated the cytotoxic ability of local lymphocyte populations showed that lymph node cells from IL-1 treated animals have enhanced cytotoxic potential when compared to lymph node

cells of animals that did not receive IL-1, as demonstrated by increased cytotoxic cell frequency. IL-1 treatment enhanced the ability of lymph node cells from animals undergoing primary tumor challenge to respond to IL-2 as judged by enhanced proliferation and increased recoverable cell numbers. IL-1 augmentation of lymph node cell responsiveness was associated temporally with increases in lymph node cell number and cytotoxic potential. Lymph node cells from IL-1 treated animals demonstrated enhanced proliferation in response to EL-4 stimulation in vitro following secondary challenge in vivo. This responsiveness was predominantly tumor-specific as indicated by the low level of proliferation in response to an antigenically distinct tumor in vitro. The presence of relevant tumor-reactive cells in the lymph nodes of IL-1 treated animals may be important in the immune response to the tumor.

Utilizing adoptive transfer of tumor resistance as an assay of in vivo activity, this study also demonstrated that systemic IL-1 treatment affects the tumor-protective capacity of the regional lymphoid tissue. Lymph node cells from IL-1 treated animals have antitumor effects against EL-4 in vivo, as demonstrated by delayed tumor development and enhanced animal survival. Spleen cells derived from these same animals had no effect on tumor development or animal survival, suggesting that responsiveness to the tumor during that time period was local and not systemic.

REFERENCES

Aiello, F.B., Longo, D.L., Overton, R., Takacs, L., and Durum, S.K. 1990. A role for cytokines in antigen presentation: IL-1 and IL-4 induce accessory functions of antigen-presenting cells. J. Immunol. 144:2572-2581.

Antoni, G., Presentini, R., Perin, F., Tagliabue, A., Ghiara, P., Censini, S., Volpini, G., Villa, L. and Boraschi, D. 1986. A short synthetic peptide fragment of human interleukin 1 with immunostimulatory but not inflammatory activity. J. Immunol. 137:3201-3204.

Aribia, M-H., Leroy, E., Lantz, O., Metivier, D., Autran, B., Charpentier, B., Hercend, T., and Senik, A. 1987. Recombinant IL-2-induced proliferation of human circulating NK cells and T lymphocytes: synergistic effects of IL-1 and IL-2. J. Immunol. 139:443-451.

Barker, P.E., Fahey, J.V., and Munck, A. 1981. Prostaglandin inhibition of T-cell proliferation is mediated at two levels. Cell. Immunol. 61:52-61.

Belardelli, F., Ciolli, V., Testa, U., Montesoro, E., Bulgarini, D., Proietti, E., Borghi, P., Sestili, P., Locardi, C., Peshle, C., and Gresser, I. 1989. Anti-tumor effects of interleukin-2 and interleukin-1 in mice transplanted with different syngeneic tumors. Int. J. Cancer 44:1108-1116.

Besedovsky, H., del Rey, A., Sorkin, E., and Dinarello, C.A. 1986. Immunoregulatory feedback between interleukin-1 and glucocorticoid hormones. Science 233:652-654.

Besedovsky, H., and del Rey, A. 1987. Neuroendocrine and metabolic responses induced by interleukin-1. J. Neurosci. Res. 18:172-178.

Bevilacqua, M.P., Pober, J.S., Wheeler, M.E., Mendrick, D., Cotran, R.S., and Giborne, M.A., Jr. 1985. Interleukin-1 acts on cultured human vascular endothelial cells to increase the adhesion of polymorphonuclear leukocytes, monocytes and related leukocyte cell lines. J. Clin. Invest. 76:2003-2011.

Boraschi, D., Nencioni, L., Villa, L., Censini, S., Bossu, P., Ghiara, P., Presentini, R., Porin, F., Frasca, D., Doria, G., Dorni, G., Musso, T., Giovarelli, M., Ghezzi, P., Bertini, R., Besedovsky, H., del Rey, A., Sipe, J., Antoni, G., Silvestri, S., and Tagliabue, A. 1988. *In vivo* stimulation and restoration of the immune response by the noninflammatory fragment 163-171 of human

interleukin-1 beta. J. Exp. Med. 168:675-686.

Cantrell, J.L., McLaughlin, C.A., and Ribi, E. 1979. Efficacy of tumor cell extracts in immunotherapy of murine EL-4 leukemia. Cancer Res. 39:1159-1167.

Chiplunkar, S., Langhorne, J., and Kaufman, S.H.E. 1986. Stimulation of B cell growth and differentiation by murine recombinant interleukin 1. J. Immunol. 137:3748-3752.

Cohen, I.R., and Livnat, S. 1976. The cell-mediated immune response: interactions of initiator and recruited T lymphocytes. Transplant. Rev. 29:24-58.

Crum, E.D. Subsets of circulating T-lymphocytes mediating resistance in in vivo growth of a carcinogen-induced syngeneic rat tumor. 1982. Cancer Res., 44:3196-3201.

Crum, E.D. Specific induction of local antitumor effector cells mediated in vivo by the circulating lymphocyte pool. 1987. Cancer Res., 47:5584-5589.

Crump, W.L., Owen-Schaub, L.B., and Grimm, E.A. 1989. Synergy of human recombinant interleukin 1 with interleukin 2 in the generation of lymphokine-activated killer cells. Cancer Res. 49:149-153.

Czuprynski, C.J. and Brown, J.F. 1987. Recombinant murine interleukin-1 alpha enhancement of nonspecific antibacterial resistance. Infect. Immun. 55:2061-2065.

Davis, T.A., Monroy, R.L., Skelly, R.R., Donahue, R.E., and MacVittie, T.J. 1990. Differential augmentation of in vivo natural killer cytotoxicity in normal primates with recombinant human interleukin-1 and granulocyte-macrophagé colony-stimulating factor. Clin. Exp. Immunol. 79:436-442.

Di Giovine, F.S., and Duff, G.W. 1990. Interleukin 1: the first interleukin. Immunol. Today 11:13-20.

Dinarello, C.A. 1984. Interleukin-1. Rev. Infect. Dis. 6:51-95.

Dinarello, C.A., Cannon, J.G., Mier, J.W., Bernheim, H.A., LoPreste, G., Lynn, D.L., Love, R.N., Webb, A.C., Auron, P.E., Reuben, R.C., Rich, A., Wolff, S.M., and Putney, S.D. 1986. Multiple biologic activities of human recombinant interleukin-1. J. Clin. Invest. 77:1734-1739.

Dinarello, C.A. The biology of interleukin 1 and comparison to tumor necrosis factor. 1987. Immunol. Let. 16:227-231.

Dresser, D.W., Taub, R.N., and Krantz, A.R. 1970. The effect of

localized injection of adjuvant material on the draining lymph node. II. Circulating lymphocytes. Immunology 18:663-670.

Durum, S.K., Higuchi, C., and Ron, Y. Accessory cells and T cell activation. 1984. The relationship between two components of macrophage accessory cell function: I-A and IL-1. Immunobiology 168:213-231.

Durum, S.K., Schmidt, J.A. and Oppenheim, J.J. 1985. Interleukin-1: An immunological perspective. Ann. Rev. Immunol. 3::263-287.

Dye, E.S., North, R.J., and Mills, C.D. 1981. Mechanisms of anti-tumor action of Corynebacterium parvum. I. Potentiated tumor-specific immunity and its therapeutic limitations. J. Exp. Med. 154:609-620.

Ebina, T., and Ishikawa, K. 1989. Antitumor effect of interleukin-1 beta in the double grafted tumor system. Jap. J. Cancer Res. 80:570-576.

Fontana, A., Kristensen, F., Dubs, R., Gemsa, D., and Weber, E. 1982. Production of prostaglandin E and an interleukin 1-like factor by cultured astrocytes and C_6 glioma cells. J. Immunol. 129:2413-2419.

Farrar, W.L., Mizel, S.P., and Farrar, J.J. 1980. Participation of lymphocyte activating factor (interleukin-1) in the induction of cytotoxic T cell responses. J. Immunol. 124:1371-1377.

Fernandez-Cruz, E., Gilman, S.C., and Feldman, J.D. 1982. Immunotherapy of a chemically-induced sarcoma in rats: characterization of the effector T cell subset and nature of suppression. J. Immunol. 128:1112-1117.

Fischer-Lindahl, K., and Wilson, D.B. 1977. Histocompatibility antigen-activated cytotoxic T lymphocytes. II. Estimates of the frequency and specificity of precursors. J. Exp. Med. 145:508-522.

Forni, G., Musso, T., Jemma, C., Boraschi, D., Tagliabue, A., and Giovarelli, M. 1989. Lymphokine-activated tumor inhibition in mice. Ability of a nonopeptide of the human IL-1 beta to recruit anti-tumor reactivity in recipient mice. J. Immunol. 142:712-718.

Frost, P., and Lance, E.M. 1978. On the mechanism of action of adjuvants. Immunology 35:63-68.

Fujiwara, H., Fukuzawa, M., Yoshioka, T., Nakajima, H., and Hamaoka, T. 1984. The role of tumor-specific Lyt-1^+2^- T cells in eradicating tumor cells in vivo. I. Lyt-1^+2^- T cells do not necessarily require recruitment of host's cytotoxic T cell

precursors for implementation of in vivo immunity. J. Immunol. 133:1671-1676.

Gao, L., Asherson, G.L., and Malkovsky, M. 1987. Increased lymphokine activated killer (LAK) activity in the regional lymph nodes of mice following immunization with contact sensitizing agents. Clin. Exp. Immunol. 70:217-221.

Gery, I., and Lepe-Zuniga, J.L. 1984. Interleukin 1: uniqueness of its production and spectrum of activities. Lymphokines 9:109-125.

Giri, J.G., Kincade, P.W., and Mizel, S.B. 1984. Interleukin 1-mediated induction of kappa-light chain synthesis and surface immunoglobulin expression on pre-B cells. J. Immunol. 132:223-228.

Gorer, P.A. 1950. Studies of antibody response of mice to tumour inoculation. Br. J. Cancer 4:372-279.

Greenberg, P.D., Cheever, M.A., and Fefer, A. 1980. Detection of early and delayed antitumor effects following curative adoptive chemoimmunotherapy of established leukemia. Cancer Res. 40:4428-4432.

Greenberg, P.D., Cheever, M.A., and Fefer, A. 1981. Eradication of disseminated murine leukemia by chemoimmunotherapy with cyclophosphamide and adoptively transferred immune syngeneic Lyt $1^{+}2^{-}$ lymphocytes. J. Exp. Med. 154:952-963.

Hagiwara, H., Su Huang, H-J., Arai, N., Herzenberg, L.A., Arai, K-I., and Zlotnik, A. 1987. Interleukin 1 modulates messenger RNA levels of lymphokines and of other molecules associated with T cell activation in the T cell lymphoma LBRM33-1A5. J. Immunol. 138:2514-2519.

Herman, J., Dinarello, C.A., Kew, M.C., and Rabson, A.R. 1985. The role of interleukin-1 in tumor NK cell interactions: Correction of defective NK cell activity in cancer patients by treating target cells with IL-1. J. Immunol. 135:2882-2886.

Ho, S.N., Abraham, R.T., Nilson, A., Handwerger, B.S., and McKean, D.J. 1987. Interleukin 1-mediated activation of interleukin 4 (IL-4)-producing T lymphocytes. Proliferation by IL-4-dependent and IL-4-independent mechanisms. J. Immunol. 139:1532-1540.

Holsti, M.A., and Raulet, D.H. 1989. Interleukin-1 and interleukin-6 synergize to stimulate IL-2 production and proliferation of peripheral T cells. J. Immunol. 143:2514-2519.

Hori, K., Mihich, E., and Ehrke, M.J. 1989. Role of tumor necrosis factor and interleukin-1 in gamma interferon promoted activation

of mouse tumoricidal macrophages. Cancer Res. 49:2606-2614.

Hornung, R.L., and Mathews, H.L. 1985. Tumor immune response modulation by interleukin-1. In: M.J. Kluger, J.J. Oppenheim, and M.C. Powanda (eds.), The Physiologic, Metabolic, and Immunologic Actions of Interleukin-1, pp. 273-284. New York, N.Y.: Alan R. Liss, Inc.

Horohov, D.W., Stocks, N.I., and Siegel, J.P. 1988. Limiting dilution analysis of human CTL differentiation. Requirement for a lymphokine-mediated differentiation signal. Immunol. 65:119-124.

Houssiau, F.A., Coulie, P.G., and Van Snick, J. 1989. Distinct roles of interleukin-1 and interleukin-6 in human T cell activation. J. Immunol. 143:2520-2524.

Hunninghake, G.W., Glazier, A.J., Monick, M.M., and Dinarello, C.A. 1986. Interleukin-1 is a chemotactic factor for human T-lymphocytes. Amer. Rev. Resp. Dis. 135:66-71.

Izumi, Y., Tsuchida, T., Okuno, K., Fujiwara, H., and Hamaoka, T. 1985. Enhanced induction of tumor-specific Lyt-$1^{+}2^{-}$ T cell-mediated protective immunity by in vivo administration of interleukin 1. Jpn. J. Cancer Res. (Gann) 76:863-870.

Johnson, C.S., Keckler, D.J., Topper, M.I., Braunschweiger, P.G., and Furmanski, P. 1989. In vivo hematopoietic effects of recombinant interleukin-1 alpha in mice: stimulation of granulocytic, monocytic, megakaryocytic, and early erythroid progenitors, suppression of late-stage erythropoiesis, and reversal of erythroid suppression with erythropoietin. Blood 73:678-683.

Kampschmidt, R.F., and Pulliam, L.A. 1978. Effect of human monocyte pyrogen on plasma iron, plasma zinc, and blood neutrophils in rabbits and rats. Proc. Soc. Exp. Bio. Med. 158:32-35.

Killar, L.M., Hatfield, C.A., Carding, S.R., Pan, M., Winterrowd, G.E., and Bottomly, K. 1989. In vivo administration of interleukin 1 elicits increased Ia antigen expression on B cells through the induction of interleukin 4. Eur. J. Immunol. 19: 2205-2210.

Koopman, W.J., Farrar, J.J., and Fuller-Bonar, J. 1978. Evidence for the identification of lymphocyte activating factor as the adherent cell-derived mediator responsible for enhanced antibody synthesis by nude mouse spleen cells. Cell. Immunol. 35:92-98.

Kreuger, J.M., Walter, J., Dinarello, C.A., Wolff, S.M., and Chedid, L. 1984. Sleep-promoting effects of endogenous pyrogen (interleukin-1). Amer. J. Physiol. 246:R994-R999.

Kunkel, S.L., Wiggins, R.C., Chensue, S.W., and Larrick, J. 1986. Regulation of macrophage tumor necrosis factor production by prostaglandin E_2. Biochem. Biophys. Res. Commun. 137:404-410.

Lachman, L.B., Dinarello, C.A., Llansa, N.D., and Fidler, I.J. 1986. Natural and recombinant human interleukin-1 beta is cytotoxic for human melanoma cells. J. Immunol. 136:3098-3102.

Lala, P.K., Parhar, P.S., and Singh, P. 1986. Indomethacin therapy abrogates the prostaglandin-mediated suppression of natural killer activity in tumor-bearing mice and prevents tumor metastases. Cell. Immunol. 99:108-118.

Larsson, E.J., Iscove, N.N., and Coutinho, A. 1980. Two distinct factors are required for induction of T-cell growth. Nature 283:664-666.

Lee, F.H., Currie, D., and Hwang, K.M. 1984. Analysis of the involvement of cells from donor and recipient mice in adoptive transfer of antitumor immunity. Cancer Res. 44:5491-5498.

Lefkovits, I., and Waldmann, H. 1984. Limiting dilution analysis of the cells of the immune system. I. The clonal basis of the immune response. Immunol. Today 5:265-268.

Libby, P., Ordovas, J.M., Birinyi, L.K., Auger, K.R., and Dinarello, C.A. 1986. Inducible interleukin-1 gene expression in human vascular smooth muscle cells. J. Clin. Invest. 78:1432-1438.

Lipsky, P.E., Thompson, P.A., Rosenwasser, L.J., and Dinarello, C.A. 1983. The role of interleukin 1 in human B cell activation: Inhibition of B cell proliferation and the generation of immunoglobulin secreting cells by antibody against human leukocytic pyrogen. J. Immunol. 130:2708-2714.

Lowenthal, J.W., Cerottini, J.C., and MacDonald, H.R. 1986. Interleukin 1-dependent induction of both interleukin 2 secretion and interleukin 2 receptor expression by thymoma cells. J. Immunol. 137:1226-1231.

Lubinski, J., Fong, T.C., Babitt, J.T., Ransone, L., Yodoi, J.J., and Bloom, E.T. 1988. Increased binding of IL-2 and increased IL-2 receptor mRNA synthesis are expressed by an NK-like cell line in response to IL-1. J. Immunol. 140:1903-1909.

Luger, T.A., Stadler, B.M., Luger, B.M., Mathieson, B.J., Mage, M., Schmidt, J.A., and Oppenheim, J.J. 1982. Murine epidermal cell-derived thymocyte-activating factor resembles murine interleukin 1. J. Immunol. 128:2147-2152.

Lynch, F., Doherty, P.C., and Ceredig, R. 1989. Phenotypic and functional analysis of the cellular response in regional lymphoid

tissue during an acute virus infection. J. Immunol. 142:3592-3598..

Mathews, H.L. 1981. Antitumor effects of a variety of non-viable bacteria against the murine EL-4 lymphoma. Cancer Immunol. Immnumother. 12:81-85.

Matsushima, K., Procopio, A., Abe, H., Scala, G., Ortaldo, J.R., and Oppenheim, J.J. 1985. Production of interleukin 1 activity by normal human peripheral blood B lymphocytes. J. Immunol. 135:1132-1136.

McCune, C.S., and Marquis, D.M. 1990. Interleukin 1 as an adjuvant for active specific immunotherapy in a murine tumor model. Cancer Res. 50: 1212-1215.

McDaniel, L.S., and Cozad, G.C. 1982. Immunoadjuvant effects of Blastomyces dermatitidis against EL-4 lymphoma in C57Bl/6J mice. J. Nat. Cancer. Inst. 69:1337-1342.

McMannis, J.D., and Plate, J.M.D. 1985. Xenogeneic antiserum to soluble products from activated lymphoid cells inhibits interleukin-1-mediated functions in helper pathway of cytolytic effector cell differentiation. Proc. Nat. Acad. Sci. USA 82:1513-1517.

Meuer, S.C., and Meyer zum Buschenfelde, K-H. 1986. T cell receptor triggering induces responsiveness to interleukin 2 but does not lead to T cell proliferation. J. Immunol. 136:4106-4112.

Miller, R.G. 1982. Clonal analysis by limiting dilution. An overview. In: C.G. Fathman and F.W. Fitch (eds.), Isolation, Characterization and Utilization of T Lymphocyte Clones, pp. 219-231. New York, N.Y.: Academic Press, Inc.

Mills, C.D., North, R.J., and Dye, E.J. 1981. Mechanisms of antitumor action of Corynebacterium parvum. II. Potentiated cytolytic T cell response and its tumor-induced suppression. J. Exp. Med. 154:621-630.

Migliori, R.J., Gruber, S.A., Sawyer, M.D., Hoffman, R., Ochoa, A., Bach, F.H., and Simmons, R.L. 1987. Lymphokine-activated killer (LAK) cells can be focused at sites of tumor growth by products of macrophage activation. Surgery 102:155-162.

Mochizuki, D.Y., Eisenman, J.R., Conlon, P.J., Larsen, A.D., and Tushinski, F.J. 1987. Interleukin 1 regulates hematopoietic activity, a role previously ascribed to hemopoietin 1. Proc. Natl. Acad. Sci. USA. 84:5267-5271.

Moore, M.A.S., and Warren, D.J. 1987. Synergy of interleukin 1 and granulocytic colony-stimulating factor: in vivo stimulation of

stem-cell recovery and hematopoietic regeneration following 5-fluorouracil treatment of mice. Proc. Natl. Acad. Sci. USA. 84:7134-7138.

Morikage, T., Mizushima, Y., Sakamoto, K., and Yano, S. 1990. Prevention of fatal infections by recombinant human interleukin 1 alpha in normal and anticancer drug-treated mice. Cancer Res. 50:2099-2104.

Mosley, B., Dower, S.K., Gillis, S., and Cosman, D. 1987. Determination of the minimum polypeptide lengths of the functionally active sites of human interleukin-1 alpha and -1 beta. Proc. Nat. Acad. Sci. USA 84:4572-4576.

Murphy, P.A., Simon, P.L., and Willoughby, W.F. 1980. Endogenous pyrogens made by rabbit peritoneal exudate cells are identical with lymphocyte-activating factors made by rabbit alveolar macrophages. J. Immunol. 124:2498-2501.

Nakamura, S., Nakata, K., Kashimoto, S., Yoshida, H., and Yamada, M. 1986. Antitumor effect of recombinant human interleukin 1 alpha against murine syngeneic tumors. Jpn. J. Cancer Res. (Gann) 77:767-773.

Neta, R., Douches, S., and Oppenheim, J.J. 1986. Interleukin 1 is a radioprotector. J. Immunol. 136:2483-2485.

Neta, R., Sztein, M., Oppenheim, J.J., Gillis, S., and Douches, S. 1987. The in vivo effects of interleukin-1. I. Bone marrow cells are induced to cycle after administration of interleukin-1. J. Immunol. 139:1861-1866.

North, R.J., Neubauer, R.H., Huang, J.J.H., Newton, R.C. and Loveless, S.E. 1988. Interleukin 1-induced, T cell-mediated regression of immunogenic murine tumors. Requirement for an adequate level of already acquired host concomitant immunity. J. Exp. Med. 168:2031-2043.

Ochoa, A.C., Gromo, G., Alter, B.J., Sondel, P.M., and Balch, F.H. 1987. Long-term growth of lymphokine-activated killer (LAK) cells: role of anti-CD3, beta-IL-1, interferon-gamma and beta. J. Immunol. 138:2728-2733.

Okusawa, S., Gelfand, J.A., Ikejima, T., Connolly, R.J., and Dinarello, C.A. 1988. Interleukin 1 induces a shock-like state in rabbits. Synergism with tumor necrosis factor and the effect of cyclooxygenase inhibition. J. Clin. Invest. 81:1162-1172.

Onozaki, K., Matsushima, K., Kleinerman, E.S., Saito, T., and Oppenheim, J.J. 1985a. Role of interleukin 1 in promoting human monocyte-mediated tumor cytotoxicity. J. Immunol. 135:314-320.

Onozaki, K., Matsushima, K., Aggarwal, B.B, and Oppenheim, J.J. 1985b. Human interleukin 1 is a cytocidal factor for several tumor cell lines. J. Immunol. 135:3962-3968.

Ostensen, M.E., Thiele, D.L., and Lipsky, P.E. 1989. Enhancement of human natural killer cell function by the combined effects of tumor necrosis factor alpha or interleukin-1 and interferon-alpha or interleukin-2. J. Biol. Res. Modif. 8:53-61.

Ozaki, Y., Ohashi, T., Minami, A. and Nakamura, S-I. 1987. Enhanced resistance of mice to bacterial infection induced by recombinant human interleukin-1 alpha. Infect. Immun. 55:1436.

Pike, B.L., and Nossal, G.J. 1985. Interleukin 1 can act as a B-cell growth and differentiation factor. Proc. Natl. Acad. Sci. USA. 82:8153-8157.

Plaut, M., Lichtenstein, L.M., Gillespie, E., and Henney, C. 1973. Studies on the mechanism of lymphocyte-mediated cytolysis. J. Immunol. 111:389-394.

Reed, S.G., Pihl, D.L., Conlon, P.J., and Grabstein, K.H. 1989. IL-1 as adjuvant. Role of T cells in the augmentation of specific antibody production by recombinant human IL-1 alpha. J. Immunol. 142:3129-3133.

Renauld, J-C., Vink, A., and Van Snick, J. 1989. Accessory signals in murine cytolytic T cell responses. Dual requirement for IL-1 and IL-6. J. Immunol. 143:1894-1898.

Rennick, D., Yang, J., Gemmel, L. and Lee, F. 1987. Control of hemopoiesis by a bone marrow stromal cell clone: lipopolysaccharide and interleukin-1 inducible production of colony stimulating factors. Blood 69:682-691.

Scala, G., Allaven, P., Djew, J.X., Kasahara, T., Ortaldo, J.R., Herberman, R.B. and Oppenheim, J.J. 1984. Human large granular lymphocytes are potent producers of interleukin-1. Nature 309:56-59.

Shu, S., and Rosenberg, S.A. 1985. Adoptive immunotherapy of a newly induced sarcoma: immunologic characteristics of effector cells. J. Immunol. 135:2895-2903.

Simon, P.L., and Willoughby, W.F. 1981. The role of subcellular factors in pulmonary immune function: physiochemical characterization of two distinct species of lymphocyte-activating factor produced by rabbit alveolar macrophages. J. Immunol. 126:1534-1541.

Sipe, J.D., Vogel, S.N., Ryan, J.L., McAdam, K.P., and Rosenstreich, D.L. 1979. Detection of a mediator derived from the endotoxin-

stimulated macrophages that induces the acute phase serum amyloid A response in mice. J. Exp. Med. 150:597-606.

Sjogren, H.O., and Hellstrom, I. 1965. Induction of polyoma specific transplantation antigenicity in Moloney leukemia cells. Exp. Cell Res. 40:208-212.

Skinner, M.A., and Marbrook, J. 1976. An estimation of the frequency of precursor cells which generate cytotoxic lymphocytes. J. Exp. Med. 143:1562-1567.

Smith, K.A. 1980. T cell growth factor. Immunol. Rev. 51:337-357.

Stephenson, K.R., Perry-Lalley, D., Griffith, K.D., Shu, S., and Chang, A.E. 1989. Development of antitumor reactivity in regional draining lymph nodes from tumor-immunized and tumor-bearing murine hosts. Surgery 105:523-528.

Tartakovsky, B., Kovacs, E.J., Takacs, L., and Durum, S.K. 1986. T cell clone producing an IL-1-like activity after stimulation by antigen-presenting B cells. J. Immunol. 137:160-166.

Taswell, C. 1981. Limiting dilution assays for the determination of immunocompetent cell frequencies. I. Data analysis. J. Immunol. 126:1614-1619.

Teh, H.-S., Harley, E., Phillips, R.A., and Miller, R.G. 1977. Quantitative studies on the precursors of cytotoxic lymphocytes. I. Characterization of a clonal assay and determination of the size of clones derived from single precursors. J. Immunol. 118:1049-1056.

Teh, H.-S., Phillips, R.A, and Miller, R.G. 1977. Quantitative studies of the precursors of cytotoxic lymphocytes. II. Specificity of precursors responsive to allo-antigens and to concanavalin A. J. Immunol. 118:1057-1062.

Tobler, I., Borbely, A.A., Schwyzer, M., and Fontana, A. 1984. Interleukin-1 derived from astrocytes enhances slow wave activity in sleep EEG of the rabbit. Eur. J. Pharm. 104:191-192.

Trenn, G., Takayama, H., Hu-Li, J., Paul, W.E., and Sitkovsky, M.V. 1988. IL-4 enhances the development of cytotoxic T cells from Lyt 2^+ resting murine T lymphocytes. J. Immunol. 140:1101-1106.

Tuttle, R.L., and North, R.J. 1976. Mechanisms of antitumor action of Corynebacterium parvum: replicating short-lived T cells as the mediators of potentiated tumor-specific immunity. J. Reticuloendothelial Soc. 20:209-216.

Van't Wout, J., Van der Meer, J., Burza, M., and Dinarello, C.A. 1988. Protection of neutropenic mice from lethal Candida al-

bicans infection by recombinant interleukin-1. Eur. J. Immunol. 18:1143-1146.

Vanhaesebroeck, B., Grooten, J., and Fiers, W. 1989. An ongoing in vivo immune response affects the abundancy and differentiation of lymphokine-activated killer cell precursors but does not influence their broad spectrum target reactivity. J. Immunol. 143:1396-1402.

Walker, C., Kristensen, F., Bettens, F., and DeWeck, A.L. 1983. Lymphokine regulation of activated (G_1) lymphocytes. I. Prostaglandin E_2-induced inhibition of interleukin 2 production. J. Immunol. 130:1770-1773.

Widmer, M.B., Acres, R.B., Sassenfield, H.M., and Grabstein, K.H. 1987. Regulation of cytotoxic cell populations from human peripheral blood by B cell stimulatory factor-1 (interleukin-4). J. Exp. Med. 166:1447-1455.

Wood, D.D. 1979. Mechanism of action of human B cell activating factor. J. Immunol. 123:2400-2407.

Yamashita, U., and Shirakawa, F. 1987. Restoration of impaired T cell functions in tumor-bearing mice by the administration of interleukin 1. Jpn. J. Cancer Res. (Gann) 78:270-278.

Zatz, M.M., and Lance, E.M. 1971. The distribution of ^{51}Cr-labeled lymphocytes into antigen-stimulated mice. J. Exp. Med. 134:224-241.

Zucali, J.R., Broxmeyer, H.E., Dinarello, C.A., Gross, M.A., and Weiner, R.S. 1987. Regulation of early human hematopoietic (BFU-E and CFU-GEMM) progenitor cells in vitro by interleukin-1 induced fibroblast conditioned medium. Blood 69:33-37.

Approval Sheet

The dissertation submitted by Sylvia M. Kiertscher has been read and approved by the following committee:

Dr. Herbert L. Mathews, Director
Associate Professor of Microbiology
Loyola University of Chicago, Maywood, Illinois

Dr. Thomas Ellis
Associate Professor of Microbiology and Medicine
Loyola University of Chicago, Maywood, Illinois

Dr. Charles F. Lange
Professor of Microbiology
Loyola University of Chicago, Maywood, Illinois

Dr. H. Terry Wepsic
Chief of Laboratory Services
Long Beach Veterans Administration Hospital,
Long Beach, California, and
Professor of Pathology
University of California, Irvine, California

Dr. M. Rita Young
Career Research Scientist
Hines Veterans Administration Hospital, Hines, Illinois, and
Associate Professor of Pathology
Loyola University of Chicago, Maywood, Illinois

The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the dissertation is now given final approval by the Committee with reference to content and form.

The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

9/26/90
Date


Director's Signature