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Modulation of the Tumor Immune Response by Interleukin 1 in the C57bl/6 Murine Model

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MODULATION OF THE TUMOR IMMUNE RESPONSE
BY INTERLEUKIN 1 IN THE C57BL/6 MURINE MODEL

by

Ronald L. Hornung

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

The author, Ronald Lee Hornung, is the second son of Melvin P. and Arlene O. Hornung. He was born March 20, 1951, in Chicago, Illinois; U.S.A.

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- Goldberg, J., Hornung, R. L., Yamashita, T., and Wehrmacher, W. 1986. Age at death and risk factors in Sudden Infant Death Syndrome. Aust. Paediatr. J. Suppl., 21-28.
- Hornung, R. L., Hanzely, L., and Lynch, D. L. 1977. Occurrence of microbodies in the green alga Bracteacoccus cinnabarinus grown heterotrophically. Protoplasma, 93:135-145.
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- Hornung, R. L., Mathews, H. L. 1985. Modulation of the murine tumor immune response by interleukin 1. Midwest Autumn Immunology Conference. Abstract.

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LIST OF ABBREVIATIONS AND SYMBOLS

2-ME	2 mercaptoethanol
ATCC	American Type Culture Collection
BAF	B cell activation factor
BCG	Bacillus Calmette-Guerin
BCGF	B cell growth factor
BDF	B cell differentiation factor
BRM	Biological response modifier
cDNAs	Complementary DNAs
Con A	Concanavalin A
CPM	Counts per minute
DIH	Delayed type hypersensitivity
E/T	Effector-to-target
EDTA	Ethylenediamine tetraacetic acid
EP	Endogenous pyrogen
FBS	Fetal bovine serum
g	Gravities
HBSS	Hank's balanced salt solution
HCl	Hydrochloric acid
HLA-DR	Human leukocyte antigen DR
HP-1	Helper peak-1
HSA	Human serum albumin
i.d.	Intradermally
i.m.	Intramuscular

i.p.	Intraperitoneally
i.t.	Intratumor
Ia	I region-associated
Ig	Immunoglobulin
IL-1	Interleukin 1
IL-2	Interleukin-2
kd	Kilodalton
LAF	Lymphocyte activating factor
LAK	Lymphokine activated killer cell
LEM	Leukocytic endogenous mediator
IGL	Large granular lymphocytes
LPS	Lipopolysaccharide
L.U.	Lytic unit
MCF	Mononuclear cell factor
ND	Not determined
MP	Mitogenic protein
NK	Natural killer cell
NP-40	Nonidet P-40
P388D1A	P388D1 (source: ATCC)
P388D1Y	P388D1 (source: Dr. Y. Yamamura)
PEC	Peritoneal exudate cells
PGE	Prostaglandin E
PGE ₂	Prostaglandin E ₂
PHA-P	Phytohemagglutinin P
pI	Isoelectric points
PIF	Proteolysis-inducing factor

PMA	Phorbol myristate acetate
PMNs	Polymorphonuclear leukocyte(s)
PSR	Percent specific release
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide electrophoresis
SI	Stimulation index
STD	Standard deviation
T _h	T helper cell
T _s	T suppressor cell
TNF	Tumor necrosis factor
TRF _m	T cell replacing factor

CHAPTER I

INTRODUCTION

The primary goal of this dissertation research has been to determine the potential role of interleukin 1 (IL-1) in the development of protective immune responses to an experimental lymphoma. This biological response modifier (BRM) is considered for study because of its many known immunoregulatory roles (64,78), including the ability to augment the immune response in defined antigenic systems (285, 312). However, no previous reports have demonstrated a direct role for IL-1 in the development of immunity to experimental neoplasia.

The experimental tumor model employed in this study is the EL-4 lymphoma which is syngeneic in C57Bl/6 mice and results in high mortality (39,100). In this system, mice can be protected from a normally fatal dose of the weakly immunogenic EL-4 lymphoma (101) by local administration of Corynebacterium parvum (183). However, these animals show no demonstrable tumor immunity since they will succumb to a second challenge of the tumor. It has been the purpose of this study to determine whether administration of IL-1 can enhance animal survival or augment the development of immunity to the EL-4 lymphoma. The remaining objective of this research was to assess the compartments of the immune system which may be modulated by IL-1 leading to the establishment of tumor immunity. To accomplish this goal, in vitro analysis of splenic and lymphatic tissues of treated animals has been performed to assess a possible mechanism(s) by which IL-1

may modulate the immune response to the EL-4 lymphoma. Several immune correlates likely to be associated with the development of tumor immunity and, therefore, under scrutiny in this project, were EL-4 tumor specific cytotoxic T cells, natural killer cells (NK), and humoral antibody response to the EL-4 lymphoma. Also, possible modulation by IL-1 of lymphoid populations and their proliferative responsiveness to the EL-4 lymphoma were investigated. All of these immunological parameters have been reported by other investigators to be augmented by IL-1 and may underlie the mechanism by which IL-1 leads to the augmentation of tumor immunity in the EL-4:C57Bl/6 experimental model.

CHAPTER II

LITERATURE REVIEW

A. Adjuvant Effects of *Corynebacterium parvum*

Over the last twenty-five years, a wide variety of immunostimulants have been shown to effect the growth of syngeneic transplantable tumors in mice. Starting in the early 1960s with zymosan (yeast cell walls) (233) and Bacillus Calmette-Guérin (BCG) (231), this list of immunostimulants has been expanded to include viable bacteria, bacterial by-products, nonviable bacteria and chemically defined compounds (17).

The first indication that *C. parvum* would become an important research tool as an immunological stimulant was demonstrated by Halpern et al. in 1964, when they found *C. parvum* to be a potent stimulator of the reticulo-endothelial system (109). Many investigators have since verified these observations (123,243,286,294) with *C. parvum* treated mice showing increases in liver and spleen weights (5,109,188,197,228) as well as an increased ability to clear intravenously injected carbon. *C. parvum* has been shown to exert adjuvant effects on antibody production, induction of delayed type hypersensitivity (5,25,223), and may be satisfactorily substituted for the mycobacterial component of Freund's complete adjuvant (223). *C. parvum* treatment enhances protection against protozoal (226), viral (143) and bacterial infection (3), as well graft-versus-host disease (122). There is abundant evidence to indicate these effects are

attributable to augmentation of macrophage function (304). Aside from enhanced macrophage function, subcutaneous (307) and systemic (62) administration of C. parvum stimulates macrophage colony production by bone marrow cells, enhances proliferation of granulopoietic cells, and increases the percentage of nondifferentiated cellular forms in the bone marrow (18,262).

Treatment with C. parvum has also been shown to retard the growth of subcutaneous and peritoneal tumors in mice (30,107,198,270,313). There is much support for the view that macrophage stimulation is the mechanism responsible for the bacterial suspensions' effectiveness in enhancing the host's defence against neoplastic disease (162). Evidence has indicated that augmentation of NK activity is a potential mechanism of C. parvum antitumor activity (200), although a more recent report has demonstrated suppression of NK activity by the bacterium (86). The role of the macrophage in the expression of immunity to tumors was initially described by studies in which peritoneal macrophage cell suspensions, taken from immunized donors, were able to exert suppressive effects on syngeneic tumors in mice (232). These macrophages were also found to inhibit the growth of tumor cells in vitro (15). Macrophage mediated tumor cell killing has been shown to be a property of highly activated macrophages (139). Peritoneal macrophages, induced by irritants such as thioglycolate, appeared to be partially activated showing increased adherence and pinocytosis (188). However, these macrophages did not become cytotoxic for tumor cells until fully activated with various bacteria or endotoxin (37). Similarly, macrophages obtained from C. parvum treated

animals, when implanted either subcutaneously or intraperitoneally (i.p.) with murine fibrosarcoma tumor cells, caused a significant reduction in tumor growth (246). These macrophages, when incubated in vitro with syngeneic tumor cells, also demonstrated cytostatic (108) as well as cytotoxic activity for tumor cells (16). Since both nude (248,315) and germ-free mice (275) demonstrate equal antitumor effectiveness, it appeared that this augmented immune function was a direct result of macrophage-C. parvum interaction. Additional evidence implicated the monocyte/macrophage precursor as the target cell for the antitumor effect of C. parvum. Whole body irradiation of mice shortly before C. parvum injection prevents the development of resistance to tumor challenge, whereas irradiation after treatment completely suppressed the humoral response to C. parvum (30), but failed to abrogate the C. parvum antitumor effect (30,200). This suggests that the relevant cell arose from a radiosensitive, rapidly dividing precursor whose mature counterpart was radioresistant. This is consistent with the characteristics of the monocyte/macrophage cell (18). Compilation of this data has led to the conclusion that the activated macrophage is the common mediator of biological effects of C. parvum (29).

Although C. parvum has been used as an immunotherapeutic anti-cancer agent, the component(s) of this organism responsible for these activities has not yet been identified. The effects of various chemical treatments on the potency of C. parvum extracts have suggested that the active component is carbohydrate in nature (4,40,250,258), but may require complete cell integrity for full activity (40) since

purified cell walls retain antigenicity, but do not retain antitumor activity (257).

Although macrophage activation may play an important role in tumor rejection, these chemical studies have shown that cytotoxic macrophages do not appear to be sufficient for the overall antitumor activity of C. parvum (40). Peritoneal exudate cells from mice treated with the whole organism or the "light residue" (primarily cell walls and cell walls containing protoplasm) were highly activated, as demonstrated by their ability to nonspecifically kill tumor cells in vitro. However, when the "light residue" was treated with metaperiodate, which reduces carbohydrate content of the residue, the high macrophage activating properties were maintained, but the anti-tumor activity diminished significantly. These results suggest that macrophage activation is not the only property of C. parvum required to induce full in vivo antitumor activity (40).

It was noted that following systemic injection (intravenous) of C. parvum in mice, there was widespread proliferation, redistribution and mobilization of lymphoid cells (42) with associated splenomegaly, hepatomegaly and increased lung (5) and lymph node (42,188,197) weights. On the other hand, there is little change in spleen and liver weights following subcutaneous injection of C. parvum, although there is an enlargement of lymph nodes draining the subcutaneous injection site (270,294), with an associated increase in lymphocyte cell number in the draining nodes (51).

Investigation of this C. parvum mediated lymphoid mobilization lead to the demonstration of C. parvum mediated adjuvant activity in

mice as demonstrated by an amplification in the antibody response to both thymus dependent IgM-IgG responses (24,130,305) and the thymus independent IgM response (60,123,130,302). Local adjuvant effects of C. parvum have been shown when the injection of sheep red blood cells admixed with C. parvum increased the number of direct plaque forming cells in draining lymph nodes (201). Enhanced antigen presentation of antigen to lymphocytes seen by C. parvum stimulated macrophages (230,307) may explain these lymphocytic stimulatory effects of C. parvum.

T cell responsiveness may be stimulated or inhibited, depending on the route or C. parvum administration. Systemic C. parvum treatment is usually associated with depression of cell-mediated immune responses (7,272), possibly due to an effective depletion of sensitized cells trapped in the C. parvum stimulated spleen (272). On the other hand, enhancement of delayed type hypersensitivity (DTH) results after subcutaneous injection with a wide range of C. parvum doses (176,271,275,294). The activation of macrophages in regional lymph nodes appears to modulate the differentiation and clonalization of nearby T cells, with the dominant effect being the inhibition of the generation of specific T suppressor cells (176). Therefore, the increase in monocyte numbers (18) and functional activity (93), both in accessory cell function and lymphocyte subset modulation, may augment antigen presentation and lymphocytic responses at the site of macrophage activation. Such mechanisms may mediate the tumor immunity associated with C. parvum therapy.

In murine systems, the efficacy of C. parvum antitumor therapy has varied greatly depending on several parameters (201), many similar to the parameters mentioned earlier in this review regarding more general modulation of immunological responses. Several important therapeutic parameters to consider when utilizing this bacterial agent are the route of injection (201), the size and location of the tumor (52,201), the amount of bacteria injected (28,223), and the immunogenicity of the tumor in the host-tumor model (17,245). Depending on the tumor model employed, systemic administration of C. parvum has been shown to be an effective therapeutic agent when single doses were given from one week prior (30,270) to two weeks (165) after tumor challenge. C. parvum therapy was also effective when tumor/bacteria admixtures were injected simultaneously (248,295).

Against established tumors, systemic C. parvum treatment is most effective against small tumor masses when injected soon after tumor inoculation; multiple C. parvum doses have generally not increased effectiveness (199,286). Although tumor regressions have been achieved with intravenous therapy, this type of effect is not usually attainable with poorly immunogenic tumors (199).

Localized tumor therapy, with direct injection of C. parvum into growing solid tumor, have resulted in complete and permanent regressions in many different tumor models (33,106,163,255,294). In some cases, metastasized tumor have been shown to regress as well (163, 165). Subcutaneous injections of C. parvum at sites distant from the tumor usually have minimal effect on tumor growth, whereas marked inhibition is seen after injection near the region of tumor

growth (271,314). Unlike systemic treatment, which appears to favor the generation of nonspecific effector mechanisms (i.e. activated macrophages (234,270), natural killer cells (229)), intralesional C. parvum therapy appears to generate T cell mediated antitumor immunity since this antitumor action requires the presence of a functional T cell system (28,80,296,315). Animals treated in this fashion are not only able to regress the treated tumor lesion, but also regress untreated tumors at distant sites which are in early stages of development (202). Rejection of distant site tumors has been associated with the augmented production of cytolytic T cells in the lymph nodes draining the site of therapeutic injection (202). Animals, which have regressed their tumors after intralesional C. parvum therapy, are subsequently able to specifically reject a similar tumor implant (273,295) and possess T cells capable of passively transferring tumor resistance to unimmunized individuals (295,296).

Mice, which have rejected tumors that have developed from tumor cells admixed with C. parvum, are similarly and specifically immune (164). However, the ability to induce T cell specific tumor immunity again appears to be limited to the more highly immunogenic tumors (80,249). Nonspecific mechanisms appear to protect animals from weakly immunogenic tumors following intralesional C. parvum therapy, consequently animals surviving a weakly immunogenic tumor challenge are unable to successfully survive a second, identical tumor challenge.

The EL-4 lymphoma, the tumor model employed in this study, is an example of a weakly immunogenic tumor (39,100). This tumor is

refractory to immunoadjuvant therapeutic agents such as viable Mycobacterium bovis, BCG, and standard regimens of nonviable C. parvum (94,289). More rigorous adjuvant therapy protocols with C. parvum (183) and Blastomyces dermatitidis (190) have enabled animals to respond to the EL-4 lymphoma yielding high survival rates following tumor challenge. However, animals surviving this initial tumor challenge are unable to demonstrate tumor specific immunity upon EL-4 rechallenge (183). These results are markedly different when highly immunogenic tumor models are treated with adjuvant type immunotherapy, where the development of tumor specific immunity is seen in protected animals (174,202). The lack of an effective tumor immune response, seen in animals protected by immunoadjuvant therapy from weakly immunogenic tumors like the EL-4 lymphoma, suggests that this therapy model may serve as an effective system to assess other means of immunomodulation which may lead to concomitant tumor immunity.

B. The Biology of Interleukin 1

IL-1 is a hormone-like protein which appears to play many roles in the inflammatory and immune responses (64,78). Table 1 briefly lists many of the wide range of attributes attributed to IL-1, some of which will be discussed in more detail later in this review. Original immunologic studies described IL-1 as a soluble product released from activated macrophages (90) capable of co-mitogenic stimulation of thymocytes (89). However, IL-1 has been rediscovered several times due to its multiple biological actions. The original discovery of such a host derived substance occurred in the late 1940s when fever was initiated by a soluble factor produced and released by

activated peritoneal exudate cells, comprised predominantly of neutrophils (20). This substance was called granulocyte pyrogen, and later endogenous pyrogen (EP), when it was found that this substance accounted for the febrile associated with bacteria, endotoxin and most other exogenous pyrogenic materials (9,69). Other terms used to (MP) (298); B cell activation, differentiation and enhancement of antibody production in vitro by B cell activation factor (BAF) (308), B cell differentiation factor (BDF) (118) or T cell replacing factor

Table 1. Actions attributed to monocyte derived IL-1.

<u>General process and target cells</u>	<u>Activity</u>
Antitumor activity	
tumor cells	directly cytostatic and cytolytic
natural killer	augmentation of cytotoxicity
macrophages	enhanced lysis of tumor targets
Growth and differentiation	
T cells	lymphokine production and release
B cells	proliferation and differentiation
fibroblasts	proliferation
Inflammation	
neutrophils	chemotaxis, degranulation,
bone-marrow release	
macrophages	PG release, chemotaxis
fibroblasts	PG and collagenase release, growth
synovium	PG and collagenase release
endothelium	PG release
hepatocytes	secretion of acute-phase reactants, control of plasma divalent cations
Tissue catabolism	
skeletal muscle	proteolysis
osteoclasts	bone resorption
chondrocytes	cartilage breakdown
Central nervous system	
hypothalamus	fever induction
unknown	sleep induction

(TRF_m) (119), and helper peak-1 (HP-1) (145) respectively. After much collaborative study of these monokines, it became apparent that these activities and associated acronyms were actually different biologic facets of a single biochemical family of molecules. Therefore, IL-1 was introduced into the nomenclature to eliminate confusion and distinguish this monokine from other immunoregulatory molecules (1).

1. The Biochemistry of IL-1

The polypeptide nature of IL-1 was shown by enzymatic digestion with a variety of proteases (269) and loss of activity following arginine residue modification (206). The possibility that IL-1 is a glycoprotein remains unresolved, although there is no direct evidence that carbohydrate residues are present or essential for IL-1 bioactivity (147,204). Early estimates concerning the molecular weight of IL-1 were often confusing and contradictory. Part of this problem arose from the molecular weight heterogeneity seen from the intracellular and extracellular forms of IL-1 (98). All reports cannot be explained by this variation since molecular weight species ranging from 60 to 250 kilodaltons (kd) have been reported for both intracellular and extracellular forms of IL-1 (211,278,292). It is likely that these IL-1 species represent either aggregates with contaminating proteins or aggregates of the IL-1 monomer resulting from mixed disulfide bridges between free cysteine residues (64). The possibility of a high molecular weight protein carrier for IL-1 may also explain these sporadic results; however, no evidence for such a carrier has been presented. Several investigations have shown that IL-1

activity may reside in polypeptide cleavage products as small as 2 kd to 4 kd in size. A 4.2 kd peptide, which co-purified with PIF from plasma of febrile human patients, exhibited biochemical characteristics and bioactivity of IL-1 (67). Also, IL-1-like peptides ranging from 2 to 4 kd have been found in human urine which retain a portion of their bioactivity (142). These reports may explain the apparent resistance of IL-1 bioactivity to certain proteases (204, 269). Only after further purification and biochemical characterization can the relationship of these low molecular weight molecules be compared to natural or recombinant forms of IL-1.

The purification and biochemical characterization of IL-1 has proven to be a difficult task. Unlike many other circulating hormones, IL-1 is not stored in any gland or tissue; and when produced using in vitro superinduction protocols, only low picomolar quantities are obtained (238). The production of large quantities of murine IL-1 by a phorbol myristate acetate (PMA) stimulated, cloned macrophage cell line, P388D1, (156) allowed for its purification to homogeneity (208). The purified murine product demonstrated a molecular weight of 14,000 and, as reported previously (84,210), exhibited microcharge heterogeneity with isoelectric points (pI) of 4.9, 5.0 and 5.1. More recent methods of IL-1 purification, utilizing immunoaffinity procedures with a heterologous antiserum prepared in goats against murine IL-1 (207), have given a much higher yield of purified IL-1, thereby allowing more detailed studies of the protein. Seven discrete species were isolated upon electrophoresis of this material with each species having a different molecular weight rang-

ing from 13 to 17 kd as analyzed in a second dimension by sodium dodecyl sulfate polyacrylamide electrophoresis (SDS-PAGE).

Early research characterizing partially purified human IL-1 reported a variety of molecular weights generally in the 11,000 to 16,000 kilodalton range (27,157,292). High yield methods of production of IL-1 secreted from human peripheral blood macrophages stimulated with heat killed, formalin-fixed Staphylococcus aureus under serum free conditions allowed for the purification to homogeneity of human IL-1 with a high yield of biological activity (149). Purified by ion exchange chromatography and affinity chromatography on Procion Red agarose, the human IL-1, like its murine counterpart, also exhibited microcharge heterogeneity with four charged species having isoelectric points ranging from 6.0 to 4.9. These values are somewhat lower than that observed for human IL-1 by other investigators (153); however, these other reports analyzed samples containing other proteins which may have influenced the isoelectric point for IL-1 by forming complexes (149). Analysis of the purified human IL-1 by two-dimensional electrophoresis-electrofocusing gels demonstrated all four charged species have an identical molecular weight of approximately 17,500. The presence of only one molecular weight species in this preparation was probably due to the serum-free culture conditions. IL-1 exhibits multiple apparent molecular weights in the presence of serum, most likely due to a reversible association with high molecular weight serum components (244,292). Complete amino acid analysis indicated an abundance of acidic residues in agreement with the low isoelectric point. No cysteine residues were found,

which was not surprising considering IL-1 activity is unaltered by reducing agents. No glucosamine or galactosamine was found. Therefore, it is unlikely that IL-1 is a glycoprotein (149).

The tremendous potency of this protein has been demonstrated on the basis of purification data. Purified murine IL-1, as demonstrated by Mizel (208), has a specific activity of 1×10^6 U/mg protein. Thus, 1 U/ml murine IL-1 is equivalent to a molar concentration of 7×10^{-11} M. Purified human IL-1 produced by mononuclear cells showed an even greater specific activity of 3.2×10^8 U/mg protein, with 1 U/ml of human IL-1 equivalent to a 1.8×10^{-13} molar concentration (149).

IL-1 mediates a diverse range of biological activities, including stimulation of thymocyte proliferation via induction of interleukin 2 (IL-2) release (159,281), stimulation of B cell maturation and proliferation (97,118,259), augmentation of NK activity (61), fibroblast growth factor activity (268), induction of acute phase reactants via stimulation of hepatocytes (287), and stimulation of prostaglandin and collagenase release from synovial cells (206) as well as fever (261). Therefore, the question arises: can all these activities reside in a single protein, or is there a family of interleukin 1's, each with separate or overlapping functions? To clarify these issues, several molecular biology laboratories have attempted to clone the genes responsible for IL-1 activity.

2. The Molecular Biology of Interleukin 1

Recently, several laboratories have isolated murine and human complementary DNAs (cDNAs) encoding proteins with characteristic IL-1

activity. Two basic strategies have been utilized to screen macrophage cDNA libraries in order to obtain cloned genes for which translation products will encode for IL-1-like molecules. Macrophage cDNA clones were hybridized to macrophage RNA, and RNA isolated from this process was injected into in vitro translation systems with the resultant product assayed for IL-1 activity. The second procedure utilized an oligonucleotide probe constructed on the basis of amino acid sequence analysis of highly purified human IL-1.

The nucleotide sequences of the first two cDNA clones coding for murine (167) or human (13) monocyte IL-1 code for polypeptide precursors of 269 and 270 amino acids respectively. The predicted primary translation products from these clones (inferred from cDNA sequence analysis) would yield proteins of 31,026 and 30,747 daltons respectively. This data strongly supports earlier work identifying the production of a high molecular weight intracellular form of IL-1 which was thought to be the precursor form of the 13,000-17,000 dalton molecular weight IL-1 molecule isolated extracellularly (211, 298). Although the 33,000 dalton murine IL-1 was the predominant intracellular form, and the 17,000-19,000 dalton IL-1, the predominant extracellular form; the 33,000 molecular weight peptide was seen in the culture supernatants indicating that IL-1 may be secreted as a 33,000 molecular weight polypeptide and then rapidly converted to lower molecular weight forms by macrophage derived proteases (98). This proteolytic activity may produce ragged amino termini, thereby providing a possible explanation for the varied molecular weights and microcharge heterogeneity often associated with IL-1. The human cDNA

IL-1 clone isolated by Auron and Dinarello (13), demonstrated only a distant relationship with the murine IL-1 of Lomedico and Mizel (76). Although the relationship of these IL-1 molecules may be distant, there does exist a significant degree of sequence homology and structural similarities. Both of these recombinant cDNAs have shown the capability of producing biologically active molecules demonstrating their functional relationship (167,303). Protein sequence data indicate that these recombinant forms of murine and human IL-1 have amino termini beginning at amino acid 115 and 117 respectively, generating IL-1 with a molecular size of 17,300 whose forms also correlate in terms of their net charge for each species. Amino acid sequence of the amino terminus and CNBr cleavage fragments of the pI 6.8 species of normal human IL-1 (36) conclusively shows that this species of IL-1 is coded for by the human cDNA isolated by Auron et al. (11). Therefore, it does appear that the IL-1 clones produced by Auron and Lomedico correspond to forms of IL-1 commonly described in the literature. The absence of strong homology between these molecules (11) suggests a possible explanation for the lack of antibody cross reactivity in spite of functional similarity and species independence observed in bioassays (65,138). Although antigenically distinct, these molecules still presumably retain a common site required for T cell activation. The C-terminal amino acids between residues 150-186 in human and 161-197 in murine IL-1 demonstrated the strongest region of homology indicating this region as a key to the functional role in IL-1 bioactivity. Statistical evaluation of protein (59) indicates these two genes appear to be members of a multigene family as opposed

to being species counterparts (11). Recent collaborative work by Immunex and Syntex Corporations has shed more light on these potential relationships. They have obtained and sequenced two different human cDNA IL-1 clones, whose gene products they have referred to as IL-1 alpha and IL-1 beta (181). Although this IL-1 nomenclature is relatively new in the field, the alpha and beta designations have become widely accepted terminology. Table 2 briefly summarizes the characteristics of human IL-1 alpha and beta. The human cDNA IL-1 clone, isolated by Auron and Dinarello (13), was found to be essentially identical to the human IL-1 beta produced by Immunex. Amino acid sequence comparison of the Lomedico recombinant murine IL-1 with human IL-1 alpha from Immunex indicates a close relationship between these two proteins which share sixty-two percent amino acid homology. Statistical analysis of sequence homology indicates Immunex's human IL-1 alpha is the murine IL-1 counterpart (12). The first 39 N-terminal amino acids sequenced from an IL-1-like protein purified from concanavalin A (Con A) stimulated human monocyte supernatants, having a molecular weight of approximately 17,000, shows extensive homology to amino acids 117 to 155 of the cDNA-derived human IL-1 precursor sequence (300). All this data strongly suggests that there are at least two genes coding for IL-1. These forms of IL-1 appear to be distinct yet distantly related.

Considering the possibility of multiple genes producing IL-1, a major issue to be investigated in IL-1 research is the range of biological activity associated with each of these gene products. Apparent differences in activity have been detected in the primary

translation products of IL-1 alpha and IL-1 beta; with IL-1 alpha demonstrating IL-1 activity, while under similar conditions, IL-1 beta does not (181). Production of large quantities of recombinant forms of IL-1, along with the production of monoclonal antibodies specific for these different forms, will allow the elucidation of the biological activities associated with each group.

Amino acid sequence analysis of IL-1 polypeptides has allowed comparison of IL-1 to several other lymphokines with no sequence homologies seen with IL-2, IL-3, gamma-interferon, and granulocyte-macrophage colony stimulating factor (11).

3. Sources and Production of IL-1

Cells of the monocyte/macrophage lineage were first thought to be the sole source of IL-1. Most tissue sources containing mononuclear phagocytes, including peripheral blood monocytes (90,91), alveolar macrophages (10,278), hepatic Kupffer's cells (66), splenic macrophages (10), peritoneal macrophages (136,216), bone marrow

Table 2. Characteristics of human recombinant IL-1 alpha and beta.

<u>Characteristic</u>	<u>IL-1 alpha</u>	<u>IL-1 beta</u>
Major secreted species by stimulated macrophage	No	Yes
Primary translation product MW (daltons)	30,606	30,749
Final cleavage product MW (daltons)	18,063	17,377
Iso-electric point (pI)	4.9 - 5.3	6.8 - 7.2

adherent cells (160), and placental mononuclear phagocytes (87), have been shown to produce IL-1 using either the endogenous pyrogen or IAF assays. The production of IL-1 has also been associated with myelomonocytic cell types, such as P388D1, J774, U937 and THP1 (237). Other cell types of non-macrophage lineage also produce IL-1 in response to a wide variety of stimuli. These non-macrophage cells include epithelial cells of the skin, epidermal keratinocytes, (171, 172, 173, 264), and cornea (104), glia and glioma cells (88), glomerular mesangial cells (169), gingival exudate cells (45), Langerhans' cells (64), lymphoblastoid B cell lines (6), stimulated B cells (267), large granular lymphocytes (LGL) (266), fibroblasts (127), and, most surprisingly, polymorphonuclear cells (PMNs) (218, 318). The strategic location and wide distribution of IL-1 producing cells supports the concept that this soluble mediator is directly involved in host defense mechanisms (64).

Initial studies (158) suggested that IL-1 was produced in vitro without stimulation. However, it was found that the macrophages under study were stimulated by picogram per milliliter levels of endotoxin, which is below the limit of detection by the limulus lysate test, but often found contaminating laboratory materials (9). Use of the cationic antibiotic polymyxin B easily rules out contamination by negatively charged lipid A containing endotoxin molecules without impairing cell function (58, 72, 256).

There is a wide variety of agents capable of inducing IL-1 production by mononuclear phagocytes. Most of these agents are pyrogenic, producing fever in humans and experimental animals (69). Aside

from gram-negative bacterial endotoxin, other microbes, microbial fractions or microbial by-products such as gram-positive bacteria (cell walls, muramyl dipeptide, exotoxins), yeast cell walls (zymosan), spirochetes (35) and viruses may also induce IL-1 production. A 20 to 30 kd toxin produced by Staphylococcus aureus, isolated from patients with toxic shock syndrome, has been shown to be equal in potency to gram-negative endotoxin in its ability to induce production of IL-1 in vitro and in vivo (126). Inflammatory agents such as bile salts, etiocholanolone, urate or silica crystals (73), C5a, and antigen antibody complexes formed in antigenic excess, also induce IL-1 production in vitro (74). Various plant lectins, such as phytohemagglutinin (PHA) and Con A (22,241), and antigens (44) including alloantigens (63), induce IL-1 stimulation via lymphokines. Macrophages activated with colony stimulating factors have also been shown to respond by increasing IL-1 production (213).

It is generally thought that surface receptors are involved in the stimulation of phagocytes to produce IL-1 (78). It becomes apparent that stimulation must occur at the macrophage membrane since blocking of phagocytic uptake of spirochetes (35) or urate crystals (73) by cytochalasin B or colchicine does not inhibit IL-1 stimulation. Serum and complement components are also thought to play a role in the eventual production of IL-1 by activated macrophages. Although not required, serum enhances the stimulation of IL-1 production in vitro by endotoxin and Staphylococcus epidermidis (154). The possible relationship of serum enhanced IL-1 production may be explained by recent findings that macrophages have receptors for the

anaphylatoxin, C5a, on their surface (46), and that purified human C5a will induce macrophage IL-1 production (99). The Ia (I region-associated) or HLA-DR (human leukocyte antigen DR) glycoproteins on the surfaces of macrophages have also been implicated in the activation process leading to IL-1 production. These surface markers, believed to participate in antigen activation of T cells which bear receptors for Ia (260,297), may perform a receptor-like role on the surface of macrophages. Several reports have demonstrated that T cells induce IL-1 release from macrophages via an Ia-restricted interaction (76) and that anti-Ia monoclonal antibodies inhibited both T cell and LPS induced IL-1 production (77,96). Incubation of mononuclear phagocytes in nonstimulating culture conditions for 24-48 hours results in the loss of Ia surface markers on these cells. Associated with this loss of Ia surface antigens is the ability to function as accessory cells for antigen processing and produce IL-1 in response to LPS (54). Treatment of these cultured cells with indomethacin prevents the loss of both Ia expression and IL-1 producing capability (284). These observations strongly suggest that Ia receives stimuli culminating in the release of IL-1.

It should be noted that not all macrophage stimulators induce IL-1 production. Levamisole, tuftsin, and NaIO₄, which perturb cell membranes, cause superoxide anion generation and activate other known macrophage functions, do not stimulate IL-1 production by human macrophages (154). A surprising result of amino acid analyses of recombinant IL-1 is the lack of a classic signal peptide (13,167,181) which directs newly synthesized proteins to their ultimate destin-

ation, whether in or out of the cell (26). This result leaves open the long-standing question of how IL-1 is excreted from the macrophage and other cell types. The finding of a slightly hydrophobic region of seventeen residues at the amino terminus of both murine (167) and human (13) IL-1 may represent a functional domain involved in transport (11), although this region bears little resemblance to signal sequences found in other secreted proteins leading to the previously described inefficient secretion of the protein (91). Dinarello has postulated that the suicidal nature of macrophages involved in their fight against infection may lead to the release of IL-1 (182). This type of mass release may be important in the inflammatory process (64). Other investigators have identified surface membrane associated IL-1 with potent thymocyte and T cell stimulatory activity on dendritic cells (217), fixed macrophage monolayers, and on membranes isolated from unfixed macrophages (151). Membrane IL-1 appears to be an integrated membrane protein since it was solubilized by detergent, but not eluted by (Ethylenedinitrilo)-tetraacetic acid (EDTA), high salt, or low pH treatment of the membranes. This report indicates that previous work demonstrating IL-1 independent T cell lines (21) are not independent of IL-1, but rather that fixed macrophages have sufficient IL-1 on their surfaces to support T cell proliferation without the addition of soluble IL-1. The authors suggest that induction of membrane IL-1 may focus the activation signal to T cells binding to the macrophage via their antigen receptors (151). This would obviously allow for the enhancement of the immune response without the necessity of mass macrophage lysis.

4. Immunologic Activity of IL-1

The ability of cells to respond to IL-1 appears to work via specific membrane receptors similar to that described for other hormones (71). Workers at Immunex have shown that human ^{125}I -IL-1 binds specifically to cells of lymphoid or fibroblast/epithelial origin; cells on which IL-1 is known to have a biological effect (71). IL-1 was found to bind with high affinity, and to associate with a membrane protein of 80,000 molecular weight, suggesting a specific membrane bound receptor for the protein.

There are a wide variety of immunologic actions modulated by IL-1 which may be shown to be important in tumor immunity. The ability of IL-1 to be mitogenic for thymocytes (89) was the earliest recognized effect of IL-1 on the immune system. It remains as the reference assay for IL-1. The importance of this lymphokine in T cell activation is demonstrated by the ability of IL-1 to restore antigen or mitogen induced T cell activation when antigen presenting cells are removed from the culture population by physical means (281) or by use of anti-Ia treatment (76,159), suggesting that IL-1 is a required component leading to expansion of T cell populations. IL-1 has been shown to stimulate the production of interleukin 2 (IL-2) in both murine and human systems (178,254). Thus, it has been proposed that IL-1 stimulates antigen or lectin activated T cells allowing the cells in the G_0 resting state to enter the G_1 stage of the cell cycle, resulting in the release of IL-2 by these cells (159,281). Antigen activated T lymphocytes bearing IL-2 receptors are in turn induced by IL-2 to enter the S phase of the cell cycle (53). In

several reports it has been shown that adjuvant active peptidoglycans can induce IL-1 production by murine and human mononuclear cells (128,242,299,310). IL-1 has demonstrated similar immunoenhancing activity for defined antigenic systems in vivo, suggesting that IL-1 may be a common mode of action of immunological adjuvants (285,312). The ability of IL-1 to up-regulate the immune response is more clearly seen by Lyt antigen phenotypic analysis of T cells (31,38) responding to IL-1. IL-1 appears to promote the generation of Lyt 1⁺ helper cells in vitro and inhibit the generation of Lyt 2⁺ suppressor cells (78). The Lyt 1⁺ T helper cells secrete IL-2 in response to IL-1 (140), augmenting the generation of cytotoxic T cells (84,276,301). Antibodies directed against helper T cells which block IL-1 function, prevent the production of IL-2 by helper T cells, but also prevent the generation of cytotoxic T cells, strongly suggesting that IL-1 stimulation of cytotoxic T cells appears to function via its ability to induce IL-2 (193). The conversion of the macrophage derived maturational signal, IL-1, into a secondary T cell derived proliferative signal, IL-2, results in the amplification of an antigen specific immune response. Another soluble macrophage factor, initially described as BAF, was shown to increase levels of antibody to sheep red blood cells released from splenocytes of nude mice (309,311). This factor was later identified as IL-1 when it co-purified with IAF (308), and its activities were inhibited with antibodies against IL-1 (166). Continued research has shown that B cells are reactive to IL-1 during at least two stages in their development. Early stage B cell maturational control was initially reported by

Hoffmann et al., (118) who described a macrophage factor inducing maturation in pre-B cells. This factor was verified as IL-1 when purified IL-1 stimulated kappa light-chain production and subsequent expression of membrane immunoglobulin (Ig) in pre-B cells (97). IL-1 has also been shown to synergize with suboptimal concentrations of anti-mu chain, inducing B cell proliferation and terminal differentiation (82). The capacity of antibodies against IL-1 to abrogate this in vitro antibody synthesis (166), strongly indicates that IL-1 may be critical for B cell activation leading to antibody production. IL-1 activation appears to be required relatively early (within twenty-four hours) after B cell stimulation by antigen or anti-immunoglobulin (117) as are other early acting cytokines such as B cell growth factor (BCGF) (124) and possibly IL-2 (161) which drive B cells through mitosis. Following early B cell activation, late acting lymphokines such as gamma-interferon (277,320) lead to further B cell differentiation and production of antibody. The source of IL-1 needed for B cell activation and differentiation was assumed to be the activated macrophage. However, recent in vitro studies indicate that B cells may play the role of accessory cells (8,47,191) in the absence of macrophages. The absence of IL-1-like activity in B cell culture supernatants produced an apparent paradox since there appeared to be no source of IL-1 available to T cells in these systems. The finding of surface membrane IL-1 on macrophages (151) leads to the resolve of this problem when the same investigators determined that B cells could be induced to express membrane IL-1 only when stimulated with both anti-immunoglobulin and T cell lympho-

kines (152). In contrast to B cells, macrophage expression of IL-1 is not dependent on T cells or their products (151). The appearance of an IL-1 inhibitor, produced by an Epstein-Barr virus-transformed human B cell line (267), may account for lack of IL-1 activity in B cell supernatants (6,267) and the necessity of cell to cell contact between accessory B cells and responding T cells. The differences in the stimuli needed to induce membrane IL-1 on the surfaces of B cells and macrophages suggests that these antigen presenting cells may serve diverse roles in the induction and maintenance of the immune response (152). Also, the apparent lack of a signal peptide in the precursor forms of recombinant IL-1 isolated to date suggests that the membrane form of IL-1 may be derived from a different family of genes than those associated with the soluble form of IL-1.

Another cell in the immune system which responds to IL-1 is the macrophage. IL-1 is a chemoattractant for these cells (170,265), it induces the release of prostaglandin E₂ (PGE₂) (68), and is involved in activation for tumor-cell killing (240). Prostaglandins have been implicated to have inhibitory effects on macrophage production of IL-1 (78). Whether this is a case of autoregulation with the same macrophage cell producing IL-1 responding to its release by producing and releasing PGE₂ has yet to be determined.

5. Other Biologic Activities of IL-1

The ability of a host derived soluble proteinaceous factor to induce fever associated with most, if not all, pyrogenic substances (6,69) initiated research into EP. This protein appears to regulate body temperature by raising the hypothalamic setpoint as a function of

EP's ability to increase levels of prostaglandins in the brain and cerebral spinal fluid (23,50). As the methodology of protein chemists improved allowing the separation and examination of partially purified factors which augment a variety of physiologic responses including fever and the acute-phase response, it became apparent that these biological activities were biochemically inseparable (131). The finding that EP and IAF activities were also biochemically inseparable in several different animal species (216,261), catalyzed the development a broad concept linking the host febrile response to immunoregulation.

It has been well established that the acute response to infectious diseases and pyrogenic substances produces neutrophilia in association with fever (56). The neutrophilic response generated by IL-1 seems due to the accelerated release of mature neutrophils from the bone marrow reserves, as there is a severe loss of mature neutrophils in the marrow following injection with EP (134). IL-1 has also been observed to dramatically increase the synthesis of acute phase reactants, a group of proteins produced by the liver in response to infection or inflammation (196,287). Those acute phase reactants normally present in the plasma; such as serum amyloid A (187), fibrinogen (133), haptoglobin (120,133), and ceruloplasmin; generally increase several fold when the host is stimulated with IL-1. Those proteins normally seen in the plasma at extremely low concentrations, such as C-reactive protein, rise several hundred fold in response to IL-1 (195,293). Whether IL-1 is acting alone or together with other factors in the induction of these acute phase reactants is still un-

clear (64). The accelerated synthesis of ceruloplasmin by IL-1 (131) accounts for the increase in the plasma level of copper ions, generally considered vital to combat bacterial diseases (251). Two other plasma divalent cations (zinc and iron), affected by the presence of IL-1, show a rapid drop in plasma concentration (132). IL-1 induced neutrophil degranulation leading to the release of lactoferrin (144) leads to the rapid drop in iron levels and is a major host defence mechanism against microbial infection. IL-1 has also been implicated in the regulation of tissue catabolism during infection or trauma when the host must mobilize a variety of components needed for synthesis or energy by inducing muscle tissue proteolysis (14,49), bone resorption by osteoclasts (102), and cartilage breakdown associated with collagenase and proteoglycanase production by human chondrocytes (103).

6. Potential Antitumor Activities of Interleukin 1

The many biologic activities of IL-1 discussed above, both in the nature of immunoregulation and physiologic regulation, demonstrate the potential usefulness of this monokine as an in vivo therapeutic agent. The activated macrophage is thought to play an important role in host defense against malignant neoplasia by exerting a cytotoxic effect on tumor cells (85). IL-1 has been shown to promote human monocyte-mediated tumor cytotoxicity possibly by prolonging the cytotoxic state of monocytes (235). Macrophage cytotoxic effects are thought to be mediated by several possible effector molecules including lysosomal enzymes (116), oxygen intermediates (221), arginase (55), a neutral proteinase (2), and tumor necrosis factor (180,319).

Purification of human IL-1 (185) has allowed Onozaki et al. to demonstrate the direct cytotoxic and growth inhibitory capability of IL-1 against a human melanoma cell line in vitro (236). Thus adding IL-1 to the list of potential macrophage cytotoxic mediators. Human monocyte derived IL-1 has been shown to augment the activity of highly purified human IGL or NK cells which are cytotoxic to a variety of malignant cells (141,225) and have been implicated as important mediators in the prevention of tumor development and metastasis (110, 137). Augmentation of this activity implies a possible role for IL-1 in immune surveillance of spontaneous or metastatic tumors (186). The previously discussed ability of IL-1 to augment the immune response to specific antigens in vivo (285,312) allows speculation that IL-1 may be able to serve an immunoadjuvant for tumor associated antigens. Several important observations can now be made from elements discussed in this review. First, it would appear that C. parvum may act as an immunoadjuvant in the C57Bl/6:EL-4 tumor model by enhancing macrophage function. Secondly, the modulatory functions of IL-1 have been shown to most likely act via lymphocytic populations. Therefore, by combining the protective C. parvum therapy with systemic IL-1 administration, this study set out to determine if IL-1 could modulate a protective immune response to the nonimmunogenic EL-4 lymphoma. The effects IL-1 may have on the involved lymphocytic populations will allow suggestions as to possible mechanisms which might lead to the development of tumor immunity in this system.

CHAPTER III

METHODS AND MATERIALS

A. The Mice

C57Bl/6 female mice, ages seven to twelve weeks obtained from Jackson Laboratory, Bar Harbor, ME, were used for all IL-1 in vivo tumor protection studies. Similarly sexed and aged C57Bl/6 mice obtained from Charles River Breeding Laboratories, Inc., Boston, MA, and Lab Supply, Plainfield, IN, were utilized for macrophage activation studies. Lymphoid tissues from DBA/2J and C3H/HeJ mice, aged seven to sixteen weeks (Jackson Laboratory), were used for IL-1 assessments.

B. The Bacteria

The Corynebacterium parvum strain 4182 (Propionibacterium acnes) was been obtained from the Pasteur Institute, Paris, France, and periodically identified by API 20 Anaerobic System (Analytab Products, Plainview, NY) (212,283) and metabolic end-products determination by gas-liquid chromatography (214). The bacteria were grown anaerobically in liquid culture using Brewer's thioglycolate medium with indicator (Difco Laboratories, Detroit, MI). Ten milliliter starter cultures were incubated at 37°C for five days, then transferred to one liter thioglycolate broth cultures. The cultures remained at 37°C for seven days under anaerobic conditions with occasional agitation. The organisms were harvested with the Beckman TU-6R centrifuge (Beckman Instruments, Inc., Palo Alto, CA) at 1500 x

gravity (g) for twenty minutes, and washed five times with sterile saline. The organisms were heat-killed at 80°C for thirty minutes with inspissation, aliquoted at 2×10^9 cells per ml and stored at -20°C until needed.

C. The Tumor Cell Lines

The EL-4 lymphoma, a 7,12 diamethylbenz(a)-anthracene induced tumor, was originated in 1945 by Gorer from C57Bl/6 mice. The EL-4 lymphoma has been shown to be a tumor of low immunogenicity (39,100), and nonsusceptible to NK lysis (114). This tumor was obtained from Dr. R. Herberman, Laboratory of Immunodiagnosis, National Cancer Institute, Bethesda, MD.

The RBL-5 lymphoma, also obtained from Dr. Herberman, is a Rausher murine virus-induced lymphoma (189), and like EL-4, is syngeneic in the C57Bl/6 mouse.

The YAC-1 lymphoma is a Moloney murine virus-induced lymphoma (280) which has been shown to be susceptible to NK lysis (114,141).

The EL-4 ascites tumor banks were maintained in vivo by, i.p., passage. A 0.1 ml suspension of EL-4 ascites cells in Hank's balanced salt solution (HBSS) at 1×10^7 cells/ml was injected, i.p., into syngeneic C57Bl/6 mice, typically at ten day intervals. EL-4, RBL-5, and YAC-1 cell lines were maintained in vitro by growth in suspension cultures in RPMI-1640 culture medium (Grand Island Biological Company, Grand Island, NY) (GIBCO), pH 7.1 to 7.3, supplemented with five or ten percent fetal bovine serum (FBS) (heat inactivated at 56°C for one hour) (GIBCO) 100 units/ml potassium penicillin G, 100 ug/ml streptomycin (sulfate), 2.5 ug/ml fungizone

(Whittaker M. A. Bioproducts, Walkersville, MD) and 2 mM L-glutamine (Whittaker M. A. Bioproducts). All in vitro cell cultures utilized for experimentation or propagation in this study were maintained at 37°C with five percent CO₂ in high humidity, unless otherwise stated. Media supplemented with L-glutamine and the antibiotics listed above will be referred to as complete medium. All routinely in vitro passaged cell lines were incubated in either 50 ml or 500 ml tissue culture flasks (Corning Glass Works, Corning, NY).

The P388D1 macrophage-like cell line, ATCC-TIB-63, (American Type Culture Collection, Tumor Immunology Bank, Rockville, MD) was derived from a methylcholanthrene-induced lymphoid neoplasm (P388) of a DBA/2 mouse (57). It has been utilized consistently as a source of endotoxin induced murine IL-1 (155,205). This cell line has been shown to retain many macrophage cell-like properties including the ability to phagocytize latex particles and zymosan, firmly adhere to glass and latex, and carry cell bound receptors for immunoglobulin (F_c) and complement (C₃) (146). This cell line has no detectable surface immunoglobulin (146), exhibits high effector activity in an antibody-dependent cell mediate cytotoxic system (252), and synthesizes lysozyme (253). This cell line was maintained in vitro as monolayer cultures in complete RPMI-1640 medium supplemented with ten percent FBS. A second P388D1 cell line, obtained from Dr. Y. Yamamura (Ponce School of Medicine, Ponce, Puerto Rico), was a cloned cell line which constitutively produces IL-1 (317). This cell line was derived by Dr. Yamamura from a cell line isolated by Dr. H. Koren (Duke University Medical Center, Durham, NC). This cell line was

maintained in vitro in complete m-alpha medium without nucleosides (Grand Island Biological Company, Grand Island, NY) supplemented with seven percent FBS. To avoid confusion, the cell lines obtained from the American Type Culture Collection (ATCC) and Dr. Y. Yamamura will be referred to as P388D1A and P388D1Y respectively.

D. IL-1 Production by P388D1 Macrophage Cell Lines

IL-1 required for in vivo experimentation was produced using the constitutive IL-1 generating P388D1Y cell line as previously described (317). P388D1Y adherent cells were removed from stationary monolayer cultures with a rubber policeman or by rigorous pipetting. The cells were pelleted by centrifugation at 400 x g for seven to ten minutes, washed once in HBSS, and then seeded in 50 ml tissue culture flasks at 2×10^5 cells/ml in 10 ml of complete m-alpha medium without nucleosides plus five percent endotoxin-free FBS (HyClone Laboratories Inc., Logan, UT). After three to five days of incubation, the culture supernatants were harvested by decantation, then centrifuged and/or filter sterilized by passage through a 0.45 um Swinnex filter unit (Millipore Corp., Bedford, MA) to remove any remaining cells. The IL-1 containing conditioned medium was aliquoted and frozen at -20°C until required for experimentation or assay. IL-1 has been shown to remain stable for at least six months under these conditions (205).

P388D1A cells utilized for production of IL-1 were prepared for seeding in the same manner as described for the P388D1Y cells. Production cultures were seeded at 2×10^5 cells/ml/well in 24-well tissue culture plates (Falcon Plastics, Los Angeles, CA). Cells were

incubated either with or without 10 ug/ml lipopolysaccharide (LPS) B from Salmonella typhosa 0901, (Difco, Detroit, MI) for three days. The culture supernatants were harvested, aliquoted, and stored as described above for P388D1Y supernatants. Conditioned supernatants from macrophage cultures not given LPS were utilized in the in vivo protection experiments as control macrophage conditioned media.

E. Recombinant IL-1

Purified recombinant murine IL-1, derived from a cloned murine cDNA gene for IL-1 in Escherichia coli (167), was generously supplied by Peter T. Lomedico (Hoffmann-LaRoche Inc., Nutley, NJ). This preparation of IL-1 had an activity of 4×10^6 units/ml and was maintained in 5M Guanidine hydrochloric acid (HCl) buffer. The recombinant IL-1 sample was diluted to the concentration desired for assaying or for in vivo experimentation in HBSS containing 250mg/ml human serum albumin (HSA) as a carrier protein. The diluted IL-1 was then aliquoted and stored at -20°C until needed. Similarly prepared HSA solutions (250mg/ml) without IL-1 were aliquoted and stored at -20°C and used as control injection mixtures for the murine recombinant IL-1 in vivo protection experiments.

F. IL-1 Assays

The standard co-mitogenic thymocyte assay was utilized to assess IL-1 activity as previously described by Mizel (209). The thymus from either a C3H/HeJ or DBA/2J mouse was aseptically removed following cervical dislocation of the animal. A single cell suspension was prepared by passage of the tissue through a 60-mesh wire screen into HBSS. The suspension was decanted into a 15 ml conical

centrifuge tube (Corning Glass Works, Corning, NY) where large cell clumps were allowed to settle for five minutes before the cell suspension was again carefully decanted to another tube. The cells were washed once in HBSS, resuspended to 1×10^7 cells/ml in complete RPMI-1640 medium, and plated at 100 ul/well in 96-microwell tissue culture plates (Microtest III, Falcon Plastics, Los Angeles, CA). The culture medium was supplemented with FBS, phytohemagglutinin P (PHA-P) (Difco, Detroit, MI), and 2 mercaptoethanol (2-ME) (Sigma Chemical Co., St. Louis, MO); such that a final concentration of ten percent FBS, 0.01 percent PHA-P and 5×10^{-5} M 2-ME was attained when final volumes were added to each well. Varying amounts of test samples, ranging from 0 ul to 50 ul, were added to each well with an appropriate amount of culture medium added bringing each well to a 200 ul total volume. All samples and controls were tested in triplicate. Following sixty-six hours of incubation, the microcultures were pulsed with 1 uCi ^3H -thymidine (7.6 Ci/mmmole), 1 mCi in 20 ml HBSS (ICN Biomedicals, Inc., Irvine, CA). After an additional six hours of incubation, the microcultures were harvested onto glass fiber filters (Cambridge Technology, Inc., Cambridge, MA) using a PHD automated cell harvester (Cambridge Technology Inc.). The degree of radiolabel incorporation for each sample was determined using liquid scintillation spectrophotometry (Unilux II, Nuclear-Chicago, Des-Plains, IL; or LS5801, Bechman Instruments, Irvine, CA).

A variation of the standard IL-1 assay described by Yamamura (317), utilizing murine splenocytes, was also incorporated into the study. Splenocyte cell suspensions were obtained from DBA/2J mice in

a similar manner as described above for the thymocyte assay. Erythrocytes in the spleen cell suspension were destroyed by rapid hypotonic lysis with distilled water. The remaining white blood cells were washed twice with HBSS, and resuspended in complete m-alpha medium supplemented with 0.01 percent PHA-P. No FBS or 2-ME were included in the incubation media. For this assay cells were resuspended to 2×10^6 cells/ml before plating 100 ul/well of the splenocyte suspension into individual wells of a 96-microwell plate. Test samples and medium were added to the wells as in the thymocyte assay. These microcultures incubated for forty-eight hours followed by a twenty-four hour pulse with ^3H -thymidine. Harvesting and radiolabel counting procedures for the splenocyte assay were the same as those described for the thymocyte assay.

Units of IL-1 were arbitrarily determined by equating one unit with the capability of stimulating thymocytes or splenocytes to one-half the maximal value obtained in the assay with a high activity P388D1Y supernatant reference lot of IL-1.

G. In Vivo Tumor Protection Studies

C57Bl/6 mice were challenged intradermally (i.d.) on the right flank with a 0.1 ml suspension of EL-4 tumor cells suspended in HBSS. The tumor dose ranged from 1×10^3 to 5×10^3 cells per injection. The EL-4 ascites cells had been previously removed from the peritoneal cavity of a murine tumor bank, washed once in HBSS, and promptly injected in the mice either alone or as an admixture with 2×10^9 heat-killed C. parvum bacterial cells. The C. parvum suspension had been lightly sonicated after removal from freezer storage to elimi-

nate bacterial clumping before admixture with the tumor cells. As previously described (121), animals receiving both tumor challenge protocols were divided into two groups. One group from each protocol was given no further treatment. The second group of animals from each protocol was given accompanying systemic IL-1 therapy. Animals receiving IL-1 therapy (either IL-1 containing conditioned culture supernatants obtained from the P388D1Y constitutive IL-1 producing cell line, murine recombinant IL-1 or LPS induced P388D1A cell line conditioned culture supernatants) were administered equal unit dosages of IL-1, i.p., everyday or on alternate days starting with the day of tumor challenge and ending nine days later. Control animals received accompanying systemic therapy (in the form of either P388D1A conditioned culture supernatants or a 250 mg/ml HSA solution) using a comparable injection protocol. A total of 100 units of IL-1 was administered to each mouse receiving this therapy. Mice were periodically checked for survival. All mice surviving this initial tumor challenge and showing no visible signs of tumor growth after thirty-five to fifty days were rechallenged with the EL-4 tumor. The rechallenge inoculum was injected i.d. on the right flank between the site of the initial tumor challenge and draining axillary lymph nodes. The EL-4 tumor dose was equal to that given for the initial challenge and no further therapy was administered to these animals. Mice were evaluated periodically for survival. In separate experiments, mice were randomly selected at successive time periods following either initial or secondary EL-4 challenge for in vitro evaluation of lymphoid immunological responsiveness.

H. Radiolabeling Tumor Target Cells

Either in vitro passaged EL-4, RBL-5, YAC-1, K562, or in vivo passaged EL-4 ascites tumor target cells were washed once after harvesting, resuspended in HBSS and counted. A suspension of between 3×10^6 and 1×10^7 tumor cells was pelleted by centrifugation at $400 \times g$ for seven to ten minutes. The supernatant was discarded and the cell pellet resuspended in the residual HBSS (150 ul). Tumor target cells were radiolabeled by incubation with 100 uCi to 200 uCi $\text{Na}_2^{51}\text{CrO}_4$ (^{51}Cr) (New England Nuclear, Boston, MA, or Amersham Corporation, Arlington Heights, IL) for forty to sixty minutes with frequent agitation. The ^{51}Cr labeled cells were pelleted, washed four times in HBSS, and resuspended in complete medium for immediate addition to effector cells at 1×10^4 target cells per well.

I. T Cell Mediated Cytotoxicity

T cell-mediated, tumor cell cytotoxic activity of lymphoid tissues taken from tumor challenged animals at various time points following antitumor therapy, was assessed following procedures described by Brunner and colleagues (34). Mice were sacrificed by cervical dislocation. The spleen and lymph nodes draining the site of the tumor challenge were aseptically removed from each animal, passed through a 60-gauge wire mesh to obtain a cell suspension, and washed once in HBSS. Erythrocytes in the splenic suspension were destroyed by rapid hypotonic lysis with distilled water, followed by several HBSS washes. Splenic and lymph node effector cells were then resuspended in complete RPMI-1640 medium with ten percent FBS. Cytotoxic activity of all cell populations were determined at four

effector-to-target (E/T) ratios by serial dilution of effector cell concentrations, ranging from 1×10^6 to 1.2×10^5 cells per well, in round bottom chambers of 96-well tissue culture plates (Falcon Plastics). Lymph node effector cells were evaluated at the highest possible cell concentration allowing for four serially diluted E/T ratios. This variability was due to the low number of cells occasionally obtained from these tissues. All E/T ratios and controls were performed in triplicate (1×10^4 ^{51}Cr labeled tumor target cells were added to each well in 100 ul of medium). Total well volume was brought to 200 ul with media when necessary. The cytotoxicity cultures were gently pelleted by centrifugation at $100 \times g$ for five minutes. Following a four-hour incubation period, the cultures were pelleted by centrifugation at $400 \times g$ for five minutes. Radiolabeled tumor target cells incubated without effector cells were used to determine the spontaneous cellular ^{51}Cr release. Maximum cellular release was obtained by lysing labeled tumor targets with Nonidet P-40 (NP-40) (Sigma Chemical Co., St. Louis, MO). Released radiolabel was harvested by removing 130 ul of supernatant from each well, and counted on a Beckman Gamma 4000 gamma radiation counter (Beckman Instruments, Inc., Irvine, CA) with data expressed as counts per minute (CPM). Results were expressed for each E/T ratio as percent specific release (PSR):

$$\text{PSR} = 100 \times \frac{(\text{CPM in experimental well}) - (\text{CPM in control well})}{(\text{CPM in NP-40 well}) - (\text{CPM in control well})}$$

PSR data were represented graphically as a function of culture dilution. Linear least square curve fitting using Curve Fitter-PC soft-

ware (Interactive Microware, Inc., Danbury, CT) determined the best straight line through the PSR data points. Using this analysis the slope of the line will vary depending on the proportion of cytolytic cells and the efficiency of cytolysis in the effector population (290). Results, extrapolated from the fitted curve, are expressed as lytic units (L.U.) per cell culture, with one L.U. defined as the inverse of the fraction of a 1×10^6 effector cell culture required to achieve ten percent specific release (43).

J. Natural Killer Cell Activity

The NK assay was performed in the same manner as that described above for the cytotoxic T cell assay, except that the (^{51}Cr) radiolabeled target cell was the NK susceptible YAC-1 tumor cell line. Since NK activity is seen in unimmunized animals, the determination of any modulation of this parameter must be measured carefully against the control protocol mice. As discussed in the previous section, data are graphically expressed as PSR, then converted to L.U. of activity. Statistical evaluation of the data was determined by t-test analysis of the PSR for the 100 to 1 E/T ratio data points.

K. Macrophage Tumoricidal Activation and Assessment

l. The Thioglycolate Inducer

Brewer's thioglycolate broth (Difco, Detroit, MI), used for, i.p., injection of mice, was prepared as described by Campbell, et al. (35). An autoclaved 100 ml aliquot of thioglycolate medium was oxidized by exposed to room air for two months. The oxidized media was distributed in 5 ml aliquots and frozen at -20°C for later use.

2. The Macrophage Activators

LPS from Salmonella typhosa 0901 (Difco, Detroit, MI), obtained in lyophilized form, was rehydrated in HBSS, distributed into 1 ml aliquots at a concentration of 100 ug/ml, and stored at -20°C until needed. Con A (Difco, Detroit, MI), obtained in crystalline form, was also rehydrated in HBSS and stored at -20°C. Preparation of C. parvum for use in this assay was the same as that described in section B. of this chapter.

3. The Macrophage Cytotoxicity Assay

The methods employed for this assay (37) are modifications of the technique originally described by Doe and Henson (70). Mice were injected with 1 ml of aged thioglycolate broth, i.p., and sacrificed by cervical dislocation four to five days later. Peritoneal exudate cells (PEC) were obtained by peritoneal lavage with 10 ml of HBSS containing ten units of heparin/ml (Difco, Detroit, MI). The PECs were washed once in HBSS and resuspended at 1.3×10^6 cells/ml in complete RPMI-1640 medium supplemented with ten percent FBS. One hundred microliters of the suspensions were plated in 96-well microtiter plates. Nonadherent cells were eliminated after two hours of incubation by removing the culture medium and replacing it with 100 ul of fresh RPMI-1640. After an additional twenty to twenty-four hours of incubation, the culture media was again removed and replaced with 80 ul of complete RPMI-1640 medium. The appropriate activators were then added in 100 ul volumes to each well in quadruplicate. Twenty to twenty-four hours later, 1×10^4 ^{51}Cr -labeled target cells were added to each well in a 100 ul volume. After eighteen to twenty

hours, the cultures were centrifuged at 400 x g for five minutes. Seventy microliters of culture supernatant were carefully removed from each well and radioactivity determined using the Gamma 4000 gamma radiation counter. Control cultures contained radiolabeled tumor targets incubated with nonactivated thioglycolate-induced, adherent PECs. Maximum possible cellular release of ^{51}Cr was obtained by adding NP-40 to control cultures, or by disturbing the pelleted target cells with rigorous pipetting before supernatant harvesting. Data are expressed as PSR: $\text{PSR} = 100 \times$

$$\frac{(\text{Mean CPM in activated well}) - (\text{Mean CPM in control well})}{(\text{Mean CPM NP-40 well}) - (\text{Mean CPM in control well})}$$

L. Lymphoid Cell Proliferative Response

Spleen and lymph node cell suspensions, processed in the same manner as described for the cytotoxic T cell assay, were resuspended in complete RPMI-1640 medium supplemented with two percent FBS. One hundred microliter aliquots of spleen and lymph node cells were plated in 96-well microtiter plates at 2×10^5 and 1×10^5 cells per well, respectively.

Mitomycin-c inactivated tumor cells were prepared by incubating 1×10^7 tumor cells with 25 ug of mitomycin-c in one ml of complete RPMI-1640 medium for one hour. The cells were washed three times in HBSS, and resuspended in complete RPMI-1640 medium with two percent FBS. Each stimulator cell population was plated in triplicate at 1×10^5 cells per well in 100 ul aliquots with spleen and lymph node effector cell populations. Spleen and lymph node effector cells were incubated alone to determine a basal level of prolifer-

ative activity. Duplicate cultures were pulsed six hours with 1 uCi ^3H -thymidine after two and five days of incubation. Cell cultures were harvested with the Cambridge PHD automated cell harvester and radiolabel incorporation for each sample was determined using liquid scintillation spectrophotometry. Data are expressed as stimulation indexes (SI):

$$\text{SI} = \frac{\text{Mean CPM (Effector Cells + Stimulator Cells)}}{\text{Mean CPM (Effector Cells)}} .$$

M. In Vitro Effect of IL-1 on EL-4 Proliferation

In vitro passaged EL-4 lymphoma cells were harvested from cell culture, washed once in HBSS, resuspended in complete RPMI-1640 medium with ten percent FBS, and incubated at 5×10^4 cells/well in 96 well microtiter plates. Various doses of IL-1, ranging from 0.1 to 25 units of IL-1 activity, were added to the tumor cell micro-cultures. All IL-1 doses were tested in triplicate. Proliferative activity was assessed with a six hour ^3H -thymidine pulse after one and two days of tumor cell incubation with IL-1. The cultures were harvested and counted as described previously for other ^3H -thymidine proliferative assays.

N. Determination of Serum Antibody Titers Against the EL-4 Lymphoma

1. Preparation and Fixation of the EL-4 Lysate

EL-4 ascites suspensions were washed three times with saline, then resuspended in 2.5 ml of 0.5 percent NP-40 lysis buffer per 1×10^7 cells. The cells were incubated for ten minutes at 4°C with occasional vortexing followed by centrifugation at $700 \times g$ for thirty minutes. The supernatant was carefully decanted with 50 ul of this

cell lysate added to each well of a flexible 96-well microtiter plate (Falcon Plastics, No. 3911). The lysate was incubated in the wells at 37°C overnight, allowing the lysate to dry in each well. The cell lysate was fixed to the wells by the addition of 100 ul of a 0.02 percent glutaraldehyde (Sigma Chemical Co., St. Louis, MO) in phosphate buffered saline (PBS) at room temperature for ten minutes. The wells were then washed three times with PBS before they were filled with a 0.1 percent gelatin (Sigma Chemical Co., St. Louis, MO) solution in PBS. After sixty minutes of incubation at room temperature the plates were again washed three times with PBS, inverted, drained, and stored dry at 4°C covered with plastic wrap.

2. Antibody Binding Assay

Blood samples were obtained from mice receiving various treatment protocol animals by cardiac puncture. The blood was allowed to clot at room temperature for several hours. Serum was separated by centrifugation at 700 x g for twenty minutes, decanted, and stored at -20°C until needed.

Fifty microliters of each serum sample were added to the EL-4 cell lysate coated microtiter plate wells in a moist chamber and incubated eighteen hours at 4°C. The wells were washed six times with 0.1 percent gelatin in PBS and drained. One hundred microliters of a 1:2000 dilution of horse radish peroxidase labeled affinity purified goat antibody to mouse IgA+IgG+IgM heavy and light chains (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD) was added to each EL-4 lysate coated well. The antibody was incubated one hour at room temperature. The wells were then washed ten times with the 0.1

percent gelatin in PBS solution by inversion, followed by four more washes with 0.1 percent gelatin in PBS by aspiration with a pasteur pipet. After thorough draining 150 ul of substrate consisting of, 0.1 mM 2,2'-Azino-di(3-ethyl-benzthiazoline sulfonic acid) as substrate and 0.1M glycine citrate buffer pH 4.0 at 25°C and 0.02 percent H₂O₂, was added to each well. Following one hour of incubation color development of the substrates was measured by optical density at 405 nm with a MR-600 Microplate Reader (Dynatech Laboratories Inc., Alexandria, VA). The antibody titer will be determined using the ABC₅₀ method described by Minden and Farr (203).

CHAPTER IV

RESULTS

A. Anti-tumor activity of C. parvum

1. In vivo Suppression of the EL-4 Lymphoma by C. parvum

Varying numbers of nonviable C. parvum cells were admixed with in vivo passaged EL-4 lymphoma cells, then injected, i.d., in the flanks of mice. In this manner, the bacterial dose required to suppress tumor growth was assessed. Pooled results of two experiments which yielded similar results, shown in Table 3, indicate that suppression of tumor growth was dependent upon the number of bacteria injected with the tumor challenge. Under these experimental conditions, optimal protection against the tumor resulted when 2×10^9 C. parvum cells were admixed with the tumor challenge. This dose of C. parvum was utilized for all further in vivo tumor protection experiments. Lower concentrations of C. parvum proved to be less effective with 1×10^9 cells protecting fifty-seven percent of the animals and 1×10^8 resulting in only a twenty percent survival rate. Animals which received the tumor challenge without local C. parvum therapy demonstrated no survival.

Animals which survived the initial tumor challenge remained free of subsequent tumor development. These animals were rechallenged with 5×10^3 EL-4 tumor cells thirty to sixty days later. As shown in Table 3, none of these animals survived. These results

suggest that no long-lasting immunity was generated as a consequence of C. parvum treatment of the EL-4 lymphoma.

2. In vitro Macrophage Activation by C. parvum

Thioglycolate induced macrophages activated by a forty-eight hours incubation with heat-killed C. parvum demonstrated tumoricidal activity against ^{51}Cr radiolabeled EL-4 and RBL-5 tumor target cells in a dose-dependent manner as shown in Figure 1, a representative example of six experiments demonstrating similar results. Previous work by Wigzell (306) has correlated ^{51}Cr release from radiolabeled tumor targets with loss of cell viability as determined by trypan blue dye exclusion. As little as 1×10^8 C. parvum cells/ml were able to activate peritoneal macrophages to a tumoricidal stage, with increasing levels of cytotoxic activity peaking at an activator dose of 1×10^9 bacterial cells/ml.

Following sixteen hours of incubation with the C. parvum activator, ^{51}Cr radiolabeled EL-4 tumor target cells demonstrated no loss of viability as determined by trypan blue dye exclusion (see Table 4). Tumor cell viability remained high after incubation with C. parvum activator concentrations ranging from 1×10^8 to 1×10^{10} bacteria/ml. Likewise, LPS demonstrated no toxicity to the tumor cell targets in the concentration range utilized in the cytotoxicity assay, although LPS was cytotoxic to these cells at a concentration of 100 ug/ml. Therefore, release of radiolabel in these experiments was not due to direct cellular toxicity by the activators in this system. These data demonstrate that C. parvum activates macrophages to become tumoricidal for experimental lymphomas, thereby suggesting

TABLE 3. Suppression of the EL-4 Lymphoma by Corynebacterium parvum.^a

Bacterial Number	<u>Survival</u>				
	<u>Primary Challenge</u>		x ²	<u>Secondary Challenge</u>	
	#Survivors/ #Challenged	(%)			#Survivors/ #Challenged
2 x 10 ⁹	13/13	(100)	p < 0.01	0/13	(0)
1 x 10 ⁹	8/14	(57)	p < 0.01	0/8	(0)
1 x 10 ⁸	2/10	(20)	N.S. ^b	0/2	(0)
EL-4 Alone	0/14	(0)		---	---

^aBacteria (0.1 ml) were admixed with EL-4 cells (0.1 ml) and injected i.d. into the right flank of each animal.

^bN.S., not significant; p > 0.05, experiemnts were compared with those for animals injected with EL-4 alone.

Figure 1. Effect of in vitro C. parvum activation of thioglycolate induced PEC on the induction of tumoricidal activity. Following a 24 hour PEC stimulation with C. Parvum, ^{51}Cr radiolabeled EL-4 (■) and RBL-5 (Δ) tumor targets were added to the culture. After 16-18 hours of incubation the PSR of ^{51}Cr was determined for each culture. Each value represents the mean PSR of triplicate cultures \pm STD.

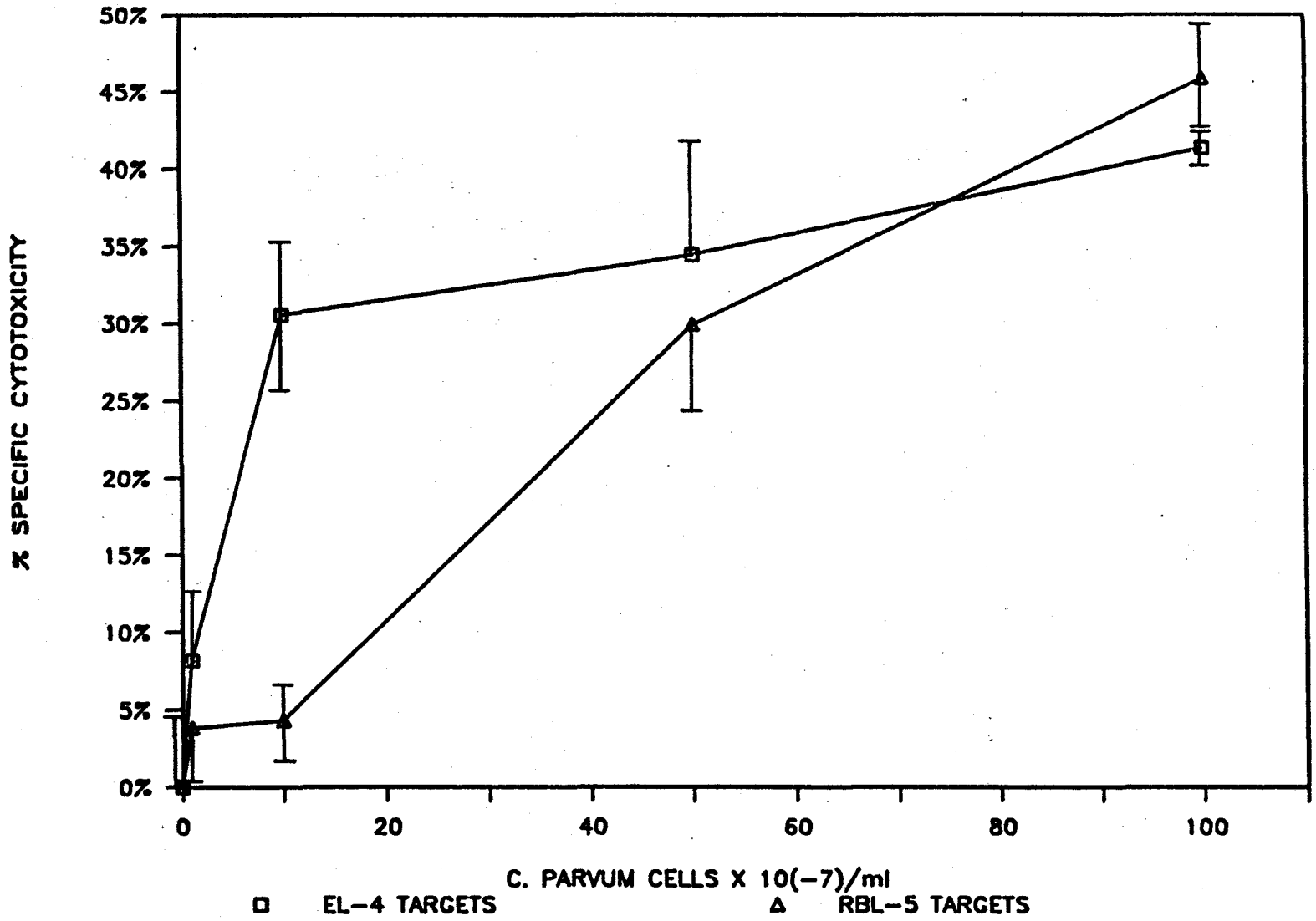


TABLE 4. EL-4 Lymphoma Cell Viability Following Incubation With C. parvum and LPS Activators.

ACTIVATOR ^a	AMOUNT/ml	VIABILITY ^b (%)
<u>C. parvum</u>	1 x 10 ¹⁰	89.6 ± 5.4
<u>C. parvum</u>	1 x 10 ⁹	90.6 ± 3.1
<u>C. parvum</u>	1 x 10 ⁸	86.4 ± 5.1
LPS	100 ug	46.2 ± 10.2
LPS	10 ug	87.4 ± 4.0
Medium	0	92.0 ± 2.4

^a2 x 10⁵ EL-4 tumor target were incubated with activators in 96-well microtiter plates for sixteen hours.

^bViability was determined by trypan blue dye exclusion. The data are expressed as the mean viability of duplicate cultures ± the standard deviation.

that the in vivo tumor protective capability of C. parvum observed in this experimental system may be a result of local activation of macrophages.

B. In vivo Protective Effect of IL-1 Against the EL-4 Lymphoma

1. Effects of P388D1 Macrophage Derived IL-1

A series of in vivo experiments were performed with IL-1 to induce protective immunity in the EL-4 lymphoma/C. parvum treatment system. Animals given the EL-4/C. parvum admixture were divided into two groups. One group received no further therapy while the other group received, i.d., P388D1 macrophage derived IL-1 therapy as described in Section G of the Methods and Materials chapter. Table 5 summarizes the results from six experiments. Approximately eighty percent of the animals in both protocol groups survived the initial challenge. Thirty-five to fifty days later, animals which survived the primary tumor challenge, were rechallenged with a similar EL-4 tumor dose with no further therapy. Animals which received i.p. injections of IL-1 at the time of the primary tumor challenge, showed a statistically significant enhanced survival rate of 53.5 percent upon rechallenge ($p < 0.02$) when compared by chi-square analysis to animals not given IL-1. Surviving animals, given the EL-4/C. parvum admixture without IL-1, exhibited a rechallenge survival rate of 20.7 percent. No statistical differences were demonstrated between re-challenged animals initially treated with the EL-4/C. parvum admixture without IL-1, EL-4 with IL-1 alone, or EL-4 without therapy.

The survival curves for these animals following the primary tumor challenge are presented in Figure 2. Animals receiving EL-4

TABLE 5. Suppression of the EL-4 Lymphoma by Corynebacterium parvum and P388D1Y Macrophage Derived IL-1.^a

Treatment Protocol	<u>Survival</u>				
	<u>Primary Challenge</u>		<u>Secondary Challenge</u>		
	#Survivors/ #Challenged	(%)	#Survivors/ #Challenged	(%)	x ²
EL-4 with <u>C. parvum</u> + MD IL-1	43/53	(81.1)	23/43	(53.5)	p < 0.02
EL-4 with <u>C. parvum</u>	29/37	(78.4)	6/29	(20.7)	N.S. ^b
EL-4 Plus MD IL-1	7/30	(23.3)	1/7	(14.3)	
EL-4 Alone	8/44	(18.2)	1/7	(14.3)	

^aBacteria (2×10^9 per 0.1 ml) were admixed with EL-4 cells (0.1 ml) and injected i.d. into the flank of each animal. IL-1 was administered i.p. (0.1 ml) from day 0 to day 9 post-tumor inoculation. A total of 100 units of IL-1 was administered per animal.

^bN.S., not significant; $p > 0.9$, experimental was compared with animals receiving EL-4 alone, IL-1 alone and surviving second challenge.

and C. parvum or EL-4 and C. parvum plus IL-1 showed nearly identical survival patterns. Animals administered EL-4 alone or EL-4 plus IL-1 therapy exhibited low survival rates of 18.2 and 23.3 percent respectively, with no statistical difference shown between these survival rates as determined by chi-square analysis.

Survival curves for animals (which had survived the initial challenge) following a secondary challenge with the tumor are depicted in Figure 3. Animals which had initially received the EL-4 challenge, alone or with accompanying IL-1 therapy, showed a rapid drop in survival to 14.3 percent by thirty days post rechallenge. Animals which initially received EL-4 and C. parvum, or EL-4 and C. parvum plus IL-1, showed an enhanced survival rate until approximately day forty-two, at which time the animals that had received EL-4 and C. parvum exhibited a drop in survival to a level (20.7 percent) not statistically higher, by t test analysis, than animals which had initially received either EL-4 alone or EL-4 and IL-1.

This data suggests that systemically administered P388D1 macrophage derived IL-1 augmented immunity to the EL-4 lymphoma such that a second tumor challenge did not progressively grow to kill the animals. In contrast, rechallenged mice which received C. parvum treatment without IL-1 demonstrated no statistically enhanced survival. Systemically administered IL-1 had no tumor suppressive effect without the addition of a local administration of C. parvum.

2. Effects of Murine Recombinant IL-1

An In vivo tumor protection experiment was performed using recombinant murine IL-1 obtained from Hoffmann-LaRoche, Inc. In this

Figure 2. Effect of P388D1Y macrophage derived IL-1 therapy on survival following the primary EL-4 tumor challenge. EL-4 challenged mice were treated with either C. parvum (X), P388D1Y macrophage derived IL-1 (◇), C. parvum and P388D1Y macrophage derived IL-1 (Δ), or no treatment (■). Treatment and tumor injection protocols were administered as described in section G of the Methods and Materials chapter.

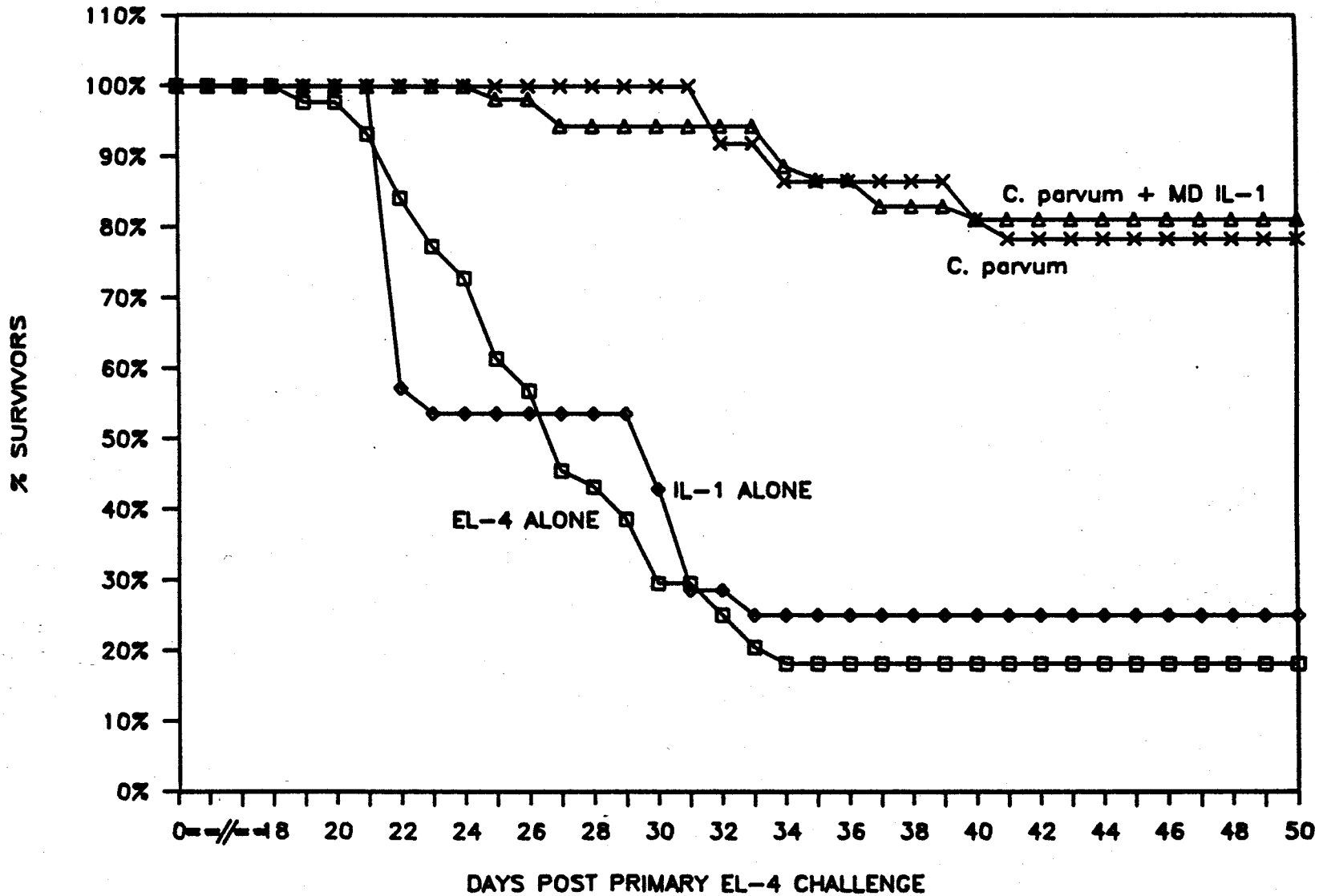
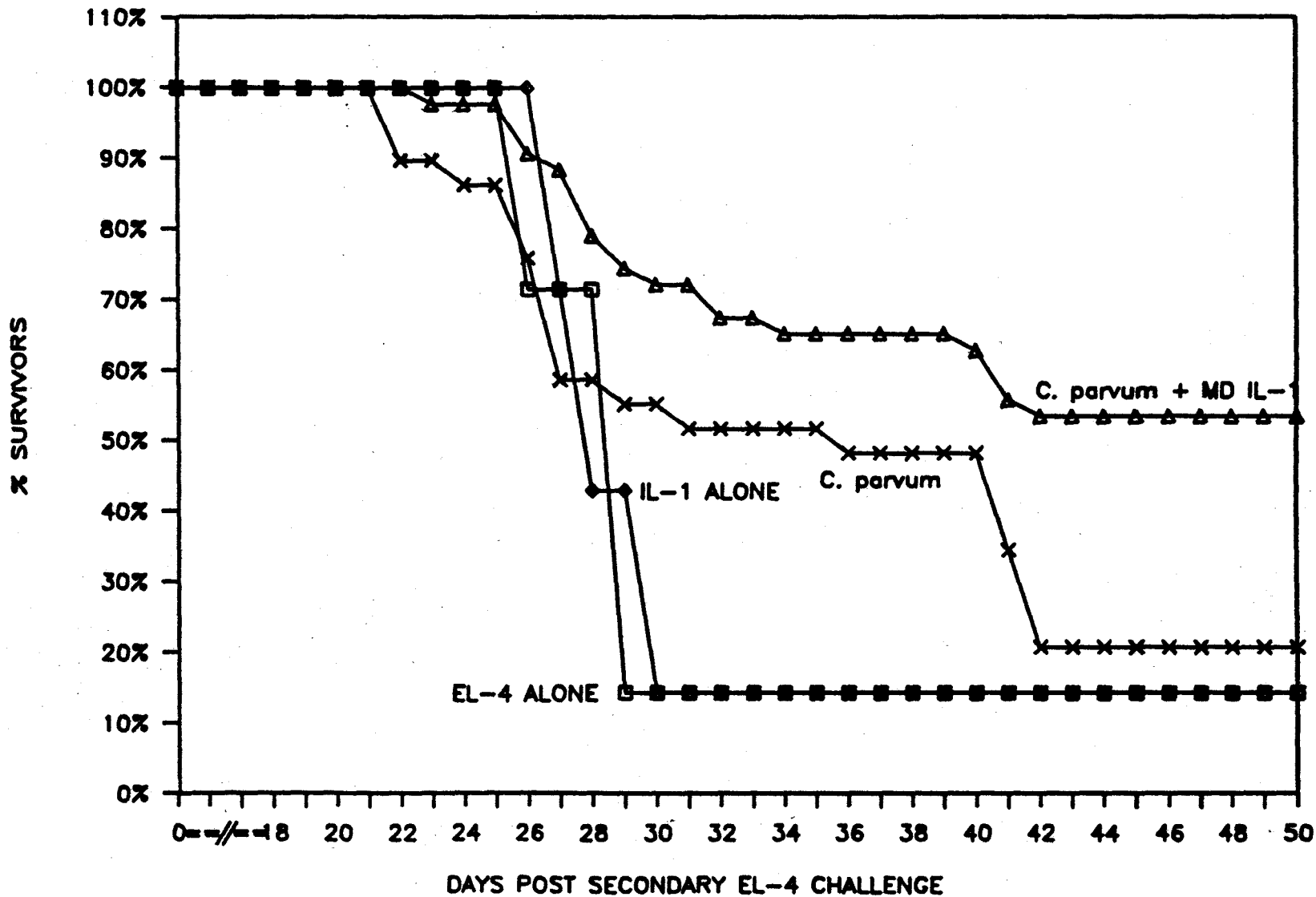


Figure 3. Effect of P388D1Y macrophage derived IL-1 therapy on survival following the secondary EL-4 tumor challenge. Mice treated at the time of the primary EL-4 challenge with either C. parvum (X), P388D1Y macrophage derived IL-1 (◇), C. parvum and P388D1Y macrophage derived IL-1 (Δ), or no treatment (■), were given a second EL-4 challenge. Treatment and tumor injection protocols were as administered as described in section G of the Methods and Materials chapter.



experiment animals received the primary EL-4 tumor challenge with local C. parvum therapy, i.d., plus i.p. therapy, with either 100 units of murine recombinant IL-1 or unstimulated P388D1A conditioned culture supernatants. Animals which survived the initial tumor challenge were given a second EL-4 challenge thirty-five days after the primary tumor dose was administered. Results from this trial are shown in Table 6. EL-4 challenged animals that received either C. parvum plus murine recombinant IL-1, or C. parvum plus unstimulated P388D1A conditioned culture supernatants demonstrated similar survival rates of 93 and 100 percent, respectively, following the primary tumor challenge. Animals that received the EL-4 challenge without therapy all died within thirty-two days (Figure 4). Following the EL-4 rechallenge, animals which received systemic recombinant IL-1 therapy demonstrated a 26.1 percent survival rate, while animals which received the non-IL-1 containing supernatant, all succumbed to the tumor (Table 6). All rechallenged animals, that received C. parvum therapy plus the unstimulated P388D1A culture supernatants rapidly died (within twenty-seven days) and showed nearly identical survival patterns with no prolongation of mean survival time when compared to animals that received the EL-4 as an initial challenge as determined by t test analysis (Figure 5). In contrast, animals that had received IL-1 upon initial challenge showed a more gradual decline in rechallenge survival, thereby generating a markedly prolonged survival curve with the last death recorded at day thirty-seven as shown in Figure 5. The mean rechallenge survival duration of 31.3 days for the IL-1 treated animals was determined to be

TABLE 6. Suppression of the EL-4 Lymphoma by Corynebacterium parvum and Murine Recombinant IL-1.^a

Treatment Protocol	<u>Primary Challenge</u>		<u>Secondary Challenge</u>	
	<u>#Survivors/ #Challenged</u>	<u>(%)</u>	<u>#Survivors/ #Challenged</u>	<u>(%)</u>
EL-4 with <u>C. parvum</u> + IL-1	40/43	(93.0)	6/23	(26.1)
EL-4 with <u>C. parvum</u> + Non-IL-1 ^b	14/14	(100)	0/4	(0)
EL-4 Alone	0/10	(0)	0/5	(0)

^aBacteria (2×10^9 per 0.1 ml) were admixed with 5×10^3 EL-4 cells (0.1 ml) and injected i.d. into the flank of each animal. IL-1 was administered i.p. (0.1 ml) on alternate days from day 0 to day 9 post primary tumor inoculation.

^bNon-IL-1, P388D1A unstimulated conditioned culture supernatant.

Figure 4. Effect of murine recombinant IL-1 therapy on survival following the primary EL-4 tumor challenge. EL-4 challenged mice were treated with either C. parvum and murine recombinant IL-1 (Δ), C. parvum and P388D1A unstimulated conditioned media (\diamond), or no treatment (\blacksquare). Treatment and tumor injection protocols were administered as described in section G of the Methods and Materials chapter.

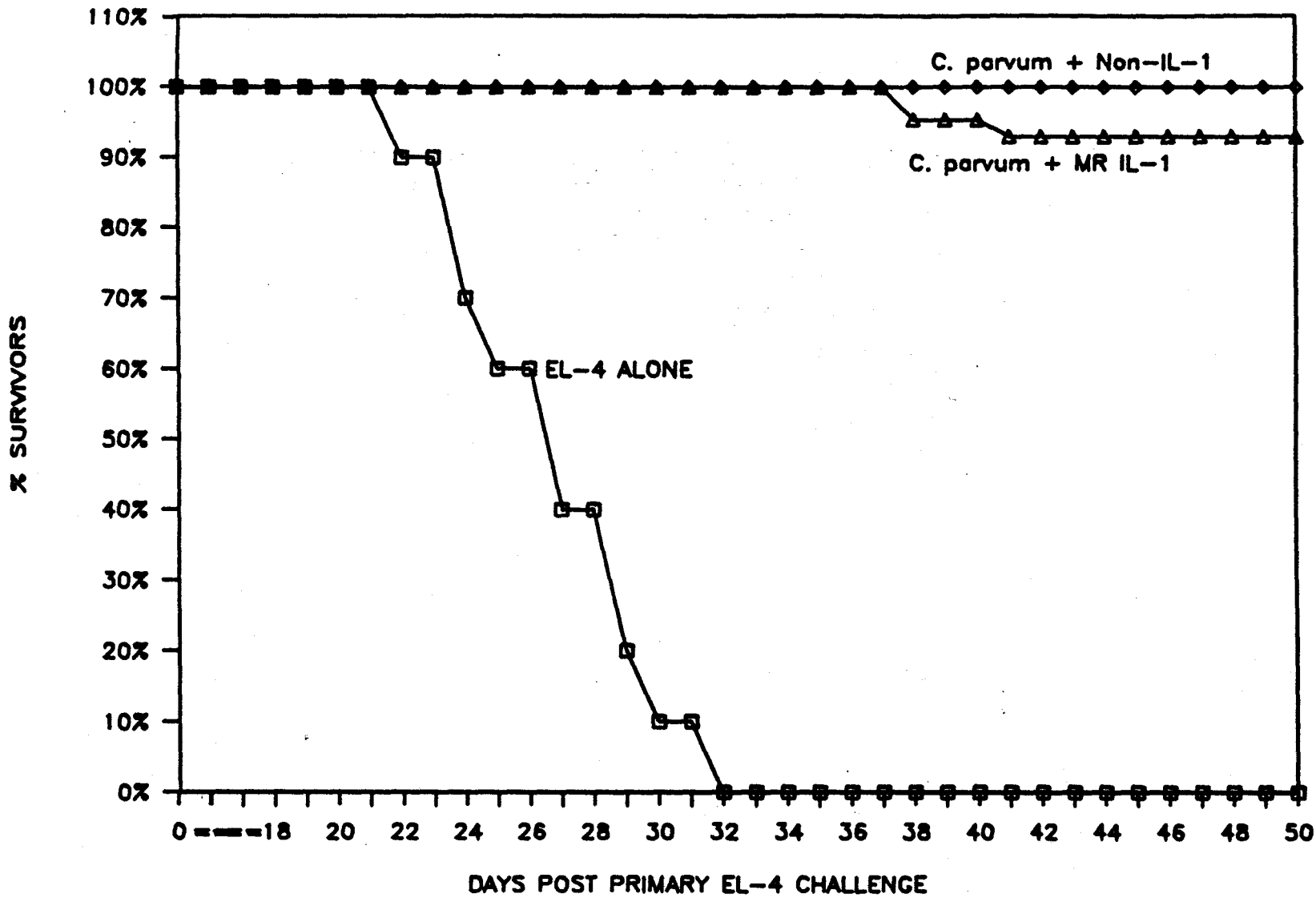
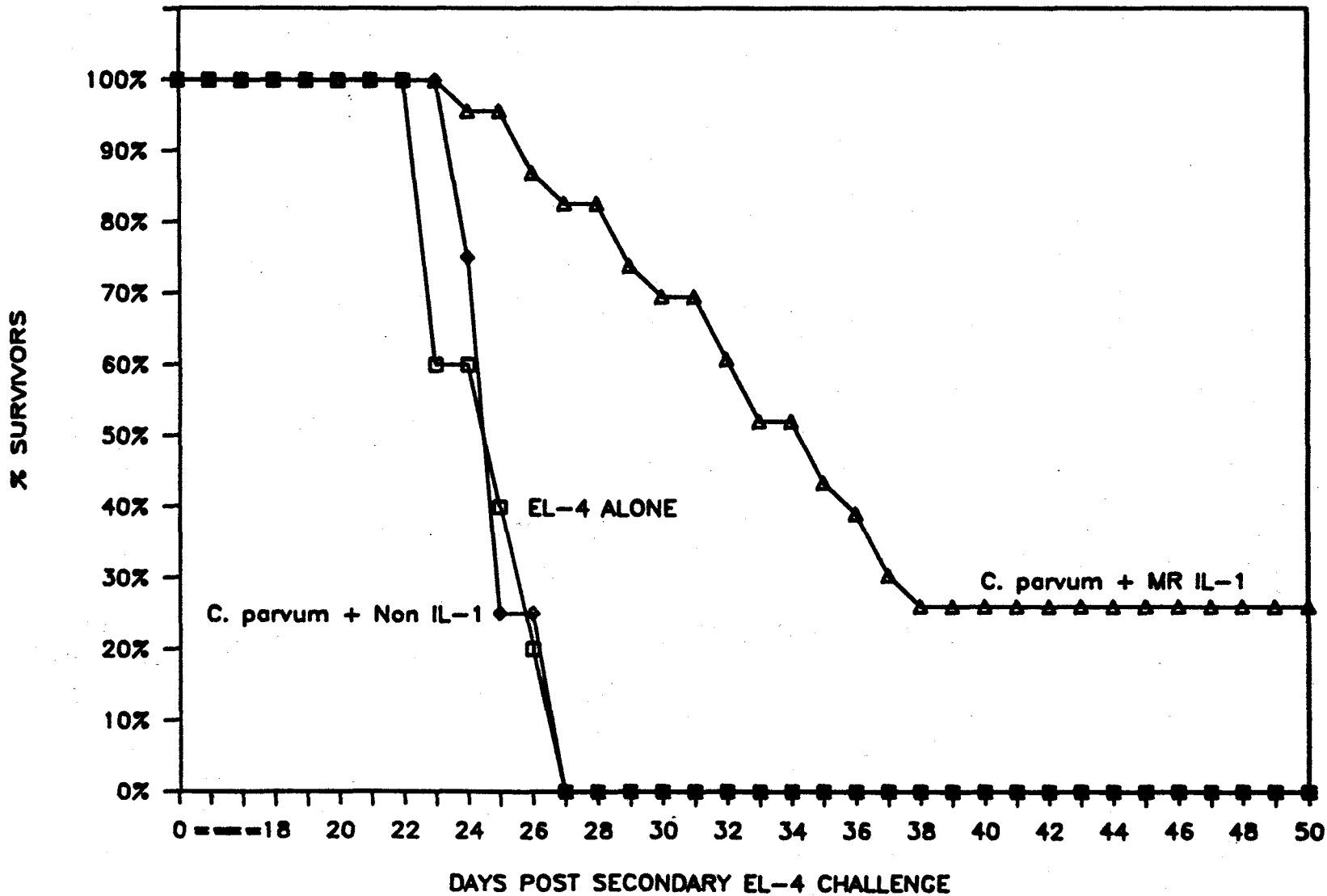


Figure 5. Effect of murine recombinant IL-1 therapy on survival following the secondary EL-4 tumor challenge. EL-4 challenged mice were treated with either C. parvum and murine recombinant IL-1 (Δ), C. parvum and P388D1A unstimulated conditioned media (\diamond), or no treatment (\blacksquare), were given a second EL-4 challenge. Treatment and tumor injection protocols were as administered as described in section G of the Methods and Materials chapter.



significantly enhanced ($p < 0.02$) when compared to the 25.2 day mean survival duration of mice which received the unstimulated P388D1 conditioned supernatant.

These data demonstrate that a combination of local adjuvant therapy (C. parvum), in conjunction with systemic recombinant IL-1 administration, resulted in a statistically enhanced survival duration for rechallenged animals. These results are similar to those obtained with macrophage derived IL-1.

C. In vitro Effect of IL-1 on the EL-4 lymphoma

Various amounts of IL-1 were added to actively proliferating in vitro EL-4 cultures as described in section M of the Methods and Materials chapter. As shown in Figure 6, from 0.75 to 12.5 units of exogenously added IL-1 had no deleterious or enhancing effects on the in vitro proliferative capacity of EL-4 cultures after twenty-four or forty-eight hours of incubation.

D. Assessment of EL-4 Reactive Antibody Titers Against the EL-4 Lymphoma

In Table 7, serum obtained from animals which received the EL-4 tumor challenge either alone, with C. parvum, or with C. parvum plus macrophage derived IL-1, were tested for EL-4 reactive antibody titers against the EL-4 lymphoma. It was apparent that no increase in antibody titers was detected in the sera of mice challenged via any of the experimental protocols when tested periodically up to eighteen days after the initial tumor challenge. Mice cured of the tumor via C. parvum plus IL-1 therapy also demonstrated no detectable levels of EL-4 reactive antibody up to 40 days after the tumor

Figure 6. Effect of murine recombinant IL-1 on the in vitro proliferation of EL-4 tumor cells. EL-4 cells were cultured with or without Murine recombinant IL-1 for 24 (◇) and 48 hours (■). EL-4 cell proliferation was determined by cellular ³H-thymidine incorporation as described in section M of the Methods and Materials. Each value represents the mean PSR of triplicate cultures ± STD.

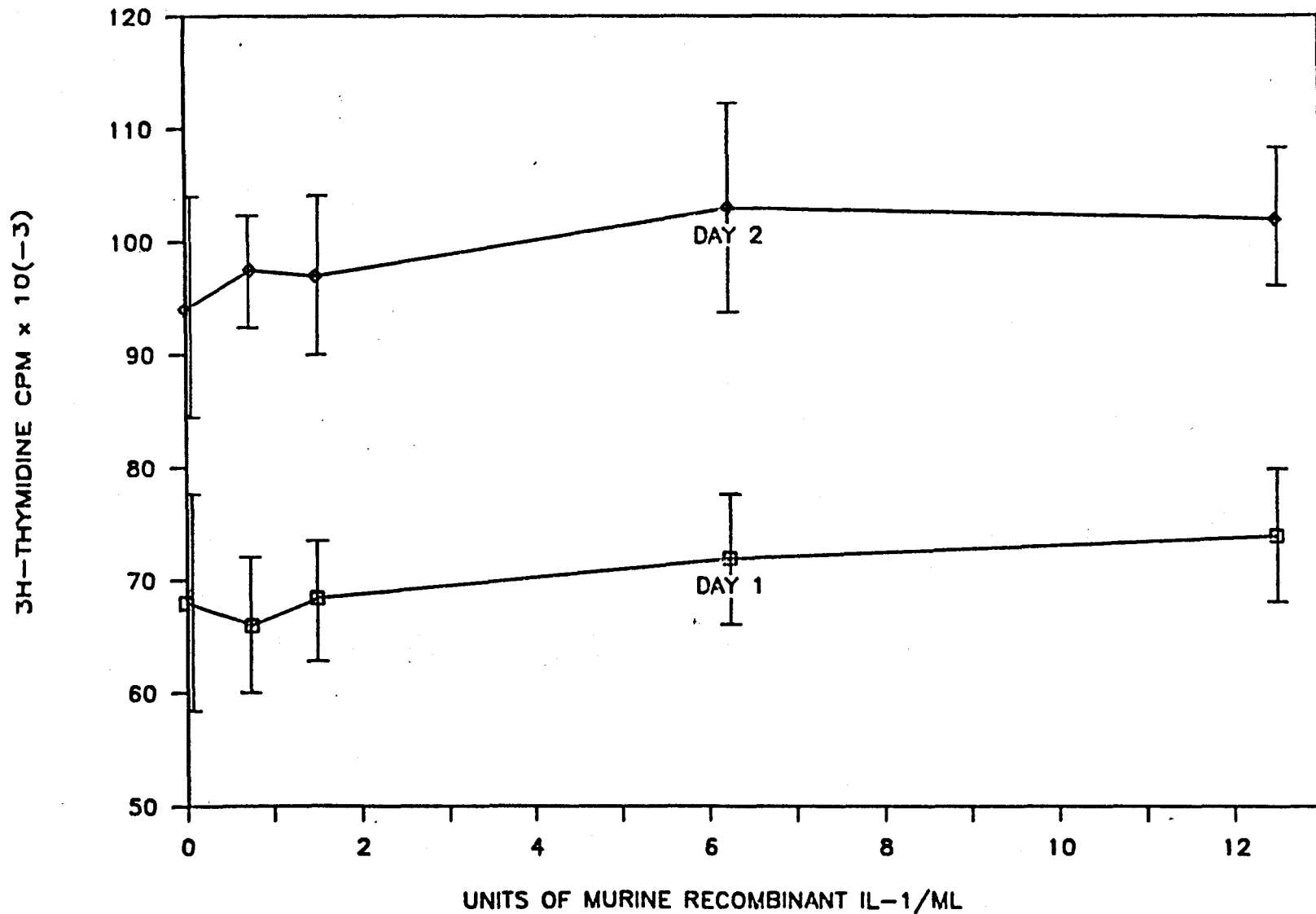


TABLE 7. Serum Antibody Levels Reactive With the EL-4 Lymphoma Following the Primary EL-4 Tumor Challenge.a

TREATMENT PROTOCOL	<u>Serum Antibody Titer</u>				
	<u>DAY POST PRIMARY TUMOR CHALLENGE</u>				
	DAY 6	DAY 9	DAY 13	DAY 18	DAY 40
EL-4 Alone	<10	<10	<10	<10	ND ^e
EL-4 with <u>C. parvum</u>	<10	<10	<10	<10	ND
EL-4 with <u>C. parvum</u> Plus MD IL-1	<10	<10	<10	<10	<10 ^b
No Treatment ^c	ND	ND	ND	ND	<10, 25
Hyperimmune ^d Against EL-4	ND	ND	ND	ND	370, 115, 25 <10, <10

^aSerum antibody titer determined by ELIZA as described in section N of the Methods and Materials chapter.

^bAnimals with no visible sign of tumor development, forty days after the EL-4 challenge, were considered cured of the tumor. Three animals were tested, all with antibody titers <10.

^cNormal C57Bl/6 serum.

^dAnimals were repeatedly immunized with mitomycin-c treated EL-4 tumor cells.

^eND, Not determined.

challenge. A majority of mice hyperimmunized by repeated injection with mitomycin-c treated EL-4 tumor cells did show an elevated antibody titer to the tumor. These data indicate that systemically administered IL-1 was unable to augment the humoral immune response against the EL-4 lymphoma, within the detectable limits of the ELISA system employed.

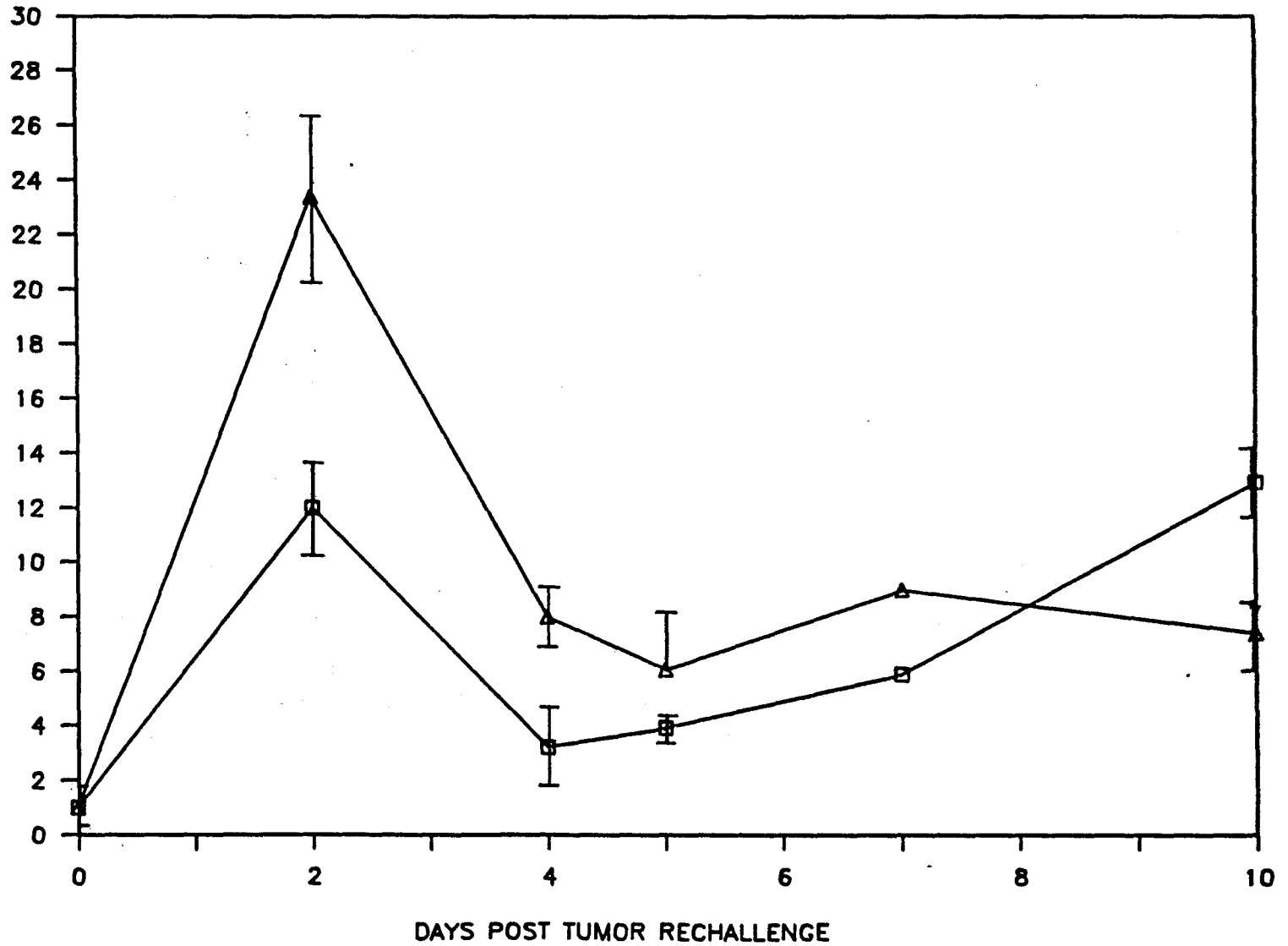
E. Effect of Systemically Administered IL-1 Upon the Number of Cells Recovered from the Lymph Nodes Draining the Site of the EL-4 Tumor Challenge

Animals which received EL-4 and C. parvum or EL-4 and C. parvum plus IL-1 were sacrificed at various time periods following both the EL-4 primary and secondary challenge. The total number of lymph node cells sequentially recovered from the regional axillary and brachial lymph nodes draining the tumor injection site following the secondary EL-4 challenge is presented in Figure 7. Mice that received both tumor protection protocols demonstrated marked increases in recovered lymph node cell numbers soon after the EL-4 rechallenge. Although, both groups demonstrated increased cell numbers, IL-1 treated animals demonstrated a significantly greater response two and four days after the rechallenge, when compared to animals not given IL-1. The lymph node cell numbers in both protocol groups decreased by day five after the rechallenge, with no differences in cell number noted between the two groups subsequently.

Initially, organ weights were determined for lymph nodes draining the site of the primary tumor challenge for IL-1 and non-IL-1 treated mice. However, at early time points lymph nodes were

Figure 7. Effect of P388D1Y macrophage derived IL-1 therapy on the lymphocyte cell number in the lymph nodes draining the site of the EL-4 tumor challenge. Mice treated at the time of the primary EL-4 challenge with either C. parvum (■) or C. parvum plus P388D1Y macrophage derived IL-1 (Δ), were given a second EL-4 challenge 35 days after the initial EL-4 challenge. The lymph nodes cell number was determined for lymph nodes draining the site of the tumor challenge at various time periods for each group following the second EL-4 tumor challenge. Each value represents the mean lymph node cell number of there mice \pm STD.

LYMPH NODE CELL NUMBER X 10⁻⁶ (6)



too small to assess weights accurately. To overcome this difficulty, total lymph node cell numbers were obtained for each treatment protocol animal group in a second series of experiments. Pooling of data obtained from both protocols was accomplished by calculating the percent change of lymph node weight or lymph node cell number between mice given C. parvum and C. parvum plus IL-1. Pooled results for days 4, 8, 13, and 17 yielded a percent change of +10%, +6%, -19%, and -3% respectively. This data did not reflect any marked modulation of lymph node mass due to IL-1 therapy following the primary tumor challenge. Only data from animals without tumor were utilized in these calculations since animals with palpable tumor (generally detectable at day 12 to 15) developed correspondingly enlarged lymph nodes draining the tumor site regardless of previous treatment (data not shown). These results suggest that, while IL-1 therapy did not modulate gross lymph node mass following the primary tumor challenge, IL-1 did induce accelerated regional lymph node cell proliferation and/or the stimulation of lymphoid cell convergence to the regional lymph nodes draining the site of the tumor rechallenge following a second exposure to the EL-4 tumor.

F. Effects of Systemically Administered IL-1 Upon Splenic and Lymph Node in vitro Correlates of Immunity Directed Against the EL-4 Lymphoma

In a series of experiments, animals were given an EL-4 tumor challenge with C. parvum therapy plus either: macrophage derived IL-1, murine recombinant IL-1, control HSA, or no accompanying i.p. therapy. These mice were assessed for either splenic or lymph node

cytotoxic T cell activity, NK activity, or lymphoproliferative responsiveness to the EL-4 lymphoma, following both primary and secondary tumor challenges.

1. Cytotoxic T Cell Activity

As shown in Table 8, it is apparent that spleen and lymph node cells derived from animals undergoing an initial EL-4 tumor challenge either with the EL-4 tumor alone, EL-4 and the protective C. parvum therapy, or EL-4 and C. parvum plus macrophage derived IL-1, demonstrated no detectable T cell cytolytic activity against ^{51}Cr radio-labeled EL-4 tumor targets greater than one L.U. The lack of lytic activity by spleen cells was demonstrated by periodic testing between day three and day seventeen following the primary tumor challenge. Lymph node cell populations, derived from these animals, were tested periodically for activity from day eight to day seventeen following the primary tumor challenge and showed a similar lack of cytolytic T cell activity against the EL-4 tumor targets.

Similar results, shown in Table 9, were obtained when lymph node and spleen cell populations derived from EL-4 rechallenged animals protected initially with C. parvum plus either murine recombinant IL-1, macrophage derived IL-1, or HSA control injection therapy, were tested for cytolytic activity against EL-4 targets thirty-five days after the primary challenge (day 0 post rechallenge). Following rechallenge with the EL-4 lymphoma, the lymph node and spleen cell populations from these groups of animals showed no cytolytic response greater than one L.U. to the EL-4 lymphoma.

TABLE 8. Effect of macrophage derived IL-1 on Splenic and Lymph Node T Cell Cytotoxicity Against the EL-4 Lymphoma Following Primary EL-4 Tumor Challenge.

DAYS POST PRIMARY CHALLENGE	SPLEEN CELL POPULATIONS ^{a,b}			LYMPH NODE CELL POPULATIONS ^{a,c}		
	EL-4	EL-4 with <u>C. parvum</u>	EL-4 with <u>C. parvum</u> Plus IL-1	EL-4	EL-4 with <u>C. parvum</u>	EL-4 with <u>C. parvum</u> Plus IL-1
3	<1 ^d	<1	<1	ND	ND	ND
6	<1	<1	<1	ND	ND	ND
8	<1	<1	<1	<1	<1	<1
10	<1	<1	<1	<1	<1	<1
13	<1	<1	<1	<1	<1	<1
17	<1	<1	<1	<1	<1	<1

^aSpleen and lymph node effector cell suspensions represent cells pooled from three animals sacrificed from each treatment protocol group.

^bRadiolabeled EL-4 YAC-1 tumor targets (1×10^4) were incubated with effector spleen cell populations at E/T ratios of 200:1, 100:1, 500:1, and 25:1 for four hours as described in the Methods chapter.

^cRadiolabeled EL-4 YAC-1 tumor targets (1×10^4) were incubated with effector lymph node cell populations at E/T ratios of 100:1, 50:1, 25:1, and 12.5:1 for four hours as described in the Methods chapter.

^dData are represented as L.U. of activity at ten % specific release per 1×10^6 effector cells as described in section I of the Methods and Materials chapter.

TABLE 9. Effect of Murine Recombinant (MR) and Macrophage Derived (MD) IL-1 on Splenic and Lymph Node T Cell Cytotoxicity Against the EL-4 Lymphoma Following the Secondary EL-4 Tumor Challenge.

DAYS POST SECONDARY CHALLENGE	SPLEEN CELL POPULATIONS ^{a, b}			LYMPH NODE CELL POPULATION ^{a, c}		
	EL-4 with <u>C. parvum</u> + HSA	EL-4 with <u>C. parvum</u> + MR IL-1	EL-4 with <u>C. parvum</u> + MD IL-1	EL-4 with <u>C. parvum</u> + HSA	EL-4 with <u>C. parvum</u> + MR IL-1	EL-4 with <u>C. parvum</u> + MD IL-1
0	<1 ^d	<1	<1	<1	<1	<1
3	<1	<1	<1	<1	<1	<1
6	<1	<1	<1	<1	<1	<1

^aSpleen and lymph node effector cell suspensions represent cells pooled from three animals sacrificed from each treatment protocol group.

^bRadiolabeled EL-4 tumor targets (1×10^4) were incubated with effector spleen cell populations at E/T ratios of 200:1, 100:1, 500:1, and 25:1 for four hours as described in section I of the Methods and Materials chapter.

^cRadiolabeled EL-4 tumor targets (1×10^4) were incubated with effector lymph node cell populations at E/T ratios of 100:1, 50:1, 25:1, and 12.5:1 for four hours as described in section I of the Methods and Materials chapter.

^dData are represented as L.U. of activity at 10% specific release per 1×10^6 effector cells as described in section I of the Methods and Materials chapter.

These data suggest that IL-1 therapy was not able to elicit the development of a notable in vitro T cell cytolytic response in the spleen or regional lymph nodes in animals following the primary EL-4 tumor challenge nor in cured mice which were treated with IL-1 and then exposed to a second tumor dose.

2. Natural Killer Cell Activity

Repeated periodic assessments were made of NK activity of spleen cell populations derived from animals undergoing an initial EL-4 tumor challenge either with the EL-4 tumor alone, EL-4 and C. parvum therapy, or EL-4 and C. parvum plus macrophage derived IL-1. Results of a typical experiment, presented in Table 10, showed a degree of fluctuation in the amount of lytic activity against YAC-1 tumor targets in tests performed from day to day. However, compared differences in L.U. between spleen cell populations on individual days shows no pattern of augmented NK activity as a result of systemic administration of macrophage derived IL-1. Results of t test analysis comparing the PSR of the 100 to 1 E/T ratio data points for the three spleen cell populations tested on each day, established that no statistical differences in NK activity were seen between these groups on days three, eight, ten, and thirteen following the primary tumor challenge. On day six, animals which received no therapy or C. parvum therapy with the EL-4 challenge, demonstrated a statistically enhanced cytolytic response when compared to mice that had received EL-4 and C. parvum plus IL-1 therapy. This result was reversed on day 17, when the IL-1 treated mice demonstrated an enhanced development of NK activity, when compared to animals not given IL-1.

TABLE 10. Effect of Macrophage Derived IL-1 on Splenic and Lymph Node Natural Killer Cell Activity Against YAC-1 Tumor Targets Following the Primary EL-4 Tumor Challenge.

DAYS POST PRIMARY CHALLENGE	SPLEEN CELL POPULATIONS ^{a, b}			LYMPH NODE CELL POPULATIONS ^{a, c}		
	EL-4	EL-4 with <u>C. parvum</u>	EL-4 with <u>C. parvum</u> + IL-1	EL-4	EL-4 with <u>C. parvum</u>	EL-4 with <u>C. parvum</u> + IL-1
3	3.22 ^d	2.70	2.30	ND	ND	ND
6	2.08	1.91	1.33	ND	ND	ND
8	3.13	2.74	1.47	<1	<1	<1
10	<1	<1	1.14	<1	<1	<1
13	2.70	3.03	3.33	<1	<1	<1
17	<1	<1	2.00	<1	<1	<1

^aSpleen and lymph node effector cell suspensions represent cells pooled from three animals sacrificed from each treatment protocol group.

^bRadiolabeled YAC-1 tumor targets (1×10^4) were incubated with effector spleen cell populations at E/T ratios of 200:1, 100:1, 500:1, and 25:1 for four hours as described in section J of the Methods and Materials chapter.

^cRadiolabeled YAC-1 tumor targets (1×10^4) were incubated with effector lymph node cell populations at E/T ratios of 100:1, 50:1, 25:1, and 12.5:1 for four hours as described in section J of the Methods and Materials chapter. E/T ratios of 25:1 and 12.5:1 only were assayed for the EL-4 alone mice on day eight.

^dData are represented as L.U. of activity at ten percent specific release per 1×10^6 effector cells as described in section I of the Methods and Materials chapter.

NK activity of lymph node cell populations were also determined. Sufficient cell numbers for assessment of NK activity were not obtained from the regional lymph nodes draining the site of the tumor challenge until the eight days after the primary EL-4 challenge. As shown in Table 10., NK activity of lymph node cells obtained from animals given the EL-4 challenge alone, or EL-4 with C. parvum or EL-4 with C. parvum plus macrophage derived IL-1, demonstrated no detectable NK activity above one L.U. from eight to seventeen days following the primary tumor challenge.

Both lymph node and spleen cell populations obtained from animals surviving the initial EL-4 challenge with C. parvum plus either murine recombinant IL-1, macrophage derived IL-1, or HSA therapy, were tested for NK activity thirty-five days after the primary challenge (day 0 post rechallenge), and then on days three and six following the secondary EL-4 challenge. As shown in Table 11., spleen cells obtained from IL-1 treated animals demonstrated no lytic activity greater than one L.U. either immediately before being given the second EL-4 tumor challenge or on days three and six after the rechallenge.

Cells obtained from regional lymph nodes draining the site of the tumor rechallenge of these mice were also assessed for NK activity. Results of these experiments, also presented in Table 11, show that the lymph node cell populations obtained from the IL-1 treated animals were equally unable to develop NK activity greater than one L.U. up to six day after the second EL-4 challenge was administered. Control animals which received HSA injections along

TABLE 11. Effect of Murine Recombinant (MR) and Macrophage Derived (MD) IL-1 on Splenic and Lymph Node Natural Killer Cell Activity Against YAC-1 Tumor Targets Following the Secondary EL-4 Tumor Challenge.

DAYS POST SECONDARY CHALLENGE	SPLEEN CELL POPULATIONS ^{a,b}			LYMPH NODE CELL POPULATIONS ^{a,c}		
	EL-4 with <u>C. parvum</u> + HSA	EL-4 with <u>C. parvum</u> + MR IL-1	EL-4 with <u>C. parvum</u> + MD IL-1	EL-4 with <u>C. parvum</u> + HSA	EL-4 with <u>C. parvum</u> + MR IL-1	EL-4 with <u>C. parvum</u> + MD IL-1
0	1.08 ^d	<1	<1	<1	<1	<1
3	<1	<1	<1	1.63	<1	<1
6	<1	<1	<1	1.90	<1	<1

^aSpleen and lymph node effector cell suspensions represent cells pooled from three animals sacrificed from each treatment protocol group.

^bRadiolabeled YAC-1 tumor targets (1×10^4) were incubated with effector spleen cell populations at E/T ratios of 200:1, 100:1, 500:1, and 25:1 for four hours as described in section J of the Methods and Materials chapter.

^cRadiolabeled YAC-1 tumor targets (1×10^4) were incubated with effector lymph node cell populations at E/T ratios of 100:1, 50:1, 25:1, and 12.5:1 for four hours as described in section J of the Methods and Materials chapter.

^dData are represented as L.U. of activity at 10% specific release per 1×10^6 effector cells as described in section I of the Methods and Materials chapter.

with the protective C. parvum therapy did demonstrate NK activity greater than one L.U. on days three and six, although in neither case did this activity exceed two L.U. These data indicate that NK activity did not appear to be enhanced in C. parvum treated animals which received IL-1, following either the primary or secondary tumor challenge. Suggesting that enhancement of NK activity does not appear to account for the IL-1 tumor protective effect.

3. Lymphoproliferative Responsiveness

In vitro splenic proliferative responsiveness to EL-4 and YAC-1 stimulator tumor targets was assessed following a secondary EL-4 tumor challenge. Splenic populations from mice that had survived the primary EL-4 challenge with the aid of protective C. parvum therapy plus either control HSA therapy, macrophage derived IL-1 therapy or murine recombinant IL-1 therapy were evaluated. Proliferative responsiveness was determined sequentially for these groups of mice on the day animals received the secondary EL-4 challenge and on days three and six following the rechallenge. The results are presented for proliferative responsiveness to the EL-4 and RBL-5 lymphoma stimulator cells in Figures 8 and 9, respectively. Both Figures have identical Y axis scales to allow easier comparisons from one Figure to the other. Animals which received macrophage derived IL-1 therapy demonstrated a modest yet significantly enhanced response to the EL-4 lymphoma (Figure 8) on day three (SI = 3.28) and day six (SI = 4.85). Such an enhancement was not seen in the IL-1 treated animals which were assessed immediately before the rechallenge (day 0). However, the spleen cells from the same IL-1 treated animals did not respond

to YAC-1 tumor cells (Figure 9) on days three or six (SI = 0.94 and 1.33 respectively) when identically derived cell populations were responding to the EL-4 stimulator cells. This response to the EL-4 lymphoma was shown to be significantly enhanced ($p < 0.001$) when compared to the responsive to YAC-1 stimulator cells on both days three and six, as determined by t test analysis.

Rechallenged mice which had received the HSA injections showed a statistically enhanced ($p < .001$) yet weak proliferative response to the EL-4 lymphoma (SI = 2.3) three days after the EL-4 rechallenge (Figure 8). However, no statistically augmented response (SI = 1.47) was elicited from HSA treated mice on day six following the rechallenge. No response to YAC-1 stimulator cells (Figure 9) was noted for these animals on either day three or day six (SI = 0.81 and 0.96 respectively). Although this weak spleen cell proliferative response to the EL-4 lymphoma was observed three days after the tumor rechallenge in HSA treated mice, the splenic proliferative response to the EL-4 lymphoma, generated by the macrophage derived IL-1 treated animals, was shown to be even statistically greater ($p < 0.002$) than the response produced by the HSA treated animals.

Spleen cells from murine recombinant IL-1 treated animals were likewise assessed for proliferative responsiveness to EL-4 and YAC-1 stimulator cells following a secondary tumor challenge. Results also presented in Figures 8 and 9 respectively, were remarkably similar to those produced by animals which received macrophage derived IL-1. Recombinant IL-1 treated mice demonstrated a modest yet significantly enhanced response to the EL-4 lymphoma on day three (SI = 3.11) and

Figure 8. Effect of P388D1Y macrophage derived IL-1 therapy on in vitro splenic proliferative responsiveness to EL-4 and YAC-1 tumor cells following the secondary EL-4 tumor challenge. Mice treated with either C. parvum plus P388D1Y macrophage derived IL-1 or C. parvum plus HSA which survived a primary EL-4 tumor challenge, were given a second EL-4 challenge. Spleen cell populations derived from these two groups of mice were cultured 48 hours with mitomycin-c treated EL-4 or YAC-1 stimulator cells. Spleen cell proliferative responsiveness to the stimulator cells was determined by cellular ³H-thymidine incorporation as described in section L of the Methods and Materials chapter. Each value represents the mean SI of triplicate cultures \pm STD.

EL-4 STIMULATOR CELLS

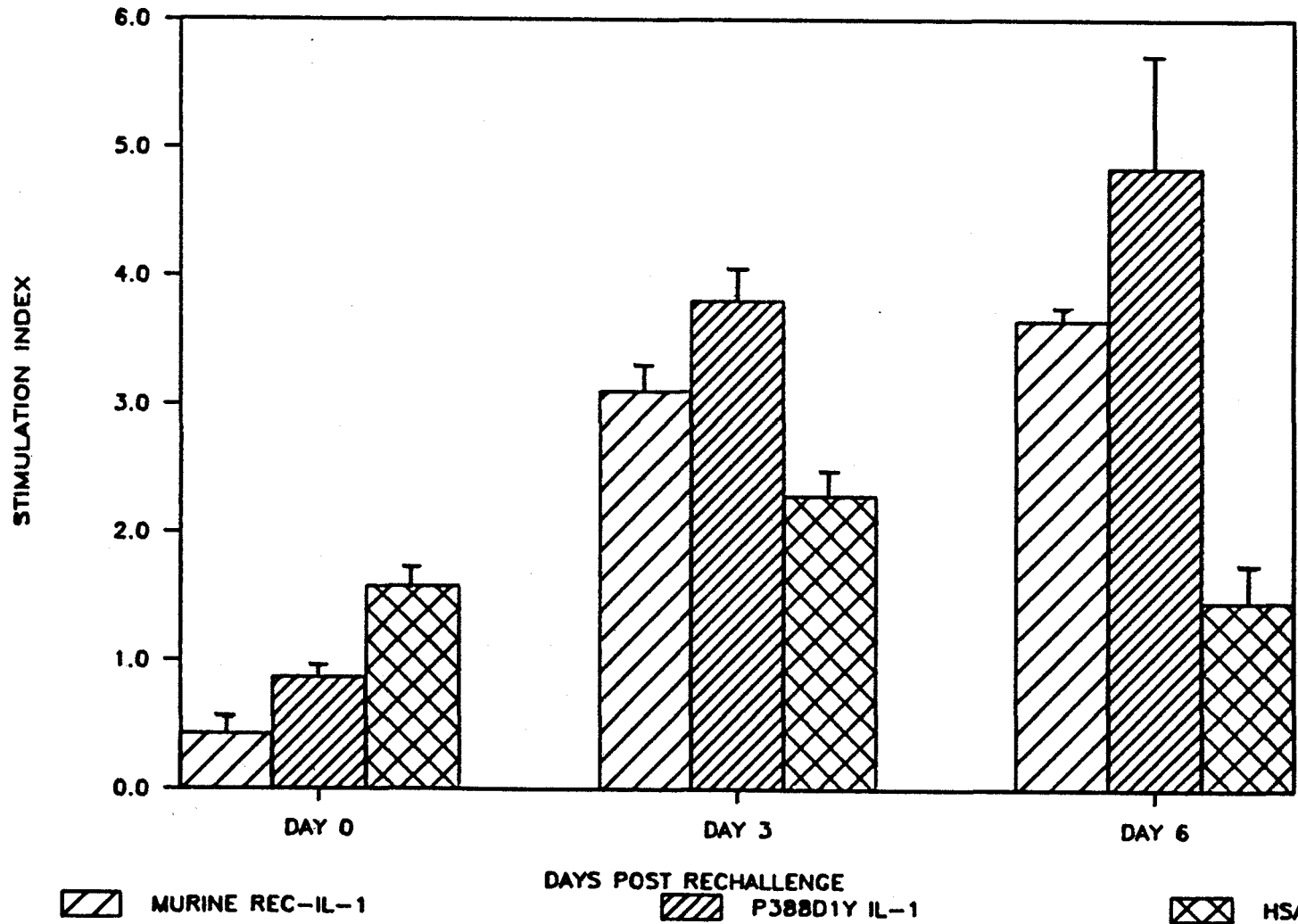
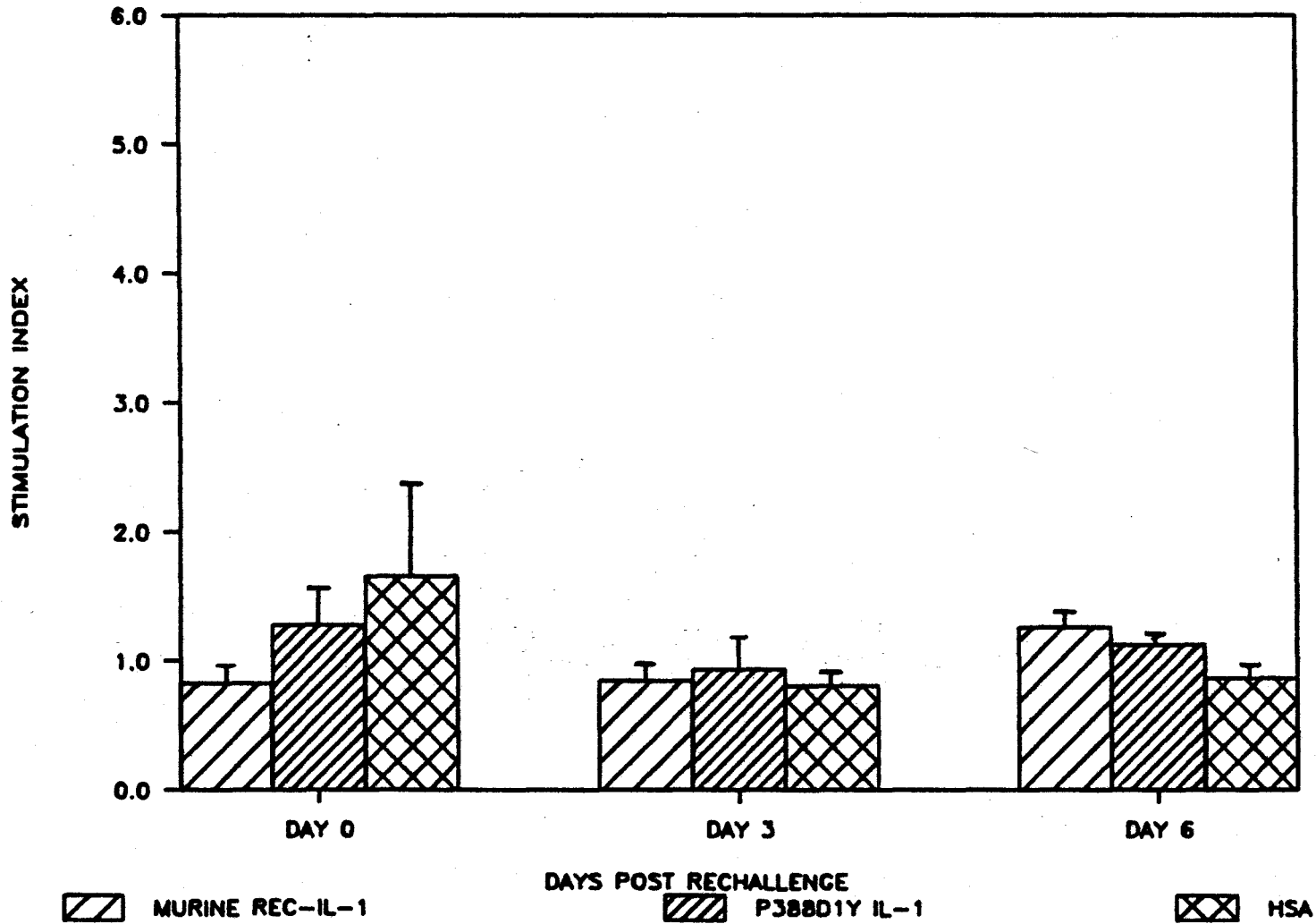


Figure 9. Effect of murine recombinant IL-1 therapy on in vitro splenic proliferative responsiveness to EL-4 and YAC-1 tumor cells following the secondary EL-4 tumor challenge. Mice treated with either C. parvum plus murine recombinant IL-1 or C. parvum plus HSA which survived a primary EL-4 tumor challenge, were given a second EL-4 challenge. Spleen cell populations derived from these two groups of mice were cultured 48 hours with mitomycin-c treated EL-4 or YAC-1 stimulator cells. Spleen cell proliferative responsiveness to the stimulator cells was determined by cellular ³H-thymidine incorporation as described in section L of the Methods and Materials chapter. Each value represents the mean SI of triplicate cultures \pm STD.

YAC-1 STIMULATOR CELLS



day six (SI = 3.66) following the EL-4 rechallenge. This response was not seen in the IL-1 treated mice which were assessed immediately before the tumor rechallenge (day 0 post rechallenge). Spleen cells from the same recombinant IL-1 treated animals did not respond to YAC-1 tumor targets on either days three or six (SI = 0.86 and 1.27, respectively). These splenic responses to the EL-4 lymphoma were shown to be significantly enhanced ($p < 0.001$) when compared to the response to YAC-1 stimulator cells both on days three and six.

Once again, the proliferative response generated by IL-1 treated animals to the EL-4 lymphoma was statistically greater, both on day three ($p < 0.05$) and day six ($p < 0.001$), when compared to the weaker response produced by the HSA treated control animals.

Following the initial EL-4 tumor challenge, as shown in Table 12, no responsiveness was demonstrated by IL-1 treated animals to the EL-4 antigen in contrast to the enhanced response seen after the secondary EL-4 challenge. This time course study, initiated three days and ending fourteen days after the primary challenge, detected no proliferative response of spleen cells from IL-1 treated animals greater than 1.63 (seen on day fourteen post rechallenge). Animals that received the EL-4 challenge without treatment also failed to generate a proliferative stimulation index to the EL-4 lymphoma greater than 1.58 as seen on day seven. Animals that received EL-4 with C. parvum alone demonstrated a weak proliferative response to the EL-4 antigen (SI = 2.43) on day fourteen following the primary challenge. This group of animals failed to generate any marked proliferative response (SI > 2.0) from day three to day ten.

TABLE 12. Effect of P388D1Y Macrophage Derived IL-1 on the Spleen Cell Proliferative Response to the EL-4 Lymphoma Following the Primary EL-4 Tumor Challenge^a.

TREATMENT PROTOCOL	STIMULATION INDEX			
	DAYS POST PRIMARY CHALLENGE			
	DAY 3	DAY 7	DAY 10	DAY 14
EL-4 Alone	0.86 ± 0.34 ^b	1.58 ± 0.10	0.70 ± 0.22	1.32 ± 0.21
EL-4 with <u>C. parvum</u>	0.79 ± 0.15	1.91 ± 0.16	0.97 ± 0.30	2.43 ± 0.11
EL-4 with <u>C. parvum</u> + IL-1	1.53 ± 0.23	1.17 ± 0.17	1.18 ± 0.39	1.63 ± 0.16

^aSpleen effector cell suspensions represent cells pooled from three animals sacrificed from each treatment protocol group. Mitomycin-c treated EL-4 lymphoma cells (1×10^5) were incubated two days with various effector cell populations (2×10^5 cells) in a 0.2ml volume. ³H-thymidine incorporation was determined as described in section L of the Methods and Materials chapter.

^bData are expressed as the mean stimulation index of triplicate cultures ± the standard deviation.

These data indicate that both macrophage derived and murine recombinant forms of systemic IL-1 therapy were able to elicit a sequential enhancement of the splenic proliferative response in EL-4 rechallenged animals. This response strongly suggests specificity for the EL-4 tumor; the tumor antigen seen by these animals at the time of IL-1 treatment. This response was shown to be markedly stronger and more prolonged than responses generated by control HSA treated mice.

Unlike the enhanced proliferative response seen in the spleen cells from rechallenged IL-1 treated mice to mitomycin-c inactivated EL-4 stimulator cells, no detectable increase in responsiveness was generated in lymphoid cells taken from regional lymph nodes draining the tumor challenge site. Results obtained for lymph node cells, obtained periodically from mice following both the primary and secondary EL-4 tumor challenge, are shown in Tables 13 and 14 respectively. Seven days after the primary EL-4 challenge, a weak proliferative response (SI = 2.10) was generated in mice that received EL-4 with C. parvum plus macrophage derived IL-1. This proliferative response was not seen when later assessed at day ten and day fourteen. Although the IL-1 treated animals did demonstrate a weak proliferative response at day seven, animals that did not receive IL-1 also demonstrated an equally weak proliferative response to the EL-4 stimulator cells. Thus, IL-1 treatment did not appear to be responsible for this weak response generated early after the initial tumor challenge. The response seen in mice treated with C. parvum alone also declined by day ten after the primary challenge.

TABLE 13. Effect of P388D1Y IL-1 on Lymph Node Cell Proliferative Responsiveness to the EL-4 Lymphoma Following the Primary EL-4 Tumor Challenge^a.

TREATMENT PROTOCOL	<u>STIMULATION INDEX</u>		
	<u>DAYS POST PRIMARY CHALLENGE</u>		
	DAY 7	DAY 10	DAY 14
EL-4 Alone	ND ^b	ND	1.65 ± 0.21
EL-4 with <u>C. parvum</u>	2.30 ± 0.16 ^c	1.00 ± 0.30	1.23 ± 0.11
EL-4 with <u>C. parvum</u> + IL-1	2.10 ± 0.17	1.10 ± 0.39	1.15 ± 0.16

^aLymph Node effector cell suspensions represent cells pooled from three animals sacrificed from each treatment protocol group. Mitomycin-c treated EL-4 lymphoma cells (1×10^5) were incubated two days with various effector cell populations (1×10^5 cells) in a 0.2ml volume. ³H-thymidine incorporation was determined as described in section L of the Methods and Materials chapter.

^bND, not determined.

^cData are expressed as the mean stimulation index of triplicate cultures ± the standard deviation.

TABLE 14. Effect of P388D1Y Macrophage Derived IL-1 on the Lymph Node Cell Proliferative Response to the EL-4 Lymphoma Following the Secondary EL-4 Tumor Challenge^a.

TREATMENT PROTOCOL	STIMULATION INDEX			
	DAYS POST SECONDARY CHALLENGE			
	DAY 5	DAY 7	DAY 10	DAY 14
EL-4 Alone	ND ^b	ND	ND	1.67 ± 0.07
EL-4 with <u>C. parvum</u>	1.04 ± 0.18 ^c	2.16 ± 0.15	0.89 ± 0.30	1.20 ± 0.14
EL-4 with <u>C. parvum</u> + IL-1	1.73 ± 0.11	1.92 ± 0.09	1.11 ± 0.20	1.20 ± 0.17

^aLymph Node effector cell suspensions represent cells pooled from three animals sacrificed from each treatment protocol group. Mitomycin-c treated EL-4 lymphoma cells (1×10^5) were incubated two days with various effector cell populations (1×10^5 cells) in a 0.2ml volume. ³H-thymidine incorporation was determined as described in section L of the Methods and Materials chapter.

^bND, not determined.

^cData are expressed as the mean stimulation index of triplicate cultures ± the standard deviation.

Lymph node proliferative responsiveness of macrophage derived IL-1 treated animals to the EL-4 lymphoma, following the secondary tumor challenge, was assessed. No pattern of lymph node proliferative responsiveness was noted following periodic assessment from five to fourteen days following the EL-4 rechallenge. The greatest response generated by these animals in this time period was seen on day seven (SI = 1.92). However, a statistically similar response was also generated by C. parvum treated animals that were not given IL-1 therapy. These data indicate that IL-1 did not elicit any detectable lymph node cell proliferative response to the EL-4 lymphoma following either the primary or secondary tumor challenge at the time periods assessed.

CHAPTER V

DISCUSSION

The use of cytokines in cancer immunotherapeutic models has become increasingly attractive as the importance of these intercellular messenger molecules becomes more and more apparent in both in vivo and in vitro studies. The monokine, IL-1, was chosen for study of immunomodulation of the tumor immune response due to the wide variety of biological activities associated with this protein (64, 78). Several lines of evidence strongly suggests that IL-1 may be able to positively modulate the host response to a neoplastic challenge. IL-1 has been shown to augment in vitro immune responses by enhancing the proliferation of T (48,177,192) and B lymphocytes (124,166,247), either directly or through the production of other lymphokines such as IL-2 and gamma-INF (94,281). IL-1 has been shown to enhance natural killer cell activity (115,239), contribute to the generation of cytotoxic T lymphocytes (281), and augment antibody production both in vitro (166) and in vivo (285). IL-1 induces macrophage production of PGE₂ (68), and is involved in the activation of macrophages to become tumoricidal (240). IL-1 has also been shown to be a chemoattractant for macrophages (170). Finally, IL-1 has demonstrated direct cytostatic and cytotoxic activity against some tumor cell lines (168,235).

Little direct evidence exists which indicates a functional role for IL-1 in the host response to neoplastic disease

or for the use of IL-1 as an immunotherapeutic agent. Therefore, IL-1 has been evaluated in this research to assess the efficacy of IL-1 as an immunomodulator in an experimental neoplastic model. In this model, IL-1 in conjunction with local adjuvant therapy, has been demonstrated to significantly enhance the tumor immune response of to the EL-4 lymphoma.

Initial in vivo immunoadjuvant tumor protection studies using intradermal injections of heat-killed C. parvum admixed with an EL-4 tumor challenge verified previous work (183,190) indicating that the use of nonviable bacterial immunoadjuvant antitumor therapy was effective in protecting C57Bl/6 mice from an initial EL-4 tumor challenge. As demonstrated previously, the tumor protective response associated with bacterial adjuvanticity was enhanced in a dose dependent manner, with 2×10^9 C. parvum cells enabling very high survival against the EL-4 tumor challenge. The ability of EL-4 cells to survive sixteen hours in in vitro suspensions of heat-killed C. parvum bacterial cells ranging in concentration from 1×10^8 to 1×10^{10} cells without loss of viability, as shown in Table 2, strongly indicates that the admixed heat-killed C. parvum cells were not toxic to the EL-4 tumor cells upon in vivo challenge. Therefore, toxicity of the bacterial immunoadjuvant utilized was not responsible for the observed protection.

All C. parvum treated animals, which survived the initial EL-4 challenge and were given a second EL-4 dose without adjuvant therapy. As shown in table 3, these rechallenged mice all succumbed to the second tumor challenge. These results are in strong agreement with

previous observations which indicate that highly nonimmunogenic tumors, such as the EL-4 lymphoma, fail to elicit protective tumor immunity in animals cured of a primary tumor challenge by adjuvant-type immunotherapy (80,249).

It is apparent by viewing Figure 1 that C. parvum has the capacity to activate macrophages to tumoricidal activity in a dose dependent manner. It is also apparent that this activation was non-specific in nature since both EL-4 and RBL-5 tumor cells were both lysed by C. parvum activated macrophages. These results are in agreement with previous work suggesting that the antitumor effect of C. parvum in vivo on relatively nonimmunogenic tumor is the result of immunologically nonspecific mechanisms (294). The C. parvum and LPS activators did not decrease the viability of the EL-4 tumor cells when incubated together without the presence of the PECs for the duration of the experiment. A decrease in target cell viability was seen only when the LPS concentration was increased to 100 g or ten times the maximum amount reported in these macrophage activation experiments. These results suggest that one possible mechanism of antitumor activity of C. parvum in this C57Bl/6 murine model is local C. parvum mediated activation of macrophages leading to tumoricidal activity against the admixed EL-4 tumor cells, and not as a result of direct toxicity to the injected tumor by the bacterial adjuvant.

There is also sufficient evidence to indicate that C. parvum can prime mice for TNF production (41,112). Serum containing TNF causes hemorrhagic necrosis of mouse and human tumors in vivo (41, 111,113,316). Abundant evidence indicates that the macrophage is the

major source of serum TNF with the demonstration that peritoneal macrophages produce TNF-like activity after stimulation with endotoxin (150,184,263). Recent studies have shown that the tumor variants which become resistant to lysis by activated macrophages have acquired resistance to TNF. These investigators also observed that TNF-neutralizing antibody totally inhibited activated macrophage mediated tumor cell lysis. These results along with the known TNF producing capability of activated macrophages strongly suggests that the C. parvum adjuvant therapy given EL-4 challenged mice may activate macrophages leading to the production of TNF. Thus indicating a nonspecific antitumor macrophage mediated mechanism which lead to the destruction of the primary tumor.

In rechallenge experiments which employed systemic treatment with macrophage derived IL-1 combined with the local administration of C. parvum, a marked enhancement in rechallenge survival rates (53.5 percent) was seen in IL-1 treated animals. A significantly lower percentage of animals (20.5) which received C. parvum therapy without IL-1 survived a similar rechallenge. Surviving animals that received only IL-1 therapy following the initial tumor challenge were also rechallenged. These animals demonstrated a rechallenge survival rate statistically similar to animals which received C. parvum therapy alone. Therefore, it appears that macrophage derived IL-1 was able to enhance rechallenge survival only in conjunction with local C. parvum therapy.

In similar experiments utilizing murine recombinant IL-1, a very high proportion of animals survived which received the EL-4

tumor challenge admixed with C. parvum, whether they received the murine recombinant IL-1 or the P388D1A culture supernatants. Upon rechallenge of survivors from each treatment group there was again an increase in survival rates among mice that had received IL-1 when compared to mice given the control P388D1A culture supernatants. Although the difference in survival rates between these two animal treatment groups was not statistically significant at $p < 0.05$, the prolonged survival curve produced by the recombinant IL-1 treated mice indicated there was an antitumor effect produced by the recombinant molecule. Statistical analysis verified the observed enhancement of survival duration ($p < 0.01$) in the recombinant IL-1 treated mice. Survival curves, shown in figure 5, generated by rechallenged mice that received the control P388D1A culture supernatants were essentially identical to control animals that received an initial tumor challenge without therapy. It therefore appears that recombinant IL-1 therapy was effective in generating an antitumor response against the EL-4 rechallenge in conjunction with local C. parvum adjuvant therapy.

In experiments utilizing macrophage derived IL-1 no differences were apparent in survival rate of animals which received IL-1 alone or in conjunction with C. parvum when compared to the corresponding group of animals not receiving IL-1, nor did IL-1 extend the survival time of these animals. Only following a secondary tumor challenge was a significantly enhanced survival rate noted for IL-1 treated animals, whereas an enhanced survival duration was noted for animals treated with recombinant IL-1. This observed antitumor

activity noted in IL-1 treated animals following the second tumor challenge was associated with a marked increase in the number of lymphocytes in the lymph nodes draining the site of the tumor challenge. The altered lymphocyte distribution seen in the lymph nodes draining the site of the EL-4 rechallenge of IL-1 treated animals, suggests that these lymph nodes may well have harbored lymphoid cells responding to the EL-4 tumor.

EL-4 specific serum antibody titer was the initial immunological parameter assessed in a series of in vitro experiments. Under the conditions of the experiments performed in this study, there did not appear to be any production of detectable antibody against the EL-4 lymphoma in IL-1 treated animals within the first several weeks following the tumor challenge. IL-1 treated mice considered cured of the primary EL-4 challenge also failed to elicit a detectable EL-4 specific antibody response 35 days after the tumor challenge.

Unlike man, the range of target cells susceptible to lysis by allogeneic leukocytes when sensitized with antibody is very restricted. Murine target cells sensitized with anti-IA antibody are generally unharmed by mouse leukocytes, as determined by the chromium release assay, although the possibility of long-term damage has not been ruled out (19). The lack of any significant IL-1 induced antibody titer against the EL-4 lymphoma, combined with the generally poor sensitivity of murine cells to antibody dependent cellular cytotoxicity (19), makes this an unlikely mechanism accounting for the anti-tumor activity associated with IL-1 treatment.

EL-4 specific T cell mediated cytotoxicity, was also shown not to be enhanced by the administration of IL-1. The induction of cytotoxic T cells in the lymph nodes and spleens of mice treated with or without IL-1 was assessed in this system, following either the primary tumor challenge or after animals cured of the initial challenge received a secondary tumor challenge. Cytotoxic T cells directed against the EL-4 lymphoma producing cytotoxic activity greater than one L.U. was not observed in any of the treatment protocol animals including the animals given IL-1. This data, taken together with the observed lack of augmented humoral immunity in IL-1 treated mice, suggests that the augmentation of antitumor activity seen in these animals may have resulted from the generation of nonspecific anti-tumor effector mechanisms.

Although recent evidence indicates that IL-1 may enhance NK activity of large granular lymphocytes (LGLs) (115,239), there was no apparent increase in NK activity after the primary tumor challenge. These results were seen for both spleen and lymph node cell populations in C. parvum treated animals given either macrophage derived or recombinant IL-1, when compared to animals which received EL-4 alone or EL-4 admixed with C. parvum. Splenic NK activity observed for each of the various treatment groups of mice following the primary tumor challenge, as expressed in lytic units, appeared to fluctuate from day to day. However, the results for the different treatment groups within each day's experiment showed less fluctuation with no pattern of any significant increased or decreased levels of NK activity for IL-1 treated animals. Very low levels of NK activity (less

than one L.U.) were noted for lymph node cell populations of C. parvum plus IL-1 treated animals as well as control animals that received EL-4 alone or EL-4 admixed with C. parvum. Thus, IL-1 treatment did not appear to augment the NK responsiveness of mice after the initial challenge. This is not surprising considering the lack of enhanced survival of IL-1 treated mice following the initial tumor challenge.

Assessment of spleen and lymph node cell NK activity, following the EL-4 rechallenge of animals treated with local C. parvum therapy plus either recombinant IL-1 or macrophage derived IL-1, demonstrated very low levels of NK activity. Control animals which received C. parvum therapy plus HSA injections showed similarly poor NK responsiveness. The lack of IL-1 enhanced NK activity may have been due to the low dosage of IL-1 utilized in this system. More recent evidence suggests that BFM-induced increases in NK activity in other organs such as the liver is partially due to an increase in IGL cell numbers which occurs by a combination of IGL proliferation and an influx of IGLs via the blood (32,125,179). Further investigation has suggested that the hyporesponsiveness of NK activity which is induced in the blood and spleen by repeated administration of some BFM, may be partially due to a redistribution or diversion of NK cells from those sites (288). Therefore, the possibility remains that IL-1 treatment may indeed have increased NK activity in this system, but at sites not relevant to those tested in this dissertation research, e.g., the localized lymph nodes draining the tumor site and the spleen.

Splenic populations from EL-4 rechallenged mice cured of the initial tumor challenge with the combination C. parvum plus either recombinant or macrophage derived P388D1Y IL-1 therapy were incubated in the presence of EL-4 stimulator cells, and assayed for proliferative responsiveness to the tumor. No proliferative responses were noted in splenic populations obtained from mice prior to the EL-4 tumor rechallenge. However, lympho-proliferation did occur in spleen cell taken from mice several days after the second exposure to tumor antigens for the second time. Proliferative responsiveness to the tumor was observed in spleen cell taken from mice up to six days after the EL-4 rechallenge. The same spleen cell populations from IL-1 treated animals which responded to the EL-4 stimulator cells did not respond to the inappropriate YAC-1 stimulator cells. Similarly rechallenged control animals which survived the initial EL-4 challenge with the aid of C. parvum therapy in combination with HSA injections were unable to respond as strongly to the EL-4 cells three days after the rechallenge, and not at all six days after the second tumor challenge. Spleen cells from these animals were likewise not able to respond to the YAC-1 stimulator cells.

This IL-1 augmented proliferative response may well be a significant indicator pointing to the role of IL-1 in the capacity as an antitumor agent (240). Studies in other syngeneic tumor model systems have shown strong correlations between acquisition of cutaneous delayed-type hypersensitivity reactivity, macrophage migration inhibition, and lymphocyte blastogenesis to tumor antigens (194). Along with the good correlation between these activities, the responses

were shown to be tumor-line specific and dose dependent (194). Therefore, this enhanced splenic response observed in IL-1 treated animals points to a possible tumor specific response capable of augmenting a variety of antitumor mechanisms. These responses may be initiated by migratory inhibition (170) and activation of monocytes, leading to enhanced antigen presentation. An enhanced presentation of EL-4 antigen may in turn lead to the induction of IL-2 or gamma-interferon. These BRMs are both capable of anti-tumor activity, and further activating immune processes via the lymphokine cascade (83).

The generation of an enhanced tumor specific response to the EL-4 lymphoma in the spleens of IL-1 treated animals which received a second tumor challenge indicates earlier IL-1 therapy at the time of the initial challenge may have induced systemic modulation of the immune system. Lack of proliferative responsiveness after the initial challenge may have been due to the very low immunogenicity of the EL-4 tumor (183,190). There is also the possibility that a tumor induced suppressive state, as seen in other systems (79,224) may have developed in these mice, thereby not allowing the detection of activated cells potentially responsive to the tumor. The lack of any detectable in vitro augmentation of tumor specific effector functions in lymphoid populations of IL-1 treated animals, along with the enhanced proliferative response noted in IL-1 treated, EL-4 rechallenged animals, suggests that the EL-4 responsive lymphoid cells may be enhancing another antitumor mechanism. Since the tumor specific proliferative responses noted in spleens of IL-1 treated mice failed to generate any detectable enhanced tumor specific effector cell

functions, the enhanced survival suggests that IL-1 may be modulating the immune system leading to a nonspecific mechanism of tumor control by the murine host. Recent studies have shown that lymphokine activated killer cells (IAK) are capable of destroying a wide spectrum of murine and human tumor cell lines in vivo and in vitro (81). The in vitro development of IAK cells requires the incubation of lymphoid cells in the presence of IL-2 (105), therefore, this type of activity would not have been detected by in vitro assessments in this study. IL-1 treated mice demonstrated an enhanced lymphoid proliferative response in the spleen, an area well separated from the site of the tumor challenge. This activation following a secondary exposure to the EL-4 tumor strongly implies lymphoid organ production of IL-2, distant from the site of tumor challenge. It has been demonstrated in mice that IL-2 administration results in the in vivo generation of IAK activity (215). Thus the likely augmentation of IL-2 production following a second exposure to the tumor at a site distant from the tumor suggests a generalized response, possibly leading to the induction of IAK cell activity. Since induction of IAK activity is associated with lymphocyte proliferation, this greater number of lymphocytes seen in the lymph nodes draining the site of the tumor challenge indicates possible IL-2 mediated proliferation and induction of IAK activity. Although this mechanism of tumor cell killing is not tumor specific in nature, antigen induction of IL-2 may well have resulted in an antigen specific manner, as indicated by the in vitro tumor specific splenic proliferation seen in IL-1 treated mice after the EL-4 rechallenge.

The lack of an enhanced proliferative response by lymph node cells obtained from IL-1 treated mice following the secondary tumor challenge may have been a result of low percentage of macrophages detected in the regional lymph node cell populations following the i.d. injection of C. parvum. Without sufficient numbers of macrophages in the in vitro incubation culture, the modest proliferative responses noted in the splenic cultures may not occur due to the lack of a critical number of antigen presenting cells in the lymph node cultures. There is also the possibility that a high proportion of EL-4 reactive lymphoid cells responsible for memory induction to the tumor may have migrated from the tumor site to the spleen following the primary tumor challenge. The possibility also exists that the massive trapping of lymphocytes in the regional lymph nodes due to the C. parvum injection may have diluted EL-4 reactive lymphocytes to the point that they would not be detected in the thymidine incorporation assay at the cell concentration utilized. This concept also leads to the possibility that an elongated time frame of testing after the secondary tumor challenge may have allowed the EL-4 reactive cells to proliferate to the point of detection.

Speculation as to why splenic proliferative responsiveness was seen in C. parvum plus IL-1 treated mice and not in mice given only C. parvum may partially be explained by the events seen in other tumor models. Many reports have shown that suppressor T cells are generated in response to growth of a variety of immunogenic tumors (220). In the P-815 tumor system studied by Robert North, mice develop Ly 1⁻²⁺ T cells capable of tumor specific lysis of P-815 target

cells in vitro, and also capable of regressing a P-815 tumor in the early stages of development established in gamma-irradiated mice (224). The development of this early cytolytic response quickly abates as a consequence of the progressive development of Ly 1⁺2⁻ suppressor T cells which were capable of inhibiting the expression of passively transferred immunity against an established. Because the EL-4 lymphoma is regarded as a weakly immunogenic tumor, the time required to generate a detectable immune response may be prolonged such that the development of suppressor T cells following the primary challenge may prevent the induction of a response sufficient to allow the development of immunological memory. However, IL-1 treated animals which survived the primary EL-4 challenge were able to generate a moderate in vitro splenic proliferative response to EL-4 stimulator cells soon after a second tumor challenge, suggesting these mice had responded to the tumor during the primary challenge and developed memory as indicated by the splenic proliferation to EL-4 tumor targets following the second tumor challenge. Evidence indicating IL-1 as an up regulator for T helper cells (T_H) in vitro while inhibiting the generation of T suppressor cells (T_S) (75,78) suggests this mechanism of action may account for the proliferative activity seen in the IL-1 treated mice. Whether (T_H) is directly enhanced or (T_S) is inhibited allowing greater expression of (T_H) has not been elucidated. The ability of IL-1 treated animals in this study to proliferate in response to EL-4 targets following the second tumor challenge indicates IL-1 may have enhanced the (T_H) population responding to the tumor at the time of the initial challenge or

inhibited the generation of (T_S) to a degree, thus allowing the development of memory to the tumor. Upon rechallenge the IL-1 treated animals were then able to generate a proliferative response sooner and with greater intensity than animals not given IL-1 or following a primary challenge. The results of splenic proliferation following the primary and secondary tumor challenge distinctly show the type of pattern one would expect if this mechanistic scenario was followed. The IL-1 treated animals demonstrated a stronger and more prolonged response to EL-4 targets following the secondary tumor challenge, while the mice not treated with IL-1 showed a significantly weaker and shorter response.

In vivo antitumor effects of human recombinant IL-1 alpha, without the aid of other corresponding adjuvant therapy have been reported against several immunogenic murine tumor cell lines (33). An intra-tumor (i.t.) single injection of a very large dose of IL-1 (2.5×10^5 units) was required to successfully regress a seven day old palpable Meth A sarcoma tumor. Intramuscular (i.m.) and i.v. therapy proved less successful, with protective results attained only following seven daily injections at 2.5×10^5 units of IL-1 per injection. Intravenous therapy at even higher doses did not lead to protection against the Meth A tumor. Despite the large IL-1 doses employed by these investigators, they surprisingly reported no sign of macroscopic inflammation at the site of the injections. Similarly high, i.t., doses of IL-1 afforded mice protection against seven day old B16 melanoma tumors, while even higher i.v. and i.m. IL-1 doses showed no protective efficacy. Several conceivable mechanisms may be

responsible for the high efficacy by the i.t. route. IL-1 may: have direct cytotoxic effects against the tumor cells, act directly on the tumor cells so as to facilitate cytolysis by IGLs, act as a chemotactic factor for monocytes, and/or augment monocyte tumoricidal activity (240).

IL-1 has been demonstrated to have in vitro cytostatic as well as cytotoxic (168,235) effects on several transformed cell lines, suggesting a possible direct in vivo therapeutic effect against tumor development. Direct in vitro cytolysis of A375 human melanoma and I929 murine fibroblast cell lines was detected with recombinant IL-1 concentrations as low as 0.125 units of IL-1 per ml, with lytic activity peaking at between one and eight units per ml. The minimal cytostatic IL-1 dose for the same cell lines was 0.5 units per ml, with the effect peaking at between two to four units/ml. The IL-1 serum levels required to achieve in vitro inhibitory effects may well have been reached with the in vivo IL-1 protocols used in this study, considering that 10 units of IL-1 were administered per mouse per injection. However, at these relative recombinant IL-1 doses, between 1 to 12.5 units per ml, no significant in vitro proliferative inhibitory activity was observed against EL-4 lymphoma cells by recombinant murine IL-1. Nor was any enhancement of proliferative activity noted by the EL-4 cells. The maximal in vitro IL-1 dosages attained in these microcultures were greater than the individual dosages injected into mice receiving IL-1 therapy. Similar results were obtain for Meth A tumor cell in vitro resistance to IL-1. These cells were also not susceptible to in vitro incubation with high

doses of the human recombinant IL-1 alpha (M. Yamada, personal communication). Hence, IL-1 did not demonstrate any direct in vitro antitumor activity against the EL-4 lymphoma utilized in this study or against tumor successfully treated in other work.

As shown in figures 4 and 6 respectively, neither recombinant or P388D1Y macrophage derived IL-1 therapy, without protective C. parvum therapy, was effective at suppressing EL-4 tumor progression leading to animal death. Taken together with the in vitro results, it appears unlikely that in vivo administered IL-1 had any direct antitumor effect upon the intradermally injected EL-4 tumor cells used in this study.

IL-1 has also been reported to have the capacity to enhance monocyte tumoricidal activity via a PGE₂ mediated mechanism (240). IL-1 apparently augments the tumoricidal activity of monocytes by enhancing the production of PGE₂, which in turn is responsible for monocyte induction of cytolytic activity (236). Since i.t. injection of IL-1 was the more successful route of administration in the Meth A and B16 models, and IL-1 did not inhibit these cell lines at high in vitro concentrations, this IL-1 mediated mechanism might better explain the observed antitumor effect. The monocyte chemoattractant ability of IL-1 might potentiate this mechanism against the tumors, simply by increasing the number of tumoricidal cells at the tumor site. However, as previously discussed for the direct IL-1 antitumor activity of IL-1, this mechanism also appears unlikely to have been an important factor contributing to the protection of C57Bl/6 mice against the EL-4 tumor. This assumption can be reached for several

reasons. Firstly, IL-1 therapy was ineffective against the primary EL-4 tumor challenge, the time at which this mechanism would most likely be effective against the tumor. Secondly, IL-1 was injected i.p. in protection protocols against the EL-4 tumor, therefore, there is little reason to suspect these injections would cause an increase in monocyte/macrophage cell numbers at the tumor site.

The ability of recombinant IL-1 alpha to protect against the weakly immunogenic colon 26 adenocarcinoma when treated with similar doses of recombinant IL-1 therapy (219) was not as dramatic as that mentioned for the immunogenic Meth A. Although a significant decrease in tumor mass was noted twenty-one days following the tumor challenge, tumor growth in these animals accelerated, eventually causing death in these animals at approximately the same time as the untreated control animals. Therefore, IL-1 was unable to cure animals of a non-immunogenic tumor.

Following IL-1 treatment, mice cured of the initial Meth A sarcoma were able to reject a rechallenge with the same tumor. In view of the concurrent development of immunity in mice protected against immunogenic tumors, it is possible to suggest that IL-1 modulated tumor specific mechanism/s lead to the destruction of immunogenic tumors. Although these authors have suggested that IL-1 is working in their system by indirect antitumor mechanisms, no in vitro assessments of immunological parameters associated with anti-tumor activity have been conducted to verify these assumptions. Also the IL-1 dosages required to obtain effective cure ratios in their system were only three to four fold under the ID₅₀ dose for naive

mice, suggesting the development of in vivo cellular toxic effects resulting from these doses (19). Furthermore, specificity of this tumor immunity was not determined, since surviving animals were not rechallenged with a tumor cell line other than that used for the primary challenge (M. Yamada, personal communication). Therefore, the mechanism of IL-1 mediated antitumor effects at these large doses have yet to be explained.

Although in vivo tumor protection studies showed that the murine recombinant IL-1 statistically enhanced the survival duration of EL-4 rechallenged mice, the increased survival rates observed for IL-1 treated animals when compared to non-IL-1 treated animals was not sufficient to determine a statistical difference. There are several possible explanations for not observing statistical differences in survival rates in these in vivo recombinant IL-1 protection studies.

The possibility that the recombinant molecule is not as biologically active as the macrophage derived IL-1 employed for study in this dissertation research. The recent purification of recombinant forms of IL-1 has stirred speculation about the similarities and dissimilarities demonstrated by monocyte or biologically derived and recombinant IL-1. The biological activity of the murine recombinant IL-1 isolated by Peter Lomedico (the same source utilized for the in vivo studies presented in this dissertation) has been reported to be pyrogenic, causes hypozincemia and leukocytosis, and demonstrates in vitro IAF activity (219). However, the same authors found that recombinant IL-1 alpha was not capable of increasing energy expend-

iture or protein catabolism or anabolism. The recombinant form of IL-1 also failed to increase the acute phase proteins (291). The pyrogenic effect of the murine recombinant IL-1 was also found to be delayed in onset, although, the effect was eventually as potent as the monocyte derived IL-1 (282). Therefore, it appears that the recombinant molecule is not identical to the monocyte derived IL-1 whose biological activities have been extensively outlined in the past. It is very possible that the recombinant IL-1 molecule represents a pure protein expressed by one gene while the monocyte derived IL-1 may represent a family of proteins each responsible for separate of overlapping biological activities. If this is the case, it is possible that the antitumor effects of host or macrophage derived IL-1 may act through more than one specific mechanism, and that these effects may lead to additive or synergistic antitumor activity.

When working with a recombinant molecule, there is also the possibility that lower level of activity associated with that recombinant molecule is due to partial denaturation or incorrect renaturation. This does seem unlikely, however, since the recombinant molecule does retain many of the biological activities associated with biologically derived IL-1.

Another precaution which must be assessed when using recombinant materials derived from E. coli is the potential of endotoxin contamination or reactivity to the recombinant protein storage buffer (in this case guanidine hydrochloride). In vivo analysis of reactivity to a similar concentration of a nonrecombinant IL-1 recombinant protein, made in E. coli and purified in an identical manner to the

mouse recombinant IL-1, caused no significant increase in any of the biological parameters assessed in murine systems (219). Similarly negative results were obtained when the guanidine hydrochloride buffer was also tested in this system. The recombinant protein and buffer concentration (0.008 M guanidine hydrochloride) tested in that system was over forty times greater than the concentrations which were injected with the recombinant IL-1 in experiments employed for this research. Therefore, the recombinant IL-1; and not potential contaminating endotoxin, bacterial proteins, or buffering reagents; appear to be responsible for the enhanced antitumor activity observed in this system.

Although recombinant IL-1 appeared to be less active in this system according to statistical analysis of survival rates, there is the possibility that other biological response modifiers in the macrophage derived IL-1 samples may be involved in producing additive or synergistic antitumor effects. The evidence produced in this research does not support the idea of additive antitumor effects of other potential BRMs in the macrophage derived IL-1 containing culture supernatants, since no antitumor response was reflected in the survival data. Control animals treated systemically with unstimulated macrophage culture supernatants died following EL-4 rechallenge while a significant number of animals that received IL-1 treatment were able to survive. Furthermore, the survival curve generated for these control animals was no different from animals that received a primary EL-4 tumor challenge without the aid of any therapy, suggesting that other BRMs in the macrophage derived culture supernatants

were solely responsible for the augmented antitumor effects.

These results do not eliminate the possibility of synergistic effects by IL-1 and other BRMs produced by the P388D1 macrophage cell line. Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF), a macrophage product, has been found to be produced by cell lines established from myelomonocytic leukemias (175). It was not surprising when work completed in Rita Young's laboratory demonstrated that CSF was found to be in the P388D1Y, IL-1 containing culture supernatants (Rita Young, personal communication, data not shown). The ability of this BRM to stimulate cell division, and ultimately the number of functionally mature granulocytes and macrophages available to the host for effector or accessory cell function, may allow a more effective response to occur in response to the EL-4 tumor challenge. This is a distinct possibility and, if true, may account for the significantly augmented survival rates not seen solely with the murine recombinant material. There is also the possibility that further experimentation with larger groups of animals may allow the expression of statistical differences in survival rates between the different treatment groups which were not apparent in these studies.

An alternative or synergistic mechanism of action which may help account for the enhanced IL-1 augmentation of immunity in this tumor system revolves around the observation that IL-1 administration elicits radioprotective capabilities in the murine system (222). Many bacterial agents, such as BCG, LPS, muramyl dipeptide and glucan have radioprotective effects and are potent inducers of IL-1, known to elicit radioprotective effects if delivered before irradiation

(314). Although the exact basis for this protective effect has not been clearly established, it has been postulated that these effects are a result of hemopoietic enhancement by IL-1, since previous work has indicated that cells in the late S phase of the cell cycle are more resistant to irradiation damage (279). The capacity of IL-1 to initiate the induction of the a cascade of lymphokines such as CSF and IL-2 may lead to the development of various lymphoid cells by inducing hematopoietic progenitor cell populations into the S phase of the cell cycle, thereby increasing the number of potential anti-tumor reactive lymphocytes or monocytes. As described earlier, macrophage derived IL-1 was more effective in protecting mice from the second tumor challenge. This effect may have resulted from synergistic activities of IL-1 with other lymphokines associated with macrophage conditioned medium. As note earlier, CSF was shown to be associated with the macrophage derived IL-1. The synergistic activity between these two monokines may have resulted in mobilization and development of either LAK effector cell or EL-4 reactive helper T cell populations, leading to an enhanced level of protection noted in the macrophage IL-1 experimentation. In contrast, the recombinant IL-1 without the associated CSF or other macrophage monokines was only able to prolong survival duration while the macrophage product was able to enhance survival. If indeed, IL-1 and other macrophage products acted synergistically against the EL-4 tumor, IL-1 appeared to be the main factor in this activation process. This is apparent, since mice which received P388D1A macrophage conditioned culture

medium containing CSF, but, little or no IL-1 demonstrated no anti-tumor activity, while purified IL-1 did prolong survival.

In view of the results obtained for this dissertation it is apparent that IL-1 is capable of modulating the tumor immune response generated against the weakly immunogenic EL-4 lymphoma, in a system where no previous protective response had been seen against a secondary tumor challenge. Although IL-1 had no discernable in vivo protective effect against the primary EL-4 tumor challenge, IL-1 was able to modulate the response to the tumor, enabling an enhanced population of mice to respond more successfully against a second challenge. This IL-1 induced antitumor activity was effective only when IL-1 therapy was provided in conjunction with the protective C. parvum adjuvant therapy. The enhanced protective effect observed against the primary EL-4 challenge appears to be a result of C. parvum mediated augmentation of macrophage tumoricidal activity (162). The enhanced number of lymphocytes observed in the regional lymph nodes of IL-1 treated animals following the second EL-4 tumor challenge, strongly suggests that the lymphoid population, originally recruited to the lymph nodes draining the tumor site by the subcutaneous adjuvant injection (201), were modulated by IL-1. IL-1's immunoadjuvant activity (285) may have allowed the murine lymphocytes to respond to the tumor antigens more rapidly and effectively before antigen removal. At the same time IL-1's ability to induce hematopoietic progenitor cells (222) may have also increased the number of potential lymphoid effector cells available for recruitment to the tumor site. The EL-4 specific splenic proliferation seen in IL-1

treated animals again suggests lymphocytic involvement, possibly in effector and/or helper capacity. The lack of detectable EL-4 specific humoral or cellular antitumor mechanisms such as, augmented serum antibody titers or T cell cytotoxicity against the EL-4 lymphoma, suggests the potentiation of non-specific antitumor mechanisms by IL-1. No detectable increase in NK activity, was noted in IL-1 treated animals. However, an alternate non-specific mechanism which would be an effective means of destroying the EL-4 tumor is the development of LAK cells, which demonstrate in vitro lytic activity against an extremely wide range of tumors (81). The splenic proliferative response to the EL-4 tumor strongly suggests splenic and possibly systemic lymphocytic production of IL-2 (83,140), the BRM necessary to generate effective LAK activity in vivo (105). The largest and most prolonged proliferative response to EL-4 following the secondary tumor challenge was seen in IL-1 treated animals, suggesting the possible induction of this effector mechanism in IL-1 treated mice. Therefore, the IL-1 treated mice would convert the enhanced tumor specific proliferative response to the non-specific, LAK mechanism, for tumor eradication.

SUMMARY

Although interleukin 1 (IL-1) has been shown to function as an immunological adjuvant in defined antigenic systems, there is little evidence suggesting a protective role for IL-1 in neoplastic diseases. This investigation demonstrates that intraperitoneal (i.p.) injection of macrophage derived IL-1 significantly enhanced immunity of C57Bl/6 mice to the weakly immunogenic EL-4 lymphoma. Mice given an intradermal injection of the EL-4 lymphoma admixed with C. parvum showed a high survival rate. Animals identically challenged, but given i.p. macrophage derived IL-1 (100 units/animal) showed similar survival. However, upon subsequent rechallenge of these mice with the EL-4 lymphoma alone, the group given IL-1 demonstrated a markedly enhanced survival rate ($p < 0.01$) compared to the group not given IL-1. Mice given murine recombinant IL-1 in conjunction with C. parvum, demonstrated a statistically enhanced survival duration following a secondary tumor challenge ($p < 0.02$) when compared to animals given C. parvum without IL-1. Survival rates of mice challenged with the EL-4 tumor and administered only i.p. IL-1 (macrophage derived and murine recombinant) showed no statistical difference to animals challenged with the tumor alone. These results strongly suggest that IL-1 may function to augment tumor immunity when administered systemically, in conjunction with local administration of C. parvum.

In vitro assessment of EL-4 specific antibody titer, natural killer cell activity, and EL-4 specific cytotoxic T cell activity of IL-1 and non-IL-1 treated mice demonstrated no detectable IL-1 induced augmentation of these humoral or cellular effector cell functions.

A marked increase in lymph node cell numbers obtained from lymph nodes draining the tumor challenge site of IL-1 treated mice following the secondary tumor challenge, suggested a lymphoid cell migration or a lymphoproliferative response in these lymph nodes following the second EL-4 tumor challenge. Both macrophage derived ($p < 0.001$) or murine recombinant ($p < 0.05$) IL-1 treated mice also demonstrated an enhanced EL-4 specific lymphoproliferative responsiveness in their splenic cell populations. This IL-1 induced, splenic proliferative response, combined with the lack of any observed IL-1 induced tumor specific effector cell response, strongly suggests an IL-1 enhanced tumor specific augmentation of a non-tumor specific mechanism of tumor killing .

REFERENCES

1. Aarden, L. A., Brunner, T. K., Cerottini, J-C., Dayer, J-M., de Weck, A. L., Dinarello, C. A., Di Sabato, G., Farrar, J. J., Gery, I., Gillis, S., Handschumacher, R. E., Henney, C. S., Hoffmann, M. K., Koopman, W. J., Krane, S. M., Lachman, L. B., Lefkowitz, I., Mishell, R. I., Mizel, S. B., Oppenheim, J. J., Paetkau, V., Plate, J., Rollinghoff, M., Rosenstreich, D., Rosenthal, A. S., Rosenwasser, L. J., Schimpl, A., Shim, H. S., Simon, P. L., Smith, K. A., Wagner, H., Watson, J. D., Wecker, E., and Wood, D. D. 1979. Revised nomenclature for antigen-nonspecific T cell proliferation and helper factors (letter). *J. Immunol.* 123:2928-2929.
2. Adams, D. O., Kao, K-J., Farb, R., and Pizzo, S. V. 1980. Effector mechanism of cytolytically activated macrophages. II. Secretion of a cytolytic factor by activated macrophages and its relationship to secreted neutral proteases. *J. Immunol.* 124:293-300.
3. Adlam, C., Broughton, E. S., and Scott, M. T. 1972. Enhanced resistance of mice to infection with bacteria following pre-treatment with Corynebacterium parvum. *Nature, New Biol.* Vol. 235, No. 59, 219-220.
4. Adlam, C., Reid, D. E., and Torkington, P. 1975. The nature of the active principle of Corynebacterium parvum. In: Halpern, B., ed., Corynebacterium parvum and its application in experimental and clinical oncology. Plenum Press, New York. Pages 33-39.
5. Adlam, C., and Scott, M. T. 1973. Lympho-reticular stimulatory properties of Corynebacterium parvum and related bacteria. *J. Med. Microbiol.* 6:261-274.
6. Arnold, A., Lipkowitz, S., Suthanthiran, M., Novogrodsky, A., and Stenzel, K. H. 1985. Human B lymphoblastoid cell lines provide an interleukin 1-like signal for mitogen-treated T lymphocytes via direct cell contact. *J. Immunol.* 134:3876-3881.
7. Asherson, G. L., and Allwood, G. G. 1971. Depression of delayed hypersensitivity by pretreatment with freund-type adjuvants. I. Description of the phenomenon. *Clin. Exp. Immunol.* 9:249-258.
8. Ashwell, J. D., DeFranco, A. L., Paul, W. E., and Schwartz, R. H. 1984. Antigen presentation by resting B cells.

Radiosensitivity of the antigen-presentation function and two distinct pathways of T cell activation. *J. Exp. Med.* 159:881-905.

9. Atkins, E. 1960. Pathogenesis of fever. *Physiol. Rev.* 40:580-646.
10. Atkins, E., Bodel, P., and Francis, L. 1967. Release of an endogenous pyrogen in vitro from rabbit mononuclear cells. *J. Exp. Med.* 126:357-384.
11. Auron, P. E., Rosenwasser, L. J., Matsushima, K., Copeland, T., Dinarello, C. A., Oppenheim, J. J., and Webb, A. C. 1985. Human and murine interleukin 1 possess sequence and structural similarities. *J. Mol. Cell. Immunol.* 2:169-176.
12. Auron, P. E., Rosenwasser, L. J., Matsushima, K., Copeland, T., Dinarello, C. A., Oppenheim, J. J., and Webb, A. C. 1985. Human and murine interleukin 1 possess sequence and structural similarities. Addendum in response to reviewer's comments. *J. Mol. Cell. Immunol.* 2:176-177.
13. Auron, P. E., Webb, A. C., Rosenwasser, L. J., Mucci, S. F., Rich, A., Wolff, S. M., and Dinarello, C. A. 1984. Nucleotide sequence of human monocytic interleukin 1 precursor cDNA. *Proc. Natl. Acad. Sci. U.S.A.* 81:7907-7911.
14. Baracos, V., Rodemann, H. P., Dinarello, C. A., and Goldberg, A. L. 1983. Stimulation of muscle protein degradation and prostaglandin E₂ release by leukocyte pyrogen (interleukin-1). A mechanism for the increased degradation of muscle proteins during fever. *N. Engl. J. Med.* 308:553-558.
15. Barski, G., and Youn, J. K. 1969. Evolution of cell-mediated immunity in mice bearing an antigenic tumor. Influence of tumor growth and surgical removal. *J. Natl. Cancer Inst.* 43:111-120.
16. Basic, I., Milas, L., Grdina, D. J., and Withers, H. R. 1975. In vitro destruction of tumor cells by macrophages from mice treated with Corynebacterium granulosum. *J. Natl. Cancer Inst.* 55:589-596.
17. Bast, R. C., Jr., and Bast, B. S. 1976. Critical review of previously reported animal studies of tumor immunotherapy with nonspecific immunostimulants. *Ann. NY Acad. Sci.* 277:60-93.
18. Baum, M., and Breese, M. 1976. Antitumour effect of Corynebacterium parvum. Possible mode of action. *Br. J. Cancer* 33:468-473.

19. Bean, M. A., Bloom, R. B., Cerottini, J-C., David, J. A., Herberman, R. B., Lawrence, H. S., MacLennan, I. C. M., Perlmann, P., and Stutman, O. 1976. Evaluation of in vitro methods for assaying tumor immunity. In: Bloom, B. R. and David, J. R., eds., In Vitro Methods in Cell-Mediated and Tumor Immunity, Academic Press, New York. Pages 27-65.
20. Beeson, P. B. 1948. Temperature-elevating effect of a substance obtained from polymorphonuclear leukocytes. *J. Clin. Invest.* [abstract] 27:524.
21. Beller, D. I. 1984. Functional significance of the regulation of macrophage Ia expression. *Eur. J. Immunol.* 14:138-143.
22. Bernheim, H. A., Block, L. H., Francis, L., and Atkins, E. 1980. Release of endogenous pyrogen activating factor from concanavalin A-stimulated human lymphocytes. *J. Exp. Med.* 152:1811-1816.
23. Bernheim, H. A., Gilbert, T. M., and Stitt, J. T. 1980. Prostaglandin E levels in third ventricular cerebrospinal fluid of rabbits during fever and changes in body temperature. *J. Physiol.* 301:69-78.
24. Biozzi, G., Stiffel, C., Mouton, D., Bouthillier, Y., and Decreusefond, C. 1968. A kinetic study of antibody producing cells in the spleen of mice immunized intravenously with sheep erythrocytes. *Immunology* 14:7-20.
25. Biozzi, G., Stiffel, C., Mouton, D., Liacopoulos-Briot, M., Decreusefond, C., and Bouthillier, Y. 1966. Etude du phenomene de l'immunocyto-adherence au cours de l'immunisation. *Ann. Inst. Pasteur, Paris* 110, suppl. 1:27-32.
26. Blobel, G, and Dobberstein, B. 1975. Transfer of proteins across membranes. II. Reconstitution of functional rough microsomes from heterologous components. *J. Cell Biol.* 67:852-862.
27. Blyden, G., and Handschumacher, R. E. 1977. Purification and properties of human lymphocyte activating factor (LAF). *J. Immunol.* 118:1631-1638.
28. Bomford, R. 1975. Active specific immunotherapy of mouse methylcholanthrene induced tumours with Corynebacterium parvum and irradiated tumour cells. *Br. J. Cancer* 32:551-557.
29. Bomford, R., and Christie, G. H. 1975. Mechanisms of macrophage activation by Corynebacterium parvum. II. In vivo experiments. *Cell. Immunol.* 17:150-155.

30. Bomford, R., and Olivotto, M. 1974. The mechanism of inhibition of Corynebacterium parvum of the growth of lung nodules from intravenously injected tumour cells. *Int. J. Cancer* 14:226-235.
31. Boyse, E. A., Miyazawa, M., Aoki, T., and Old, L. J. 1968. Ly-A and Ly-B: two systems of lymphocyte isoantigens in the mouse. *Proc. R. Soc. London B170*:175-193.
32. Bruley-Rosset, M., and Rappaport, H. 1983. Natural killer cell activity and spontaneous development of lymphoma. Effects of single and multiple injections into young and aged C57Bl/6 mice. *Int. J. Cancer* 31:381-389.
33. Brunda, M. J., Mathews, H. L., Ferguson, H. R., McClatchy, J. K., and Minden, P. 1980. Immunotherapy of the guinea pig line 10 hepatocarcinoma with a variety of non viable bacteria. *Cancer Res.* 40:3211-3213.
34. Brunner, K. T., Mauel, J., Cerottini, J-C., and Chapuis, B. 1968. Quantitative assay of the lytic action of immune lymphoid cells on ⁵¹Cr-labelled allogeneic target cells in vitro; inhibition by isoantibody and by drugs. *Immunol.* 14:181-196.
35. Butler, T., Spagnuolo, P. J., Goldsmith, G. H., and Aikawa, M. 1982. Interaction of borrelia spirochetes with human mononuclear leukocytes causes production of leukocytic pyrogen and thromboplastin. *J. Lab. Clin. Med.* 99:709-721.
36. Cameron, P., Limjuco, G., Rodkey, J., Bennett, C., and Schmidt, J. A. 1985. Amino acid sequence analysis of human interleukin 1 (IL-1). Evidence for biochemically distinct forms of IL-1. *J. Exp. Med.* 162:790-801.
37. Campbell, P. A., Caldwell, J. E., and Hartman, A. L. 1979. Use of a macrophage cytotoxicity system to show macrophage activation by Listeria monocytogenes cell wall fraction. *Scand. J. Immunol.* 10:1-9.
38. Cantor, H., and Boyse, E. A. 1975. Functional subclasses of T lymphocytes bearing different Ly antigens. I. The generation of functionally distinct T-cell subclasses is a differentiative process independent of antigen. *J. Exp. Med.* 141:1376-1389.
39. Cantrell, J. L., McLaughlin, C. A., and Ribic, E. 1979. Efficacy of tumor cell extracts in immunotherapy of murine EL-4 leukemia. *Cancer Res.* 39:1159-1167.

40. Cantrell, J. L., and Wheat, R. W. 1979. Antitumor activity and lymphoreticular stimulation properties of fractions isolated from Corynebacterium parvum. *Cancer Res.* 39:3554-3563.
41. Carswell, E. A., Old, L. J., Kassel, R. L., Green, S., Fiore, N., and Williamson, B. D. 1975. An endotoxin-induced serum factor that causes necrosis of tumors. *Proc. Natl. Acad. Sci. U.S.A.* 72:3666-3670.
42. Castro, J. E. 1974. The effect of Corynebacterium parvum on the structure and function of the lymphoid system in mice. *Eur. J. Cancer* 10:115-120.
43. Cerottini, J-C., Engers, H. D., MacDonald, H. R., Brunner, K. T. 1974. Generation of cytotoxic T lymphocytes in vitro. I. Response of normal and immune mouse spleen cells in mixed leukocyte cultures. *J. Exp. Med.* 140:703-717.
44. Chao, P., Francis, L., and Atkins, E. 1977. The release of an endogenous pyrogen from guinea pig leukocytes in vitro. A new model for investigating the role of lymphocytes in fevers induced by antigens in hosts with delayed hypersensitivity. *J. Exp. Med.* 145:1288-1298.
45. Charon, J. A., Luger, T. A., Mergenhagen, S. E., and Oppenheim, J. J. 1982. Increased thymocyte-activating factor in human gingival fluid during gingival inflammation. *Infect. Immun.* 38:1190-1195.
46. Chenoweth, D. E., Goodman, M. G., and Weigle, W. O. 1982. Demonstration of a specific receptor for human C5a anaphylatoxin on murine macrophages. *J. Exp. Med.* 156:68-78.
47. Chestnut, R. W., and Grey, H. M. 1981. Studies on the capacity of B cells to serve as antigen-presenting cells. *J. Immunol.* 126:1075-1079.
48. Chu, E. T., Lareau, M., Rosenwasser, L. J., Dinarello, C. A., and Geha, R. S. 1985. Antigen presentation by EBV-B to resting T cells: role of interleukin 1. *J. Immunol.* 134:1676-1681.
49. Clowes, G. H. A., Jr., George, B. C., Vilee, C. A., Jr., and Saravis, C. A. 1983. Muscle proteolysis induced by a circulating peptide in patients with sepsis or trauma. *N. Engl. J. Med.* 308:545-552.
50. Coceani, F., Bishai, I., Dinarello, C. A., and Fitzpatrick, F. A. 1983. Prostaglandin E₂ and thromboxane B₂ in cerebrospinal fluid of afebrile and febrile cat. *Am. J. Physiol.* 244:R785-R793.

51. Collet, A. J. 1971. Experimental stimulation of alveolar macrophage production by Corynebacterium anaerobium and its quantitative evaluation. *J. Reticuloendothel. Soc.* 9:424-446.
52. Conley, F. K., and Remington, J. S. 1978. Effect of Corynebacterium parvum on tumor growth in the central nervous system of mice. *J. Natl. Cancer. Inst.* 61:827-832.
53. Conlon, P. J., Henney, C. S., and Gillis, S. 1982. Cytokine-dependent thymocyte responses: characterization of IL-1 and IL-2 target subpopulations and mechanism of action. *J. Immunol.* 128:797-801.
54. Crawford, G. D., Dinarello, C. A., Wyler, D. J., and Wolff, S. M. 1983. Interleukin-1 (IL-1) production and leishmaniasis. [abstract] *Clin. Res.* 31:360A.
55. Currie, G. A. 1978. Activated macrophages kill tumor cells by releasing arginase. *Nature* 273:758-759.
56. Dale, D. C., Fauci, A. S., Guerry, D., IV, and Wolff, S. M. 1975. Comparison of agents producing a neutrophilic leukocytosis in man. Hydrocortisone, prednisone, endotoxin, and etiocholanolone. *J. Clin. Invest.* 56:808-813.
57. Dawe, C. J., and Potter, M. 1957. Morphologic and biologic progression of a lymphoid neoplasm of the mouse in vivo and in vitro. *Am. J. Pathol.* [abstract] 33:603.
58. Dayer, J-M., Passwell, J. H., Schneeberger, E. E., and Krane, S. M. 1980. Interactions among rheumatoid synovial cells and monocyte-macrophages: production of collagenase-stimulating factor by human monocytes exposed to concanavalin A or immunoglobulin Fc fragments. *J. Immunol.* 124:1712-1720.
59. Dayhoff, M. O. 1978. Survey of new data and computer methods of analysis. In: Dayhoff, M. O., ed., Atlas of protein sequence and structure. National Biomedical Research Foundation, Washington, D.C. Vol. 5, Suppl. 3. Pages 1-8.
60. Del Guercio, P. 1972. Effect of adjuvants on the antibody response to a hapten on a thymus-independent carrier. *Nature* 238:213-214.
61. Dempsey, R. A., Dinarello, C. A., Mier, J. W., Rosenwasser, L. J., Allegretta, J., Brown, T. E., and Parkinson, D. R. 1982. The differential effects of human leukocyte pyrogen/lymphocyte-activating factor, T cell growth factor, and interferon on human natural killer activity. *J. Immunol.* 129:2504-2510.

62. Dimitrov, N. V., Andre, S., Eliopoulos, G., and Halpern, B. 1975. Effect of C. parvum on bone marrow cell cultures. Proc. Soc. Expl. Biol. & Med. 148:440-442.
63. Dinarello, C. A. 1981. Demonstration of a human pyrogen-inducing factor during mixed leukocyte reactions. J. Exp. Med. 153:1215-1224.
64. Dinarello, C. A. 1984. Interleukin-1. Rev. Infect. Dis. 6:51-95.
65. Dinarello, C. A. 1984. Interleukin-1 and the pathogenesis of the acute-phase response. N. Engl. J. Med. 311:1413-1418.
66. Dinarello, C. A., Bodel, P. T., and Atkins, E. 1968. The role of the liver in the production of fever and in pyrogenic tolerance. Trans. Assoc. Am. Physicians 81:334-344.
67. Dinarello, C. A., Clowes, G. H. A., Jr., Gordon, A. H., Saravis, C. A., and Wolff, S. M. 1984. Cleavage of human interleukin 1: isolation of a peptide fragment from plasma of febrile humans and activated monocytes. J. Immunol. 133:1332-1338.
68. Dinarello, C. A., Marnoy, S. O., and Rosenwasser, L. J. 1983. Role of arachidonate metabolism in the immunoregulatory function of human leukocytic pyrogen/lymphocyte-activating factor/interleukin 1. J. Immunol. 130:890-895.
69. Dinarello, C. A., and Wolff, S. M. 1982. Exogenous pyrogens. In: Milton A. S., ed., Pyretics and antipyretics. Springer-Verlag, Berlin. Pages 73-112.
70. Doe, W. F., and Henson, P. M. 1978. Macrophage stimulation by bacterial lipopolysaccharides. I. Cytolytic effect on tumor target cells. J. Exp. Med. 148:544-556.
71. Dower, S. K., Kronheim, S. R., March, C. J., Conlon, P. J., Hopp, T. P., Gillis, S., and Urdal, D. L. 1985. Detection and characterization of high affinity plasma membrane receptors for human interleukin 1. J. Exp. Med. 162:501-515.
72. Duff, G. W., and Atkins, E. 1982. The inhibitory effect of polymyxin B on endotoxin-induced endogenous pyrogen production. J. Immunol. Methods 52:333-340.
73. Duff, G. W., Atkins, E., and Malawista, S. E. 1983. The fever of gout: urate crystals activate endogenous pyrogen production from human and rabbit mononuclear phagocytes. Trans. Assoc. Am. Physicians 46:234-245.

74. Duff, G. W., Gekowski, K. M., and Atkins, E. 1982. Endogenous pyrogen production stimulated by immune complexes. [abstract] Clin. Res. 30:364A.
75. Durum, S. K., Eardley, D. D., Green, D. R., Yamauchi, K., Murphy, D. B., Cantor, H., and Gershon, R. K. 1980. Contr suppression, a newly characterized immunoregulatory circuit: the inducer and acceptor. [Abstract] Fed. Proc. 39:353.
76. Durum, S., and Gershon, R. K. 1982. Interleukin 1 can replace the requirement for I-A-positive cells in the proliferation of antigen-primed T cells. Proc. Natl. Acad. Sci. U.S.A. 79:4747-4750.
77. Durum, S. K., Higuchi, C., and Ron, Y. 1984. Accessory cells and T cell activation. The relationship between two components of macrophage accessory cell function: I-A and III. Immunobiol. 168:213-231.
78. Durum, S. K., Schmidt, J. A., and Oppenheim, J. J. 1985. Interleukin 1: an immunological perspective. Ann Rev. Immunol. 3:263-287.
79. Dye, E. S. and North, R. J. 1981. T cell-mediated immunosuppression as an obstacle to adoptive immunotherapy of the P815 mastocytoma and its metastases. J. Exp. Med. 154:1033-1042.
80. 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.
81. Ettinghausen, E. S., and Rosenberg, S. A. 1986. The adoptive immunotherapy of cancer using lymphokine activated killer cells and recombinant interleukin-2. Springer Semin. Immunopathol. 9:51-71.
82. Falkoff, R. J. M., Muraguchi, A., Hong, J-X., Butler, J. L., Dinarello, C. A., and Fauci, A. S. 1983. The effects of interleukin 1 on human B cell activation and proliferation. J. Immunol. 131:801-805.
83. Farrar, J. J., Benjamin, W. R., Hilfiker, M. L., Howard, M., Farrar, W. L., and Fuller-Farrar, J. 1982. The biochemistry, biology, and role of interleukin 2 in the induction of cytotoxic T cell and antibody-forming B cell responses. Immunol. Rev. 63:129-166.
84. Farrar, W. L., Mizel, S. B., 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.
85. Fidler, I. J., Barnes, Z., Fogler, W. E., Kirsh, R., Bugelski P., and Poste, G. 1982. Involvement of macrophages in the eradication of established metastases following intravenous injection of liposomes containing macrophage activators. *Cancer Res.* 42:496-501.
 86. Flexman, J. P., and Shellam, G. R. 1980. Factors affecting stimulation of natural cytotoxicity to a rat lymphoma by Corynebacterium parvum. *Br. J. Cancer* 42:41-51.
 87. Flynn, A., Finke, J. H., and Hilfiker, M. L. 1982. Placental mononuclear phagocytes as a source of interleukin-1. *Science* 218:475-477.
 88. 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 C₆ glioma cells. *J. Immunol.* 129:2413-2419.
 89. Gery, I., Gershon, R. K., and Waksman, B. H. 1972. Potentiation of the T-lymphocyte response to mitogens. I. The responding cell. *J. Exp. Med.* 136:128-142.
 90. Gery, I., Gershon, R. K., and Waksman, B. H. 1971. Potentiation of cultured mouse thymocyte responses by factors released by peripheral leucocytes. *J. Immunol.* 107:1778-1780.
 91. Gery, I., and Lepe-Zuniga, J. L. 1984. Interleukin 1: uniqueness of its production and spectrum of activities. *Lymphokines* 9:109-125.
 92. Gery, I., and Waksman, B. H. 1972. Potentiation of the T-lymphocyte response to mitogens. II. The cellular source of potentiating mediator(s). *J. Exp. Med.* 136:143-155.
 93. Ghaffar, A., Cullen, R. T., Dunbar, N., and Woodruff, M. F. A. 1974. Anti-tumour effect in vitro of lymphocytes and macrophages from mice treated with Corynebacterium parvum. *Br. J. Cancer* 29:199-205.
 94. Ghose, T., Guclu, A., Tai, J., Norvell, S. T., and MacDonald, A. S. 1976. Active immunoprophylaxis and immunotherapy in two mouse lymphoma models. *J. Natl. Cancer Inst.* 57:303-315.
 95. Gillis, S., and Mizel, S. B. 1981. T cell lymphoma model for the analysis of interleukin 1-mediated T cell activation. *Proc. Natl. Acad. Sci. U.S.A.* 78:1133-1137.

96. Gilman, S. C., Rosenberg, J. S., and Feldman, J. D. 1983. Inhibition of interleukin synthesis and T cell proliferation by a monoclonal anti-Ia antibody. *J. Immunol.* 130:1236-1240.
97. 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.
98. Giri, J. G., Lomedico, P. T., and Mizel, S. B. 1985. Studies on the synthesis and secretion of interleukin 1. I. A 33,000 molecular weight precursor for interleukin 1. *J. Immunol.* 134:343-349.
99. Goodman, M. G., Chenoweth, D. E., and Weigle, W. O. 1982. Induction of interleukin 1 secretion and enhancement of humoral immunity by binding of human C5a to macrophage surface C5a receptors. *J. Exp. Med.* 156:912-917.
100. Gorer, P. A. 1950. Studies of antibody response of mice to tumour inoculation. *Br. J. Cancer* 4:372-379.
101. Gorer, P. A., and Amos, D. B. 1956. Passive immunity in mice against C57BL leukemia EL-4 by means of iso-immune serum. *Cancer Res.* 16:338-343.
102. Gowan, M., Wood, D. D., Ihrie, E. J., McGuire, M. K. B., and Russell, R. G. G. 1983. An interleukin 1 like factor stimulates bone resorption in vitro. *Nature* 306:378-380.
103. Gowan, M., Wood, D. D., Ihrie, E. J., Meats J. E., and Russell, R. G. G. 1984. Stimulation by human interleukin 1 of cartilage breakdown and production of collagenase and proteoglycanase by human chondrocytes but not by human osteoblasts in vitro. *Biochim. Biophys. Acta.* 797:186-193.
104. Grabner, G., Luger, T. A., Smolin, G., and Oppenheim, J. J. 1982. Corneal epithelial cell-derived thymocyte-activating factor (CETAF). *Invest. Ophthalmol. Vis. Sci.* 23:757-763.
105. Grimm, E. A., Robb, R. J., Roth, J. A., Neckers, L. M., Lachman, L. B., Wilson, D. J., and Rosenberg, S. A. 1983. Lymphokine-activated killer cell phenomenon. III. Evidence that IL-2 alone is sufficient for direct activation of peripheral blood lymphocytes into lymphokine-activated killer cells. *J. Expl Med.* 158:1356-1361.
106. Gupta, J. D., Morahan, P. S., and Kaplan, A. M. 1978. Corynebacterium parvum-induced resistance to a methylcholanthrene fibrosarcoma. *J. Reticuloendothel. Soc.* 23:1-9.

107. Halpern, B. N., Biozzi, G., Stiffel, C., and Mouton, D. 1966. Inhibition of tumour growth by administration of killed Corynebacterium parvum. *Nature* 212:853-854.
108. Halpern, B., Lorinet, A-M., Sparros, L., and Fray, A. 1975. Inhibition of thymidine incorporation of tumoral YC8 cells cultured in vitro by peritoneal macrophages activated with Corynebacterium parvum. In: B. Halpern ed., Corynebacterium parvum: application in experimental and clinical oncology. Plenum Press, New York. Pages 132-136.
109. Halpern, B. N., Prevot, A-R., Biozzi, G., Stiffel, C., Mouton, D., Morard, J. C., Bouthillier, Y., and Decreusefond, C. 1964. Stimulation de l'activite phagocytaire du systeme reticuloendothelial provoquee par Corynebacterium parvum. *J. Reticuloendothel. Soc.* 1:77-96.
110. Hanna, N., and Burton, R. C. 1981. Definitive evidence that natural killer (NK) cells inhibit experimental tumor metastasis in vivo. *J. Immunol.* 127:1754-1758.
111. Haranaka, K., and Satomi, N. 1981. Cytotoxic activity of tumor necrosis factor TNF on the human cancer cells in vitro. *Jpn. J. Exp. Med.* 51:191-194.
112. Haranaka, K., Satomi, N., and Sakurai, A. 1984. Antitumor activity of murine tumor necrosis factor (TNF) against transplanted murine tumors and heterotransplanted human tumors in nude mice. *Int. J. Cancer* 34:263-267.
113. Helson, L., Green, S., Carswell, E. A., and Old, L. J. 1975. Effect of tumor necrosis factor on cultured human melanoma cells. *Nature.* 258:732-732.
114. Herberman, R. B., Munn, M. E., and Lavrin, D. H. 1975. Natural cytotoxic reactivity of mouse lymphoid cells against syngenic and allogeneic tumors. I. Distribution of reactivity and specificity. *Int. J. Cancer* 16:216-229.
115. Herman, J. Dinarello, C. A., Kew, M. C., and Rabson, A. R. 1985. The role of interleukin 1 (IL-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.
116. Hibbs, J. B., Jr. 1974. Heterocytolysis by macrophages activated by Bacillus Calmette-Guerin: lysosome endocytosis into tumor cells. *Science* 184:468-471.
117. Hoffmann, M. K. 1980. Macrophages and T cell control distinct phases of B cell differentiation in the humoral immune response in vitro. *J. Immunol.* 125:2076-2081.

118. Hoffmann, M. K., Koenig, S., Mittler, R. S., Oettgen, H. F., Ralph, P., Galanos, C., and Hammerling, U. 1979. Macrophage factor controlling differentiation of B cells. *J. Immunol.* 122:497-502.
119. Hoffmann, M. K., and Watson, J. 1979. Helper T cell-replacing factors secreted by thymus-derived cells and macrophages: cellular requirements for B cell activation and synergistic properties. *J. Immunol.* 122:1371-1375.
120. Hooper, D. C., Steer, C. J., Dinarello, C. A., and Peacock, A. C. 1981. Haptoglobin and albumin synthesis in isolated rat hepatocytes. Response to potential mediators of the acute-phase reaction. *Biochim. Biophys. Acta.* 653:118-129.
121. Hornung, R. L., and Mathews, H. L. 1985. Tumor immune response modulation by interleukin 1. In: Kluger, M. J., Oppenheim, J. J., and Powanda, M. C., eds., Progress in leukocyte biology. The physiologic, metabolic, and immunologic actions of interleukin-1. Pages 273-284.
122. Howard, J. G., Biozzi, G., Stiffel, C., Mouton, D., and Liacopoulos, P. 1967. An analysis of the inhibitory effect of Corynebacterium parvum on graft-versus-host disease. *Transplantation* 5:1510-1524.
123. Howard, J. G., Christie, G. H., and Scott, M. T. 1973. Biological effects of Corynebacterium parvum. IV. Adjuvant and inhibitory activities on B lymphocytes. *Cell. Immunol.* 7:290-301.
124. Howard, M., and Paul, W. E. 1983. Regulation of B-cell growth and differentiation by soluble factors. *Ann. Rev. Immunol.* 1:307-333.
125. Huddlestone, J. R., Merigan, T. C., Jr., and Oldstone, M. B. A. 1979. Induction and kinetics of natural killer cells in humans following interferon therapy. *Nature* 282:417-419.
126. Ikejima, T., Dinarello, C. A., Gill, M. D., and Wolff, S. M. 1983. Toxic shock(TS) strains of Staphylococcus aureus are potent inducers of human leukocytic pyrogen(LP) and lymphocyte activating factor (IAF). [abstract] *Clin. Res.* 31:496A.
127. Iribe, H., Koga, T., Kotani, S., Kusumoto, S., and Shiba, T. 1983. Stimulating effect of MDP and its adjuvant-active analogues on guinea pig fibroblasts for the production of thymocyte-activating factor. *J. Exp. Med.* 157:2190-2195.
128. Iribe, H., Koga, T., Onoue, K., Kotani, S., Kusumoto, S., and Shiba, T. 1981. Macrophage-stimulating effect of a synth-

etic muramyl dipeptide and its adjuvant-active and inactive analogs for the production of T-cell activating monokines. *Cell. Immunol.* 64:73-83.

129. 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.
130. James, K., Ghaffar, A., and Milne, I. 1974. The effect of transplanted methylcholanthrene induced fibrosarcomata and Corynebacterium parvum on the immune response of CBA and A/HeJ mice to thymus dependent and independent antigens. *Br. J. Cancer* 29:11-20.
131. Kampschmidt, R. F. 1981. Leukocytic endogenous mediator/endogenous pyrogen. In: Powanda, M. C., and Canonico, P. G., eds., The physiologic and metabolic responses of the host. Elsevier/North-Holland, Amsterdam. Pages 55-74.
132. 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. Biol. Med.* 158:32-35.
133. Kampschmidt, R. F., and Upchurch, H. F. 1974. Effect of leukocytic endogenous mediator on plasma fibrinogen and haptoglobin. *Proc Soc. Exp. Biol. Med.* 146:904-907.
134. Kampschmidt, R. F., and Upchurch, H. F. 1980. Neutrophil release after injections of endotoxin or leukocytic endogenous mediator into rats. *J. Reticuloendothel. Soc.* 28:191-201.
135. Kampschmidt, R. F., Upchurch, H. F., Eddington, C. L., and Pulliam, L. A. 1973. Multiple biological activities of a partially purified leukocytic endogenous mediator. *Am. J. Physiol.* 224:530-533.
136. Kampschmidt, R. F., Upchurch, H. F., and Worthington, M. L., III. 1983. Further comparisons of endogenous pyrogens and leukocytic endogenous mediators. *Infect. Immun.* 41:6-10.
137. Kasai, M., Leclerc, J. C., McVay-Boudreau, L., Shen, F. W., and Cantor, H. 1979. Direct evidence that natural killer cells in non immune spleen cell populations prevent tumor growth in vivo. *J. Exp. Med.* 149:1260-1264.
138. Kaye, J., Gillis, S., Mizel, S. B., Shevach, E. M., Malek, T. R., Dinarello, C. A., Lachman, L. B., and Janeway, C. A., Jr. 1984. Growth of a cloned helper T cell line induced by a monoclonal antibody specific for the antigen receptor:

interleukin 1 is required for the expression of the receptors for interleukin 2. *J. Immunol.* 133:1339-1345.

139. Keller, R. 1973. Cytostatic elimination of syngeneic rat tumor cells in vitro by nonspecifically activated macrophages. *J. Exp. Med.* 138:625-644.
140. Kelso, A., and MacDonald, H. R. 1982. Precursor frequency analysis of lymphokine-secreting allo-reactive T lymphocytes. Dissociation of subsets producing interleukin 2, macrophage-activating factor, and granulocyte-macrophage colony-stimulating factor on the basis of lyt-2 phenotype. *J. Exp. Med.* 156:1366-1379.
141. Kiessling, R., Klein, E., and Wigzell, H. 1975. "Natural" killer cells in the mouse. I. Cytotoxic cells with specificity for mouse moloney leukemia cells. Specificity and distribution according to genotype. *Eur. J. Immunol.* 5:112-117.
142. Kimball, E. S., Pickeral, S. F., Oppenheim, J. J., and Rossio, J. L. 1984. Interleukin 1 activity in normal human urine. *J. Immunol.* 133:256-260.
143. Kirchner, H., Hirt, H. M., and Munk, K. 1977. Protection against Herpes simplex virus infection in mice by Corynebacterium parvum. *Infect. & Immun.* 16:9-11.
144. Klempner, M. S., Dinarello, C. A., and Gallin, J. I. 1978. Human leukocytic pyrogen induces release of specific granule contents from human neutrophils. *J. Clin. Invest.* 61:1330-1336.
145. 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.
146. Koren, H. S., Handwerker, B. S., and Wunderlich, J. R. 1975. Identification of macrophage-like characteristics in a cultured murine tumor line. *J. Immunol.* 114:894-897.
147. Krakauer, T. 1984. Structural analysis of the charge heterogeneity of human interleukin 1. *Arch. Biochem. Biophys.* 234:371-376.
148. Krane, S. M., Goldring, S. R., and Dayer, J-M. 1982. Interactions among lymphocytes, monocytes, and other synovial cells in the rheumatoid synovium. *Lymphokines* 7:75-136.

149. Kronheim, S. R., March, C. J., Erb, S. K., Conlon, P. J., Mochizuki, D. Y., and Hopp, T. P. 1985. Human interleukin 1: purification to homogeneity. *J. Exp. Med.* 161:490-502.
150. Kull, F. C., Jr., and Cuatrecasas, P. 1984. Necrosin: purification and properties of a cytotoxin derived from a murine macrophage-like cell line. *Proc. Natl. Acad. Sci. U.S.A.* 81:7932-7936.
151. Kurt-Jones, E. A., Beller, D. I., Mizel, S. B., and Unanue, E. R. 1985. Identification of a membrane-associated interleukin 1 in macrophages. *Proc. Natl. Acad. Sci. U.S.A.* 82:1204-1208.
152. Kurt-Jones, E. A., Kiely, J-M., and Unanue, E. R. 1985. Communications. Conditions required for expression of membrane IL 1 on B cells. *J. Immunol.* 135:1548-1550.
153. Lachman, L. B. 1983. Human interleukin 1: purification and properties. *Fed. Proc.* 42:2639-2645.
154. Lachman, L. B. 1983. Interleukin-1; release from LPS-stimulated mononuclear phagocytes. In: Nowotny A, ed., Beneficial effects of endotoxin. Plenum Press, New York. Pages 283-305.
155. Lachman, L. B., Hacker, M. P., Blyden, G. T., and Handschumacher, R. E. 1976. Preparation of lymphocyte-activating factor from continuous murine macrophage cell lines. *Cell. Immunol.* 34:416-419.
156. Lachman, L. B., Hacker, M. P., Blyden, G. T., and Handschumacher, R. E. 1977. Preparations of lymphocyte-activating factor from continuous murine macrophage cell lines. *Cell. Immunol.* 34:416-419.
157. Lachman, L. B., Hacker, M. P., and Handschumacher, R. E. 1977. Partial purification of human lymphocyte-activating factor (LAF) by ultrafiltration and electrophoretic techniques. *J. Immunol.* 119:2019-2023.
158. Lachman, L. B., and Metzgar, R. S. 1980. Purification and characterization of human lymphocyte activating factor. In: de Weck A. L., Kristensen, F., and Landy, M. eds., Biochemical Characterization of Lymphokines. Proceedings of the 2nd International Lymphokine Workshop. Academic Press, New York. Pages 405-407.
159. Larsson, E-L., Iscove, N. N., and Coutinho, A. 1980. Two distinct factors are required for induction of T-cell growth. *Nature* 283:664-666.

160. Lee, K-C., Wong, M., and McIntyre, D. 1981. Characterization of macrophage subpopulations responsive to activation by endotoxin and lymphokines. *J. Immunol.* 126:2474-2479.
161. Leibson, J. H., Marrack, P., and Kappler, J. 1982. B cell helper factors. II. Synergy among three helper factors in the response of T cell- and macrophage-depleted B cells. *J. Immunol.* 129:1398-1402.
162. Levy, M. H., and Wheelock, E. F. 1974. The role of macrophages in defense against neoplastic disease. *Adv. Cancer Res.* 20:131-163.
163. Likhite, V. V. 1974. Rejection of tumors and metastases in Fischer 344 rats following intratumor administration of killed Corynebacterium parvum. *Int. J. Cancer* 14:684-690.
164. Likhite, V. V. 1976. Suppression of the incidence of death with spontaneous tumours in DBA/2 mice after Corynebacterium parvum-mediated rejection of syngeneic tumours. *Nature* 259:397-399.
165. Likhite, V. V., and Halpern, B. N. 1974. Lasting rejection of mammary adenocarcinoma cell tumors in DBA/2 mice with intratumor injection of killed Corynebacterium parvum. *Cancer Res.* 34:341-344.
166. Lipsky, P. E., Thompson, P. A., Rosenwasser, L. J., and Dinarullo, 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 an antibody against human leukocytic pyrogen. *J. Immunol.* 130:2708-2714.
167. Lomedico, P. T., Gubler, U., Hellmann, C. P., Dukovich, M., Giri, J. G., Pan, Y-C. E., Collier, K., Semionow, R., Chua, A. O., and Mizel, S. B. 1984. Cloning and expression of murine interleukin-1 cDNA in Escherichia coli. *Nature* 312:458-462.
168. Lovett, D., Kozan, B., Hadam, M., Resch, K., and Gemsa, D. 1986. Macrophage cytotoxicity: interleukin 1 as a mediator of tumor cytostasis. *J. Immunol.* 136:340-347.
169. Lovett, D. H., Ryan, J. L., and Sterzel, R. B. 1983. A thymocyte-activating factor derived from glomerular mesangial cells. *J. Immunol.* 130:1796-1801.
170. Luger, T. A., Charon, J. A., Colot, M., Micksche, M., and Oppenheim, J. J. 1983. Chemotactic properties of partially purified human epidermal cell-derived thymocyte-activating factor (ETAf) for polymorphonuclear and mononuclear cells. *J. Immunol.* 131:816-820.

171. Luger, T. A., and Oppenheim, J. J. 1983. Characteristics of interleukin 1 and epidermal-cell-derived thymocyte activating factor. *Adv. Inflammation Res.* 5:1-25.
172. Luger, T. A., Stadler, B. M., Katz, S. I., and Oppenheim, J. J. 1981. Epidermal cell (keratinocyte)-derived thymocyte-activating factor (ETAf). *J. Immunol.* 127:1493-1498.
173. 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.
174. Mackaness, G. B., Auclair, D. J., and Lagrange, P. H. 1973. Immunopotential with BCG. I. Immune response to different strains and preparations. *J. Natl. Cancer Inst.* 51:1655-1667.
175. Maeda, M., and Ichikawa, Y. 1980. Production of a colony-stimulating factor following differentiation of leukemic myeloblasts to macrophages. *J. Cell. Physiol.* 102:323-331.
176. Maguire, H. C., Jr., and Cipriano, D. 1983. Immunopotential of cell-mediated hypersensitivity by Corynebacterium parvum (Propionibacterium acnes). *Int. Archs. Allergy Appl. Immun.* 70:34-39.
177. Mainel, D. N., Mizel, S. B., Diamantstein, T., and Falk, W. 1985. Induction of interleukin 2 responsiveness in thymocytes by synergistic action of interleukin 1 and interleukin 2. *J. Immunol.* 134:3108-3110.
178. Maizel, A. L., Mehta, S. R., Ford, R. J., and Lachman, L. B. 1981. Effect of interleukin 1 on human thymocytes and purified human T cells. *J. Exp. Med.* 153:470-475.
179. Maluish, A. E., Ortaldo, J. R., Conlon, J. C., Sherwin, S. A., Leavitt, R., Strong, D. M., Weirnik, P., Oldham, R. K., and Herberman, R. B. 1983. Depression of natural killer cytotoxicity after in vivo administration of recombinant leukocyte interferon. *J. Immunol.* 131:503-507.
180. Mannel, D. N., Moore, R. N., and Mergenhagen, S. E. 1980. Macrophages as a source of tumoricidal activity (tumor necrotizing factor). *Infect. Immun.* 30:523-530.
181. March, C. J., Mosley, B., Larsen, A., Cerretti, D. P., Braedt, G., Price, V., Gillis, S., Henney, C. S., Kronheim, S. R., Grabstein, K., Conlon, P. J., Hopp, T. P., and Cosman, D. 1985. Cloning, sequence and expression of two distinct human interleukin-1 complementary DNAs. *Nature* 315:641-647.

182. Marx, J. L. 1985. Interleukin-1 genes are cloned. *Science* 228:1076-1077.
183. Mathews, H. L. 1981. Antitumor effects of a variety of non-viable bacteria against the murine EL-4 lymphoma. *Cancer Immunol. Immunother.* 12:81-85.
184. Mathews, N. 1978. Tumor-necrosis factor from the rabbit. II. Production by monocytes. *Br. J. Cancer.* 38:310-315.
185. Matsushima, K., Durum, S. K., Kimball, E. S., and Oppenheim, J. J. 1985. Purification of human interleukin 1 from human monocyte culture supernatants and identity of thymocyte co-mitogenic factor, fibroblast-proliferation factor, acute-phase protein-inducing factor, and endogenous pyrogen. *Cell. Immunol.* 92:290-301.
186. Matsushima, K., Onozaki, K., Benzur, M., and Oppenheim, J. J. 1985. Interleukin-1 as a potential anti-tumor factor. [abstract] *J. Leukocyte Biol.* 37:725.
187. McAdam, K. P. W. J., Elin, R. J., Sipe J. D., and Wolff, S. M. 1978. Changes in human serum amyloid A and C-reactive protein after etiocholanolone-induced inflammation. *J. Clin. Invest.* 61:390-394.
188. McBride, W. H., Jones, J. T., and Weir, D. M. 1974. Increased phagocytic cell activity and anaemia in Corynebacterium parvum treated mice. *Br. J. Exp. Pathol.* 55:38-46.
189. McCoy, J. L., Fefer, A., and Glynn, J. P. 1967. Comparative studies on the induction of transplantation resistance in BALB/c and C57BL/6 mice in three murine leukemia systems. *Cancer Res.* 27:1743-1748.
190. McDaniel, L. S., and Cozad, G. C. 1982. Immunoadjuvant effects of Blastomyces dermatitidis against EL 4 lymphoma in C57BL/6J mice. *J. Natl. Cancer Inst.* 69:1337-1342.
191. McKean, D. J., Infante, A. J., Nilson, A., Kimoto, M., Fathman, C. G., Walker, E., and Warner, N. 1981. Major histocompatibility complex-restricted antigen presentation to antigen-reactive T cells by B lymphocyte tumor cells. *J. Exp. Med.* 154:1419-1431.
192. McKean, D. J., Nilson, A., Beck, B. N., Giri, J., Mizel, S. B., and Handwerker, B. S. 1985. The role of IL-1 in the antigen-specific activation of murine class II-restricted T lymphocyte clones. *J. Immunol.* 135:3205-3216.
193. McMannis, J. D., and Plate, J. M. D. 1985. Xenogeneic anti-serum to soluble products from activated lymphoid cells

- inhibits interleukin 1-mediated functions in helper pathway of cytolytic-effector-cell differentiation. Proc. Natl. Acad. Sci. U.S.A. 82:1513-1517.
194. Meltzer, M. S., Oppenheim, J. J., Littman, B. H., Leonard, E. J., and Rapp, H. J. Cell-mediated tumor immunity measured in vitro and in vivo with soluble tumor-specific antigens. 1972. J. Natl. Cancer Inst. 49:727-734.
195. Merriman, C. R., Pulliam, L. A., and Kampschmidt, R. F. 1975. Effect of leukocytic endogenous mediator on C-reactive protein in rabbits. Proc. Soc. Exp. Biol. Med. 149:782-784.
196. Merriman, C. R., Pulliam, L. A., and Kampschmidt, R. F. 1977. Comparison of leukocytic pyrogen and leukocytic endogenous mediator. Proc. Soc. Exp. Biol. Med. 154:224-227.
197. Milas, L., Basic, I., Kogelnik, H. D., and Withers, H. R. 1975. Effects of Corynebacterium granulosum on weight and histology of lymphoid organs, response to mitogens, skin allografts, and a syngeneic fibrosarcoma in mice. Cancer Res. 35:2365-2374.
198. Milas, L., Gutterman, J. U., Basic, I., Hunter, N., Mavligit, G. M., Hersh, E. M., and Withers, H. R. 1974. Immunoprophylaxis and immunotherapy for a murine fibrosarcoma with C. granulosum and C. parvum. Int. J. Cancer 14:493-503.
199. Milas, L., Hunter, N., Basic, I., Mason, K., Grdina, D. J., and Withers, H. R. 1975. Nonspecific immunotherapy of murine solid tumors with Corynebacterium granulosum. J. Natl. Cancer Inst. 54:895-902.
200. Milas, L., Hunter, N., Basic, I., and Withers, H. R. 1974. Protection by Corynebacterium granulosum against radiation-induced enhancement of artificial pulmonary metastases of a murine fibrosarcoma. J. Natl. Cancer Inst. 52:1875-1880.
201. Milas, L., and Scott, M. T. 1978. Antitumor activity of Corynebacterium parvum. Adv. Cancer Res. 26:257-306.
202. Mills, C. D., North, R. J., and Dye, E. S. 1981. Mechanisms of anti-tumor action of Corynebacterium parvum. II. Potentiated cytolytic T cell response and its tumor-induced suppression. J. Exp. Med. 154:621-630.
203. Minden, P., and Farr, R. S. 1978. The ammonium sulfate method to measure antigen-binding capacity. In: Weir, D. M., ed., Handbook of experimental immunology, 3rd ed., Chapter 13. Blackwell Scientific Publications, London.

204. Mizel, S. B. 1979. Physicochemical characterization of lymphocyte-activating factor (LAF). *J. Immunol.* 122:2167-2172.
205. Mizel, S. B. 1981. Production and quantitation of lymphocyte-activating factor (interleukin 1). In: Herscovitz, H. B., Holden, H. T., Bellanti, J. A., and Ghaffar, A., eds., *Manual of macrophage methodology: collection, characterization, and function.* Marcel Dekker, New York. Pages 407-416.
206. Mizel, S. B., Dayer, J-M., Krane, S. M., and Mergenhagen, S. E. 1981. Stimulation of rheumatoid synovial cell collagenase and prostaglandin production by partially purified lymphocyte-activation factor (interleukin 1). *Proc. Natl. Acad. Sci. U.S.A.* 78:2474-2477.
207. Mizel, S. B., Dukovich, M., and Rothstein, J. 1983. Preparation of goat antibodies against interleukin 1: use of an immunoadsorbent to purify interleukin 1. *J. Immunol.* 131:1834-1837.
208. Mizel, S. B., and Mizel, D. 1981. Purification to apparent homogeneity of murine interleukin 1. *J. Immunol.* 126:834-837.
209. Mizel, S. B., Oppenheim, J. J., and Rosenstreich, D. L. 1978. Characterization of lymphocyte-activating factor (LAF) produced by the macrophage cell line, P388D₁. I. Enhancement of LAF production by activated T lymphocytes. *J. Immunol.* 120:1497-1503.
210. Mizel, S. B., Oppenheim, J. J., and Rosenstreich, D. L. 1978. Characterization of lymphocyte-activating factor (LAF) produced by the macrophage cell line, P388D₁. II. Biochemical characterization of LAF induced by activated T cells and LPS. *J. Immunol.* 120:1504-1508.
211. Mizel, S. B., and Rosenstreich, D. L. 1979. Regulation of lymphocyte-activating factor (LAF) production and secretion in P388D₁ cells: identification of high molecular weight precursors of LAF. *J. Immunol.* 122:2173-2179.
212. Moore, H. B., Sutter, V. L., and Finegold, S. M. 1975. Comparison of three procedures for biochemical testing of anaerobic bacteria. *J. Clin. Microbiol.* 1:15-24.
213. Moore, R. N., Oppenheim, J. J., Farrar, J. J., Carter, C. S., Jr., Waheed, A., and Shadduck, R. K. 1980. Production of lymphocyte-activating factor (interleukin 1) by macrophages activated with colony-stimulating factors. *J. Immunol.* 125:1302-1305.

214. Moore, W. E. C., and Holdeman, L. V. 1972. Identification of anaerobic bacteria. *Amer. J. Clin. Nutr.* 25:1306-1313.
215. Mule, J. J., Shu, S., Schwarz, S. L., and Rosenberg, S. A. 1984. Adoptive immunotherapy of established pulmonary metastases with LAK cells and recombinant interleukin-2. *Science* 225:1487-1489.
216. 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.
217. Nagelkerken, L. M., and van Breda Vriesman, P. J. C. 1986. Membrane-associated IL 1-like activity on rat dendritic cells. *J. Immunol.* 136:2164-2170.
218. Nakamura, S., Goto, F., Goto, K., and Yoshinaga, M. 1982. Physicochemical characterization of a PMN-derived soluble factor that enhances lymphocyte DNA synthesis. *J. Immunol.* 128:2614-2621.
219. 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.
220. Naor, D. 1979. Suppressor cells: permitters and promoters of malignancy. *Adv. Cancer Res.* 29:45-125.
221. Nathan, C. F., Silverstein, S. C., Brukner, L. H., and Cohn, Z. A. 1979. Extracellular cytotoxicity by activated macrophages and granulocytes. II. Hydrogen peroxide as a mediator of cytotoxicity. *J. Exp. Med.* 149:100-113.
222. Neta, R., Douches, S., and Oppenheim, J. J. 1986. Interleukin 1 is a radioprotector. *J. Immunol.* 136:2483-2485.
223. Neveu, T., Branellec, A., and Biozzi, G. 1964. Adjuvant effect of *Corynebacterium parvum* on antibody production and on induction of delayed hypersensitivity to conjugated proteins. *Ann. Inst. Pasteur, Paris* 106:771-777.
224. North, R. J., and Dye, E. S. 1984. Ly 1⁺2⁻ suppressor T cells down-regulate the generation of Ly 1⁻2⁺ effector T cells during progressive growth of the P815 mastocytoma. *Immunol.* 54:47-56.
225. Nunn, M. E., Herberman, R. B., and Holden, H. T. 1977. Natural cell-mediated cytotoxicity in mice against non-lymphoid tumor cells and some normal cells. *Int. J. Cancer* 20:381-387.

226. Nussenzweig, R. S. 1967. Increased non-specific resistance to malaria produced by administration of killed Corynebacterium parvum. *Exp. Parasit.* 21:224-231.
227. O'Neill, G. J., Henderson, D. C., and White, R. G. 1973. The role of anaerobic coryneforms on specific and non-specific immunological reactions. I. Effect on particle clearance and humoral and cell-mediated immunological responses. *Immunology* 24:977-995.
228. Oehler, J. R., Lindsay, L. R., Nunn, M. E., Holden, H. T., and Herberman, R. B. 1978. Natural cell-mediated cytotoxicity in rats. II. In vivo augmentation of NK-cell activity. *Int. J. Cancer* 21:210-219.
229. Ojo, E. 1979. Positive correlation between the levels of natural killer cells and the in vivo resistance to syngeneic tumor transplants as influenced by various routes of administration of Corynebacterium parvum bacteria. *Cell. Immunol.* 45:182-187.
230. Okuda, S., Taniguchi, K., Kubo, C., and Nomoto, K. 1982. Accessory cell function in tumor-bearing mice and effects of Corynebacterium parvum. *J. Natl. Cancer Inst.* 69:1293-1297.
231. Old, L. J., Benacerraf, B., Clarke, D. A., Carswell, E. A., and Stockert, E. 1961. The role of the reticuloendothelial system in the host reaction to neoplasia. *Cancer Res.* 21:1281-1300.
232. Old, L. J., Boyse, E. A., Clarke, D. A., and Carswell, E. A. 1962. Antigenic properties of chemically induced tumors. *Ann. N.Y. Acad. Sci.* 101:80-106.
233. Old, L. J., Clarke, D. A., Benacerraf, B., and Goldsmith, M. 1960. The reticuloendothelial system and the neoplastic process. *Ann. N. Y. Acad. Sci.* 88:264-280.
234. Olivetto, M., and Bomford, R. 1974. In vitro inhibition of tumour cell growth and DNA synthesis by peritoneal and lung macrophages from mice injected with Corynebacterium parvum. *Int. J. Cancer* 13:478-488.
235. Onozaki, K., Matsushima, K., Aggarwal, B. B., and Oppenheim, J. J. 1985. Human interleukin 1 is a cytotoxic factor for several tumor cell lines. *J. Immunol.* 135:3962-3968.
236. Onozaki, K., Matsushima, K., Kleinerman, E. S., Saito, T., and Oppenheim, J. J. 1985. Role of interleukin 1 in promoting human monocyte-mediated tumor cytotoxicity. *J. Immunol.* 135:314-320.

237. Oppenheim, J. J., and Gery, I. 1982. Interleukin 1 is more than an interleukin. *Immunol. Today* 3:113-119.
238. Oppenheim, J. J., Mizel, S. B., and Meltzer, S. M. 1978. Biological effects of lymphocyte and macrophage-derived mitogenic "amplification" factors. In: Cohen, S., Pick, E., and Oppenheim, J. J., eds., Biology of the Lymphokines. Academic Press, New York. Pages 291-321.
239. Oppenheim, J. J., Onozaki, K., Benezur, M., and Matsushima, K. 1985. Biological activities of interleukin 1 with potential antitumor effects. In: Ishigami, J., ed., Recent advances in chemotherapy. Anticancer section 1. University of Tokyo Press, Tokyo. Pages 72-74.
240. Oppenheim, J. J., Onozaki, K., and Matsushima, K. 1984. Auto-regulatory effects of interleukin 1 on human monocytes. In: van Furth, R., ed., Mononuclear phagocytes: Characteristics, physiology and function. Martinus Nijhoff, Boston. Pages 287-292.
241. Oppenheim, J. J., Stadler, R. P., Siraganian, R. P., Mage, M., and Mathieson, B. 1982. Lymphokines: their role in lymphocyte responses. Properties of interleukin 1. *Fed. Proc.* 41:257-262.
242. Oppenheim, J. J., Togawa, A., Chedid, L., and Mizel, S. 1980. Components of mycobacteria and muramyl dipeptide with adjuvant activity induce lymphocyte activating factor. *Cell. Immunol.* 50:71-81.
243. Otu, A. A., Russell, R. J., and White, R. G. 1977. Biphasic pattern of activation of reticuloendothelial system by anaerobic coryneforms in mice. *Immunology* 32:255-264.
244. Pacak, F., and Siegert, R. 1982. Chemical characterization of a rabbit leukocytic pyrogen. *Eur. J. Biochem.* 127:375-380.
245. Parr, I. 1972. Response of syngeneic murine lymphomata to immunotherapy in relation to the antigenicity of the tumour. *Br. J. Cancer.* 26:174-182.
246. Peters, L. J., McBride, W. H., Mason, K. A., Hunter, N., Basic, I., and Milas, L. 1977. In vivo transfer of antitumour activity by peritoneal exudate cells from mice treated with Corynebacterium parvum: reduced effect in irradiated recipients. *J. Natl. Cancer Inst.* 59:881-887.
247. Pike, B. L., and Nossal, G. J. V. 1985. Interleukin 1 can act as a B-cell growth and differentiation. *Proc. Natl. Acad. Sci. U.S.A.* 82:8153-8157.

248. Pimm, M. V., and Baldwin, R. W. 1976. C. parvum suppression of rat tumours in athymic nude mice. *Br. J. Cancer* 34:453-455.
249. Pimm, M. V., and Baldwin, R. W. 1977. Corynebacterium parvum immunotherapy of transplanted rat tumours. *Int. J. Cancer* 20:923-932.
250. Pringle, A. T., and Cummins, C. S. 1982. The cell surface of Propionibacterium acnes: effect of specific chemical modifications on the ability of vaccines to produce splenomegaly in mice. *Can. J. Microbiol.* 28:375-382.
251. Prohaska, F. R., and Lukasewycz, O. A. 1981. Copper deficiency suppresses the immune response of mice. *Science* 213:559-561.
252. Ralph, P., and Nakoinz, I. 1977. Antibody-dependent killing of erythrocyte and tumor targets by macrophage-related cell lines: enhancement by PPD and LPS. *J. Immunol.* 119:950-954.
253. Ralph, P., and Nakoinz, I. 1977. Direct toxic effects of immunopotentiators on monocytic, myelomonocytic, and histiocytic or macrophage tumor cells in culture. *Cancer Res.* 37:546-550.
254. Rao, A., Mizel, S. B., and Cantor, H. 1983. Disparate functional properties of two interleukin 1-responsive Ly-1⁺2⁺T cell clones: distinction of T cell growth factor and T cell-replacing factor activities. *J. Immunol.* 130:1743-1748.
255. Ribi, E., Milner, K. C., Granger, D. L., Kelly, M. T., Yamamoto, K., Brehmer, W., Parker, R., Smith, R. F., and Strain, S. M. 1976. Immunotherapy with nonviable microbial components. *Ann. N. Y. Acad. Sci.* 277:228-238.
256. Rifkind, D., and Palmer, J. D. 1966. Neutralization of endotoxin toxicity in chick embryos by antibiotics. *J. Bacteriol.* 92:815-819.
257. Riveros-Moreno, V., Bomford, R., and Scott, M. T. 1978. Antitumor activity of purified cell walls from Corynebacterium parvum. *J. Natl. Cancer Inst.* 60:653-658.
258. Riveros-Moreno, V., and Niblock, A. 1979. Chemical properties of the principle in C. parvum that produces splenomegaly in mice. *Immunology* 36:495-499.
259. Rosenberg, S. A., and Lipsky, P. E. 1980. The role of monocyte factors in the differentiation of immunoglobulin secreting cells from human peripheral blood B cells. *J. Immunol.* 125:232-237.

260. Rosenthal, A. S. 1980. Regulation of the immune response: role of the macrophage. *N. Engl. J. Med.* 303:1153-1156.
261. Rosenwasser, L. J., Dinarello, C. A., and Rosenthal, A. S. 1979. Adherent cell function in murine T-lymphocyte antigen recognition. IV. Enhancement of murine T-cell antigen recognition by human leukocyte pyrogen. *J. Exp. Med.* 150:709-714.
262. Roszkowski, W., Szmigielski, S., Ko, H.L., Janiak, M., Wrembel, J. K., Pulverer, G., and Jeljaszewicz, J. 1980. Effect of Three Strains of Propionibacteria (*P. granulosum*, *P. avidum*, *P. acnes*) and cell-wall preparations on lymphocytes and macrophages. *Zbl. Bakt. Hyg., I. Abt. Orig. A* 246:393-404.
263. Ruff, M. R., and Gifford, G. E. 1981. Tumor necrosis factor. In: Pick, E., ed., *Lymphokines*, Academic Press, New York, Vol. 2. Pages 235-272.
264. Sauder, D. N., Carter, C. S., Katz, S. I., and Oppenheim, J. J. 1982. Epidermal cell production of thymocyte activating factor (ETAf). *J. Invest. Dermatol.* 79:34-39.
265. Sauder, D. N., Mounessa, N. L., Katz, S. I., Dinarello, C. A., and Gallin, J. A. 1984. Chemotactic cytokines: the role of leukocytic pyrogen and epidermal cell thymocyte-activating factor in neutrophil chemotaxis. *J. Immunol.* 132:828-832.
266. Scala, G., Allavena, P., Djeu, J. Y., 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.
267. Scala, G., Kuang, Y. D., Hall, R. E., Muchmore, A. V., and Oppenheim, J. J. 1984. Accessory cell function of human B cells. I. Production of both interleukin 1-like activity and an interleukin 1 inhibitory factor by an EBV-transformed human B cell line. *J. Exp. Med.* 159:1637-1652.
268. Schmidt, J. A., Mizel, S. B., Cohen, D., and Green, I. 1982. Interleukin 1, a potential regulator of fibroblast proliferation. *J. Immunol.* 128:2177-2182.
269. Schmidt, J. A., Oliver, C. N., Lepe-Zuniga, J. L., Green, I., and Gery, I. 1984. Silica-stimulated monocytes release fibroblast proliferation factors identical to interleukin 1. A potential role for interleukin 1 in the pathogenesis of silicosis. *J. Clin. Invest.* 73:1462-1472.
270. Scott, M. T. 1974. *Corynebacterium parvum* as a therapeutic antitumor agent in mice. I. Systemic effects from intravenous injection. *J. Natl. Cancer Inst.* 53:855-860.

271. Scott, M. T. 1974. Corynebacterium parvum as a therapeutic antitumor agent in mice. II. Local injection. J. Natl. Cancer Inst. 53:861-865.
272. Scott, M. T. 1974. Depression of delayed-type hypersensitivity by Corynebacterium parvum: mandatory role of the spleen. Cell. Immunol. 13:251-263.
273. Scott, M. T. 1975. Potentiation of the tumor-specific immune response by Corynebacterium parvum. J. Natl. Cancer Inst. 55:65-72.
274. Scott, M. T. 1976. Failure of Corynebacterium parvum sensitization to modify the antitumor effects of systemic and local therapeutic injections of C. parvum in mice. J. Natl. Cancer Inst. 56:675-677.
275. Scott, M. T., MacDonald, T., and Carta, P. 1978. C. parvum in germ-free mice. Cancer Immunol. Immunother. 4:135.
276. Shaw, J., Caplan, B., Paetkau, V., Pilarsky, L. M., Delovitch, T. L., and McKenzie, I. F. C. 1980. Cellular origin of co-stimulator (IL-2) and its activity in cytotoxic T lymphocytic responses. J. Immunol. 124:2231-2239.
277. Sidman, C. L., Paige, C. J., and Schreier, M. H. 1984. B cell maturation factor (BMF): a lymphokine or family of lymphokines promoting the maturation of B lymphocytes. J. Immunol. 132:209-222.
278. Simon, P. L., and Willoughby, W. F. 1981. The role of subcellular factors in pulmonary immune functions: physicochemical characterization of two distinct species of lymphocyte-activating factor produced by rabbit alveolar macrophages. J. Immunol. 126:1534-1541.
279. Sinclair, W. K., and Morton, R. A. 1966. X-ray sensitivity during the cell generation cycle of cultured Chinese hamster cells. Radiat. Res. 29:450-474.
280. Sjogren, H. O., and Hellstrom, I. 1965. Induction of polyoma specific transplantation antigenicity in Moloney leukemia cells. Exp. Cell Res. 40:208-212.
281. Smith, K. A., Lachman, L. B., Oppenheim, J. J., and Favata, M. F. 1980. The functional relationship of the interleukins. J. Exp. Med. 151:1551-1556.
282. Sobrado, J., Moldawer, L. L., Bistrain, B. R., Dinarello, C. A., and Blackburn, G. L. 1983. Effect of ibuprofen on fever and metabolic changes induced by continuous infusion of leuko-

- cytic pyrogen (Interleukin-1) or endotoxin. *Infect. Immun.* 42:997-1005.
283. Starr, S. E., Thompson, F. S., Dowell, V. R., Jr., and Balows, A. 1973. Micromethod system for identification of anaerobic bacteria. *Appl. Microbiol.* 25:713-717.
284. Steeg, P. S., Johnson, H. M., and Oppenheim, J. J. 1982. Regulation of murine macrophage Ia antigen expression by an immune interferon-like lymphokine: inhibitory effect of endotoxin. *J. Immunol.* 129:2402-2406.
285. Staruch, M. J., and Wood, D. D. 1983. The adjuvanticity of interleukin 1 in vivo. *J. Immunol.* 130:2191-2194.
286. Suit, H. D., Sedlacek, R., Wagner, M., Orsi, L., Silobrcic, V., and Rothman, K. J. 1976. Effect of Corynebacterium parvum on the response to irradiation of a C3H fibrosarcoma. *Cancer Res.* 36:1305-1314.
287. Sztein, M. B., Vogel, S. N., Sipe, J. D., Murphy, P. A., Mizel, S. B., Oppenheim, J. J., and Rosenstreich, D. L. 1981. The role of macrophages in the acute-phase response: SAA inducer is closely related to lymphocyte activating factor and endogenous pyrogen. *Cell. Immunol.* 63:164-176.
288. Talmadge, J. E., Herberman, R. B., Chirigos, M. A., Maluish, A. E., Schneider, M. A., Adams, J. S., Philips, H., Thurman, G. B., Varesio, L., Long, C., Oldham, R. K., and Wiltrout, R. H. 1985. Hyporesponsiveness to augmentation of murine natural killer cell activity in different anatomical compartments by multiple injectins of various immunomodulators including recombinant interferons and interleukin 2. *J. Immunol.* 135:2483-2491.
289. Tarnowski, G. S., Ralph, P., and Stock, C. C. 1979. Sensitivity of chemotherapeutic and immunomodulating agents of two mouse lymphomas and of a macrophage tumor. *Cancer Res.* 39:3964-3967.
290. Thorn, R. M., and Henney, C. S. 1976. Kinetic analysis of target cell destruction by effector T cells. I. Delineation of parameters related to the frequency and lytic efficiency of killer cells. *J. Immunol.* 117:2213-2219.
291. Tocco-Bradley, R., Moldawer, L. L., Jones, C. T., Gerson, B., Blackburn, G. L., and Bistrrian, B. R. 1986. The biological activity in vivo of recombinant murine interleukin 1 in the rat. *Proc. Soc. Exp. Biol. Med.* 182:263-271.
292. Togawa, A., Oppenheim, J. J., and Mizel, S. B. 1979. Characterization of lymphocyte-activating factor (LAF) produced by

- human mononuclear cells: biochemical relationship of high and low molecular weight forms of LAF. *J. Immunol.* 122:2112-2118.
293. Turchik, J. B., and Bornstein, D. L. 1980. Role of the central nervous system in acute-phase responses to leukocytic pyrogen. *Infect. Immun.* 30:439-444.
294. Tuttle, R. L., and North, R. J. 1975. Mechanisms of antitumor action of Corynebacterium parvum: nonspecific tumor cell destruction at site of an immunologically mediated sensitivity reaction to C. parvum. *J. Natl. Cancer Inst.* 55:1403-1411.
295. Tuttle, R. L., and North, R. J. 1976. Mechanisms of antitumor action of Corynebacterium parvum: the generation of cell-mediated tumor specific immunity. *J. Reticuloendothel. Soc.* 20:197-208.
296. 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. Reticuloendothel. Soc.* 20:209-216.
297. Unanue, E. R. 1978. The regulation of lymphocyte functions by the macrophage. *Immunol. Rev.* 40:227-255.
298. Unanue, E. R., and Kiely, J-M. 1977. Synthesis and secretion of a mitogenic protein by macrophages: description of a superinduction phenomenon. *J. Immunol.* 119:925-931.
299. Vacheron, F., Guenounou, M., and Nauciel, C. 1983. Induction of interleukin 1 secretion by adjuvant-active peptidoglycans. *Infect. and Immun.* 42:1049-1054.
300. Van Damme, J., De Ley, M., Opdenakker, G., Billiau, A., and De Somer, P. 1985. Homogeneous interferon-inducing 22K factor is related to endogenous pyrogen and interleukin-1. *Nature* 314:266-268.
301. Wagner, H., Hardt, C., Bartlett, R., Rollinghoff, M., and Pfizermaier, K. 1980. Intrathymic differentiation of cytotoxic T lymphocyte (CTL) precursors. I. The CTL immunocompetence of peanut agglutinin-positive (cortical) and negative (medullary) lyt 123 thymocytes. *J. Immunol.* 125:2532-2538.
302. Warr, G. W., and Sljivic, V. S. 1974. Enhancement and depression of the antibody response in mice caused by Corynebacterium parvum. *Clin. Exp. Immunol.* 17:519-532.
303. Webb, A. C., Auron, P. E., Rich, A., Rosenwasser, L. J., Wolff, S. M., and Dinarello, C. A. 1985. In: Sorg, C., and

Schimpl, A., eds., Cellular and molecular biology of lymphokines. Academic Press, New York.

304. Wiener, E. 1975. The role of macrophages in the amplified in vitro response to sheep red blood cells by spleen cells from Corynebacterium parvum treated mice. *Cell. Immunol.* 19:1-7.
305. Wiener, E., and Bandieri, A. 1975. Modifications in the handling in vitro of 125I-labeled keyhole limpet haemocyanin by peritoneal macrophages from mice pretreated with the adjuvant Corynebacterium parvum. *Immunology* 29:265-274.
306. Wigzell, H. 1965. Quantitative titrations of mouse H-2 antibodies using ⁵¹Cr-labelled target cells. *Transplantation* 3:423-431.
307. Wolmark, N., and Fisher, B. 1974. The Effect of a single and repeated administration of C. parvum on bone marrow macrophage colony production in syngeneic tumor-bearing mice. *Cancer Res.* 34:2869-2872.
308. Wood, D. D. 1979. Mechanism of action of human B cell-activating factor. I. Comparison of the plaque-stimulating activity with thymocyte-stimulating activity. *J. Immunol.* 123:2400-2407.
309. Wood, D. D., and Cameron, P. M. 1976. Stimulation of the release of a B cell-activating factor from human monocytes. *Cell. Immunol.* 21:133-145.
310. Wood, D. D., and Cameron, P. M. 1978. The relationship between bacterial endotoxin and human B cell-activating factor. *J. Immunol.* 121:53-60.
311. Wood, D. D., Cameron, P. M., Poe, M. T., and Morris, C. A. 1976. Resolution of a factor that enhances the antibody response of T cell-depleted murine splenocytes from several other monocyte products. *Cell. Immunol.* 21:88-96.
312. Wood, D. D., Staruch, M. J., Durette, P. L., Melvin, W. V., III, and Graham, B. K. 1983. Role of interleukin-1 in the adjuvanticity of muramyl dipeptide in vivo. In: Interleukins, Lymphokines, and Cytokines. Academic Press, New York. Pages 691-696.
313. Woodruff, M. F. A., and Boak, J. L. 1966. Inhibitory effect of injection of Corynebacterium parvum on the growth of tumour transplants in isogenic hosts. *Br. J. Cancer* 20:345-355.
314. Woodruff, M. F. A., and Dunbar, N. 1975. Effect of local injection of Corynebacterium parvum on the growth of a murine fibrosarcoma. *Br. J. Cancer* 32:34-41.

315. Woodruff, M. F. A., and Warner, N. L. 1977. Effect of Corynebacterium parvum on tumor growth in normal and athymic (nude) mice. *J. Natl. Cancer Inst.* 58:111-116.
316. Yamamoto, A., Williamson, B. D., Carswell, E. A., Fiore, N., and Old, L. J. 1985. In: Homma, J. Y., Kanegasaki, S., Luderwicz, O., Shiba, T., and Westphal, O., eds., Bacterial endotoxin: Chemical, biological and clinical aspects. Verlag-Chemie, Weinheim, F.R.G. Pages 223-234.
317. Yamamura, Y., Procter, J. W., and Fischer, B. C. 1981. Mediator production and tumorigenesis of murine monocyte lines. *JNCI* 66:1067-1073.
318. Yoshinaga, M., Nishime, K., Nakamura, S., and Goto, F. 1980. A PMN-derived factor that enhances DNA-synthesis in PHA or antigen-stimulated lymphocytes. *J. Immunol.* 124:94-99.
319. Zacharchuk, C. M., Drysdale, B-E., Mayer, M. M., and Shin, H. S. 1983. Macrophage-mediated cytotoxicity: role of a soluble macrophage cytotoxic factor similar to lymphotoxin and tumor necrosis factor. *Proc. Natl. Acad. Sci. U.S.A.* 80:6341-6345.
320. Zlotnik, A., Roberts, W. K., Vasil, A., Blumenthal, E., Larosa, F., Leibson, H. J., Endres, R. O., Graham, S. D., Jr., White, J., Hill, J., Henson, P., Klein, J. R., Bevan, M. J., Marrack, P., and Kappler, J. W. 1983. Coordinate production by a T cell hybridoma of gamma interferon and three other lymphokine activities: multiple activities of a single lymphokine? *J. Immunol.* 131:794-800.

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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.

11/22/84

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