

20030115186

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

AD-A157 543

DTIC FILE COPY

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER 2	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Mechanisms of Rapid Nonspecific Resistance Induced by Immunomodulators: Delineation Using Selective Depletion of Cells		5. TYPE OF REPORT & PERIOD COVERED 7/1/84 - 6/30/85
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Page S. Morahan, Ph.D. Alvin Volkman, M.D.		8. CONTRACT OR GRANT NUMBER(s) N00014-82-K-0669
9. PERFORMING ORGANIZATION NAME AND ADDRESS Dept. Microbiology & Immuno. 3300 Henry Ave., Medical College of Penn. Phila. PA 19129 and Dept. Pathology, E.C.U.M.S., Greenville, NC 27834		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS NR 666-008
11. CONTROLLING OFFICE NAME AND ADDRESS Jeannine A. Maijda, Ph.D., Scientific Officer Immunology Codd 441, Cellular Biosystems Group Dept. of the Navy, ONR, Arlington, VA 22217		12. REPORT DATE 7/30/85
		13. NUMBER OF PAGES 12
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office) Same as 11		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) Unlimited		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report) Unlimited		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) Macrophages, monocytes, PMN, NK cells, herpes simplex type 2, encephalomy ocarditis virus, Listeria, host defense, immunotherapy, interferon, immunomodulators, <u>C. parvum</u> , MVE-2, Muramyl dipeptide, lipoidal amines, ⁸⁹ Sr, bone marrow depletion.		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Pyrimidinone, muramyl dipeptide and tripeptide, detoxified lipopolysaccharide and trehalose dimycolate immunomodulators have been evaluated for ability to increase nonspecific resistance of CD-1 mice to <u>Listeria monocytogenes</u> , encephalomyocarditis virus and herpes simplex virus type 2. The brominated pyrimidinone was very effective in prophylactic treatment against the viral infections, but not against listeria infection by the regimens tested. Of the bacterial cell wall type immunomodulators, detoxified endotoxin and trehalose		

DTIC
ELECTE
AUG 8 1985
S A D

DD FORM 1 JAN 73 1473

EDITION OF 1 NOV 65 IS OBSOLETE

S/N 0102-LS-014-6601

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

85 8 1 147

dimycolate appeared to be the most effective. Additional experiments to directly evaluate the same doses, regimens and vehicles are planned in normal CD-1 mice. The most effective agents will be tested for ability to enhance resistance in the face of ^{89}Sr -induced monocytopenia, granulocytopenia and depressed NK cell activity.

The mechanism of activation, and effects of the C. parvum immunomodulator have been investigated in ^{89}Sr treated or congenitally defective Sl/Sld mice; both provide models for deficient bone marrow dependent functions. Our studies in the ^{89}Sr system have established that C. parvum activation of peritoneal macrophages (M ϕ) is associated with a marked acute and persistent PMN inflammatory response. If this inflammatory response is decreased sufficiently by ^{89}Sr treatment, there is reduced M ϕ activation. Studies with C. parvum effects on M ϕ in ^{89}Sr treated and Sl/Sld mice have documented unexpected additional complexity among M ϕ populations. The data suggest at least three functionally definable populations on mononuclear phagocytes which appear to be independently regulated.

In the CD-1 mouse, we have extended our observations with C. parvum to several other biologic response modifiers known to activate M ϕ for antitumor activity (pyran copolymer and M. bovis strain BCG). BCG appears to activate peritoneal M ϕ relatively normally (as assessed by ectoenzyme phenotype and antitumor activity) whether the mice are able to mount a Mo-M ϕ rich peritoneal exudate or not. These data lend additional support to the concept that certain immunomodulators can activate resident M ϕ in situ without need for a Mo-M ϕ inflammatory influx. Additional studies have shown that ^{89}Sr treatment also does not appear to affect markedly the ability of resident, thiglycollate or C. parvum activated peritoneal M ϕ to proliferate in response to CSF. The two activated M ϕ populations had increased proliferative capacity, whether there was an inflammatory exudate of immature monocytes or not.

We have also begun developing liposome methodology for depletion of tissue M ϕ . Studies in this year have been devoted to optimizing the liposome constitution, and establishing the types of interaction of liposomes with M ϕ . Studies in the coming year with focus on the effects of toxins incorporated in liposomes on M ϕ function in vitro and in vivo.

Accession No.	
DTIC CLASS	☒
DTIC TAB	☐
Unannounced	☐
Justification	
By	
Distribution/	
Availability Codes	
Avail and/or	
Dist	Special
A	

SPW 0702 LP 078-8601

PROGRESS REPORT - 1 July 1984 - 30 June 1985

1.0 Experimental Objectives

1.1 Continue to evaluate the efficacy of immunomodulators in our battery of microbial infections.

1.2 Define the effects of immunomodulators on various nonspecific effector cell populations in the 89Sr system.

1.3 Develop effective methods to decrease tissue M0 in normal and 89Sr treated mice.

2.0 Detailed Progress Report.

2.1 Evaluation of immunomodulators in a battery of microbial infections. The results reported in the 1st Annual Report (August 1984) have been submitted for publication, and document: (i) CD-1 mice express relatively normal resistance to infection with EMC and HSV-2 viruses and to Listeria monocytogenes during the first 30 days after 89Sr treatment, despite profound monocytopenia, granulocytopenia and decreased NK cell activity; and (ii) a variety of immunomodulators, including C. parvum, avridine, and MVE-2 show relatively normal ability to enhance microbial resistance in 89Sr treated mice. During this year, studies have been performed in normal CD-1 mice with additional immunomodulators.

Progress was delayed during renovation of our animal facilities into an isolation area. We now have an excellent isolation system that should assure us of having clean animals. We believe that this is essential for meaningful experiments with immunomodulators. In last year's report we documented that inapparent infection with mouse hepatitis virus increased natural resistance of mice to subsequent infection with EMC virus. In collaboration with Dr. Abigail Smith at Yale, we have now documented that overt infection of mice with mouse hepatitis virus can activate peritoneal macrophages, as assessed by changes in ectoenzyme phenotype (Table 1).

During this year we have concentrated on testing two classes of immunomodulators: the pyrimidinones and bacterial cell wall derivatives or synthetic analogs. Single i.p. or oral prophylactic treatment of mice with 2-amino-5-bromo-6-phenyl-4-pyrimidinol (ABPP, Upjohn) was quite effective in increasing resistance to EMC, while i.p. or oral multiple dose prophylactic regimens were effective against HSV-2 infection (Table 2). There may be a b.i.d. b.i.d. response to ABPP; in both infections, the higher dose systemic regimens of ABPP treatment appeared to be less effective than lower dose regimens. No gross drug toxicity was apparent, however. In contrast to the effectiveness against the viral infections, single or multiple dose prophylactic regimens of ABPP had little effect against listeria infection. No exacerbation of infection, however, was noted as has been observed with some other immunomodulators. Future studies will be directed at oral treatment, and will assess prophylactic and/or therapeutic treatment regimens.

Single i.p. or oral, prophylactic or therapeutic, treatment with

muramyl tripeptide-phosphatidylethanolamine (MTP-PE, Ciba-Geigy) was not markedly effective against either EMC or listeria infections (Table 3). Multiple dose, combined prophylactic/therapeutic treatment with MTP-PE was also ineffective against EMC virus, while a similar regimen with muramyl dipeptide (MDP, Choay Chimie Reactifs) was slightly effective. The more lipophilic derivative, MDP-glyceryldipalmitate (MDP-GDP, Choay) showed marginal activity. Single prophylactic treatment with detoxified bacterial lipopolysaccharide (detox LPS, Ribi), trehalose dimycolate (TDM), or the combination, in a 2% squalene vehicle, was markedly effective against listeria infection, and somewhat effective against EMC virus (Table 3). More direct comparisons with these bacterial cell wall types of immunomodulators, with the same doses, vehicles and regimens, need to be performed before comparative efficacy can be established.

Dependent upon availability, other immunomodulators which we are particularly interested in testing for broad nonspecific antimicrobial activity includes gamma interferon, macrophage colony stimulating factor, IL-1, and several new synthetic materials.

2.2 Effects of immunomodulators in mice that have been selectively depleted of various effector cell populations.

2.21 Heterogeneity of M0 activation by *C. parvum*. The following section briefly summarizes results that are currently in press. In normal CBA/J mice, i.p. injection of *C. parvum* increases M0 colony forming precursors (M0 CFU) in the spleen, as well as increases the number of phagocytic M0 that bind IgG2a and IgG2b immune complexes. These phagocytic M0 suppress Con A induced lymphocyte proliferation, probably reflecting a 10-fold increase in prostaglandin E (PGE) in the splenic M0. The 89Sr system was used to establish that these splenic suppressor M0 are derivatives of bone marrow M0 CFU. Treatment of mice with 89Sr reduced bone marrow M0 CFU to less than 1% of the normal levels for more than 30 days, and reduced circulating monocytes (Mo) to 5% by day 10 and 30% by day 30. Splenic M0 CFU increased 20-fold in 89Sr as compared with normal controls. However, *C. parvum* induced splenic suppressor M0 activity was sharply reduced in 89Sr treated mice despite the striking increase in splenic M0 CFU. The kinetics of recovery of splenic suppressor M0 showed a steady increase between 20 and 50 days after 89Sr, correlating with recovery of significant levels of bone marrow M0 CFU. These results suggest that radiosensitive bone marrow stem cells and/or the bone marrow microenvironment are necessary for the generation of both Mo and the splenic suppressor M0, and that one stem cell might be common to both types of mononuclear phagocytes. This notion was explored further by employing congenitally anemic mice of the Sl/Sl^d genotype in which the hemopoietic microenvironment is genetically defective in supporting stem cell proliferation, differentiation and function.

The congenital defect in Sl/Sl^d mice was found to be additionally expressed by a monocytopenia of less than 10% of the levels in normal congenic littermate controls, and by failure of splenic M0 CFU to increase in response to *C. parvum*. PGE producing suppressor M0, however, were induced normally by *C. parvum* in Sl/Sl^d mice. These data establish that significant impairment of the formation of Mo is part of the overall hemopoietic defect in Sl/Sl^d mice. The fact that PGE producing suppressor splenic M0, however, were inducible in the presence of profound monocytopenia indicates that these suppressor M0 are independent of

mechanisms that regulate blood Mo production. The data in 89Sr mice showed the additional independence of the suppressor splenic M0 from splenic M0 CFU, because the latter increase above normal levels in 89Sr treated mice. Overall, these findings show a complexity among M0 populations that requires reclassification. At the simplest level, M0 can be classified as bone marrow dependent (Mo and splenic suppressor M0) and independent (resident tissue M0 and splenic M0 CFU). The splenic suppressor M0, however, are Mo independent although bone marrow dependent. The data suggest at least three functionally definable populations of mononuclear phagocytes which appear to be independently regulated.

2.22 Role of PMN in M0 activation by *C. parvum*.

Haskill and his colleagues have hypothesized that phagocytosis of *C. parvum* by PMN is required for M0 activation by this immunomodulator. The 89 Sr system was used to render B6C3F1 and CD-1 mice granulocytopenic prior to i.p. injection of *C. parvum*. The subsequent granulocyte response in the peritoneal cavity was measured during both the acute (5 hr after *C. parvum*) and the chronic (7 day) inflammatory reactions. The 5 hr PMN influx was reduced by 85 and 86% in CD-1 mice given *C. parvum* 3 or 7 days respectively after 89Sr treatment. The 5 hr acute PMN influx in B6C3F1 mice was inhibited by 86% when *C. parvum* was administered 3 days after 89Sr, and 41% when *C. parvum* was administered at 7 days after 89Sr. The chronic, 7 day, PMN response was also depressed. PMN levels were reduced by 66-76% in CD-1 mice and by 83-89% in B6C3F1 mice. If an acute PMN influx is required for *C. parvum* complete activation of M0, then M0 from 89Sr treated mice tested 7 days after *C. parvum* should possess lower alkaline phosphodiesterase I (APD-I) ectoenzyme activity indexes and lower cytotoxicity against tumor cells. We found that *C. parvum* M0 from normal or 89Sr treated mice showed normal activation as assessed by reduced 5'nucleotidase ectoenzyme activity (data not shown). *C. parvum* M0 from 89Sr treated mice in both mouse strains, however, exhibited markedly lower APD-I activity indexes than did activated M0 from normal mice (Table 4). *C. parvum* M0 from 89Sr treated B6C3F1 mice also exhibited markedly reduced cytotoxicity against the B16F10 melanoma tumor target cells (Figure 1). In these experiments, unfortunately, M0 from *C. parvum* activated normal CD-1 mice were not cytotoxic for B16F10 cells, so antitumor activity of M0 from the 89Sr mice could not be assessed. However, the APD-I activity indexes suggest that *C. parvum* M0 from 89Sr treated CD-1 mice would exhibit reduced antitumor activity against the B16F10 tumor target.

The present results differ somewhat from our previous data indicating completely normal activation of peritoneal M0 by *C. parvum* in 89Sr treated CD-1 mice (Infect. Immun., 1982; Lab. Invest. 1983). At present we have not completely established the reasons for the differences in results. However, the APD-I activity indexes in the previous work showed no difference between *C. parvum* activated M0 from 89Sr or control mice, correlating with their positive antitumor activity against Lewis lung carcinoma target cells. It is possible that the *C. parvum* preparations have changed; we, as well as other investigators, have noted increased chronic PMN exudation with *C. parvum* over the past few years. On the other hand, the differences in antitumor activity of *C. parvum* M0 from 89Sr treated mice may be related to the tumor target used (Lewis lung vs. B16F10); we have previously noted that Lewis lung cells are more sensitive to M0 antitumor activity than are B16 cells. Direct comparative experiments are planned to address these issues.

2.23 Effect of 89Sr on resident M0 and M0 activation by a variety of immunomodulators in CD-1 mice. We investigated the effect of 89Sr on M0 activation in CD-1 mice by C. parvum, thioglycollate broth (TG), pyran, and M. bovis BCG strain. In contrast to the results obtained with C. parvum, PMN and M0 levels in the peritoneal cavity after pyran (7 days) or BCG (14 days) administration were not reduced in the 89Sr treated CD-1 mice. Lymphocyte levels were reduced, however. BCG M0 from 89Sr treated mice exhibited the same ectoenzyme phenotype (reduced 5'N and APD activity) as did BCG M0 from normal mice, and both M0 populations were cytotoxic for Lewis lung carcinoma cells. With pyran M0 from normal or 89Sr treated mice, 5'N activity was markedly reduced in both; the APD-I and antitumor results were inconclusive. These results and those from the C. parvum studies point out that there may be significant differences among immunomodulators in mechanisms of M0 activation.

The effect of 89Sr on the ability of resident peritoneal M0 to proliferate in response to CSF, and the effect of TG and C. parvum were also investigated. Table 5 shows that treatment with 89Sr did not appear to affect markedly the proliferative capacity of Res M0, nor the enhanced proliferative capacity of TG or CP M0. These data in the CD-1 mouse, together with the M0 protein profiles, ectoenzyme phenotypes, antitumor activity, and intrinsic antiviral activity to HSV-1, indicate that at least in this mouse strain 89Sr treatment does not alter a number of resident M0 functions. The experimental results also support the concept that certain immunomodulators can activate the Res peritoneal M0 in CD-1 mice without a necessary influx of inflammatory monocytes.

2.24 Effect of specific activity of 89Sr, route of administration, and mouse strain on cellular depletion. The CD-1 mouse appears to be particularly sensitive to 89Sr, in that circulating M0, PMN, and NK cells are profoundly suppressed within a week after treatment with 89Sr of either high (6000 Ci/g) or low (100 mCi/g) specific activity. Data with the B6C3F1 mouse have emphasized the importance of mouse strain in cellular suppression; NK cell activity in the B6C3F1 mouse remains high for at least one month after treatment with either high or low SA 89Sr (data not shown). Other experiments in CBA/J mice have shown a close dependence of both the magnitude and duration of suppression of circulating M0 and bone marrow M0 CFU with the specific activity of 89Sr. In contrast to these variations with mouse strain and SA of 89Sr, there are insignificant differences between 89Sr administered i.v. or i.p. to CD-1 or B6C3F1 mice (data not shown).

2.3 Development of liposome methodology to decrease tissue M0 in normal and 89Sr treated mice.

2.31 Liposome preparation. Liposomes have been prepared by the method of solvent exchange starting with lipids dissolved in chloroform, then evaporated to dryness under a stream of nitrogen. The lipids are then dispersed in saline and dispersed to form liposomes in an ultrasonic field. Preparations have included liposomes prepared from phosphatidylcholine (PC), phosphatidic acid (PA), phosphatidyl serine (PS), cholesterol and iodcholesterol. The reference preparation is PC:PS:cholesterol in a 7:1:3 molar ratio. This has yielded a negatively charged multilamellar liposome of 200-600 Å as estimated by electron microscopy. We employed two markers to quantitate liposome uptake by

M0: iodocholesterol which is quantitated by x-ray fluorospectrometry (courtesy of Dr. John Bray, ECU School of Medicine), and 3H-cholesterol which is quantitated by liquid scintillation counting.

2.32 Liposome uptake. Studies have employed two sources of M0 for determining optimum conditions for liposomal uptake: mouse resident peritoneal M0 and the P388D1 continuous mouse M0 cell line.

2.321 Effect of chemical constitution.

Constitution of liposomes with PA and PS results in a negative charge on the vesicles; deletion of PA and PS results in a neutral vesicle. Uptake of negatively charged liposomes by P388D1 and peritoneal M0 was significantly greater than of neutral liposomes. The uptake of negatively charged liposomes by P388D1 cells varied inversely with the cholesterol content of the liposomes. The levels of cholesterol used as carrier in the 3H-cholesterol studies, however, were too low to affect the results negatively. Uptake of liposomes constituted with equimolar amounts of either PA or PS phospholipids was comparable.

2.322 Uptake by suspended or adherent M0. M0 (2×10^6 in 1 ml Ca and Mg free PBS containing 0.2 ml liposome suspension) were tumbled for varying times at 37C. After washing twice, the cells were pelleted and counted. Similar numbers of M0 adherent to 35mm petri dishes after 24 hr were washed and liposomes (0.2 ml diluted to 1 ml in PBS) were added. After varying intervals the cells were lysed with NP40, the lysate washed out with PBS and counted. Cell associated radioactivity was ten-fold higher in the suspension preparations than among adherent cells, suggesting a higher efficiency of the liposome-M0 contact events.

2.323 Effect of temperature on uptake. One serious question is whether cell-associated activity of marked liposomes signifies binding to the cell membrane, endocytosis or both. Reduction of temperature to 4 C essentially halts endocytosis. Parallel preparations of P388D1 cells in suspension and adherent to petri dishes were incubated for increasing intervals from 5-180 min. The steepest increase in radioactivity was found to take place in the first 15-30 min. Cell associated activity increased linearly at lower rates at 37C from these points to the last sampling at 180 min. At 4 C the increase among suspensions was 50% less than at 37C (Fig. 1). In the adherent cells at 4C, no progressive increase was seen after 15 min. The data suggest that a substantial amount of cell associated radioactivity represents liposomes on the cell surface and not within the cell. This could be an important consideration in preparing liposomes with which to transport agents into cells. We are currently planning to test the effects of liposomes containing the ricin A chain on M0 in vitro and in vivo. The results of these experiments should provide additional information concerning liposome uptake and processing in M0.

3.0 Publications/presentations related to this project.

3.1 Manuscripts

Shibata, Y. and A. Volkman. Ontogeny and differentiation of mononuclear phagocytes. I. The effect of bone marrow depletion on prostaglandin E producing suppressor macrophages in mouse spleen. J. Immunol., in press 1985.

Shibata, Y. and A. Volkman. Ontogeny and differentiation of mononuclear phagocytes. II. The effect of hemopoietic microenvironment on suppressor macrophages in the congenitally anemic mice of the *gld* strain.

Shibata, Y., W.L. Dempsey, P.S. Morahan and A. Volkman. Selectively eliminated blood monocytes and splenic suppressor macrophages in mice depleted of bone marrow by strontium 89. J. Leuk. Biol., in press 1985.

Morahan, P.S., A. Volkman, W.L. Dempsey, and J. Connor. Antimicrobial activity of various immunomodulators: independence from normal levels of circulating monocytes and NK cells. Submitted.

Dempsey, W.L., A. Smith, A. Volkman and P.S. Morahan. Effect of inapparent murine hepatitis virus infection on mononuclear phagocytes and resistance to another virus infection. In preparation.

3.2 Abstracts and presentations

Ackermann, M.F., W.L. Dempsey, P. Hwu, D. Tenney, E. Leake and P.S. Morahan. Effect of PMN depletion on M0 activation by P. acnes. Eastern Pennsylvania Branch of the American Society of Microbiology, February 1985.

Tenney, D.J., M.F. Sit, E.R. Leake, H.T. Largent and P.S. Morahan. Effect of 89Sr on protein synthesis and intrinsic resistance of peritoneal macrophages to HSV-1. Reticuloendothelial Society annual meeting, August 1985.

4.0 Research Plan

4.1 Continue to evaluate immunomodulators for antimicrobial efficacy in normal CD-1 mice and in mice with selective defects in various effector cell populations. We will complete our studies with the pyrimidinone, MDP-PE, MTP, detoxified endotoxin, and trehalose dimycolate, and continue efforts to obtain other potentially interesting compounds to test. Depending upon the results, one or more of these compounds will be selected for test in 89Sr treated mice.

4.2 Define the effects of selected immunomodulators on effector cell populations in mice depleted of various cells. The studies with C. parvum as a prototype will be continued in order to determine the contributions of ontogenetic and environmental factors to M0 activation, by infusing lethally irradiated mice with spleen from 89Sr treated syngeneic donors. As interesting compounds are identified in the host resistance studies, the mechanisms of immunomodulatory activity of these may also be explored in the 89Sr or SI/Sld mouse systems. However, we expect that most of our efforts will be focused on Objectives 1 and 3 during our final contract period.

4.3 Develop effective methods to decrease tissue M0 in normal and 89Sr treated mice. Experiments will be focused on in vivo approaches, particularly the delivery of ricin (and possibly other toxic agents) and later, astatine-211, in normal and 89Sr treated mice. We will study the fate of the liposome inoculum, and the consequences of liposome-toxin injection on resident M0 populations at the site and remote from injection in regard to cell survival and function. We will also assess effects on host resistance to infection. Effects on non-M0 populations such as NK cells, PMN and lymphocytes may also be investigated. Finally, the effects of liposome-toxin treatment on the capacity for cell recovery and modulation by selected biologic response modifiers will be addressed.

TABLE 1
Effect of Mouse Hepatitis Virus (MHV) Infection of Peritoneal MØ
Ectoenzyme Levels^a

Day after infection	5'N	APD
3	19.8 ± 8.8	14.1 ± 0.8 ^b
5A	14.2 ± 4.8	10.9 ± 1.5 ^b
B	12.4 ± 2.4 ^b	9.1 ± 0.6 ^b
7-8	10.9 ± 1.2 ^b	20.8 ± 1.4
10	1.1 ± 1.0 ^b	18.0 ± 1.5
Control 1 ^c	19.5 ± 3.5	22.8 ± 0.2
Control 2 ^c	24.2 ± 2.8	18.1 ± 2.8

^aBalb/c mice were infected with the JHM strain of murine hepatitis virus.

^b Represents a significant reduction ($p < 0.05$) in activity as compared with the combined controls.

^c Controls were taken at the beginning (1) and the end (2) of the study.

TABLE 2

Protective Effects of ABPP Immunomodulator Against Infections in Normal CD-1 Mice

∞	Treatment			EMC			Listeria monocytogenes			HSV-2		
	Drug	Dose (mg/kg)	Route	Regimen (day)	Mortality		Survival		Median Day	Mortality		Survival
					Dead/ Total	%	Dead/ Total	%		Dead/ Total	%	Median Day
	CMC	1.0%	I.P.	-1 or -7	14/15	(93%)	4.4	15/15	(100%)	3.0	14/15 (93%)	10.8
	MVE-2	50	I.P.	-1	3/10	(30%)	> 14.0*	ND	0/10 (0%)*	> 14.0*	0/10 (0%)*	> 21.0*
	C. Parvum	35	I.P.	-7	ND			0/10 (0%)			ND	
	ABPP	200	I.P.	-7	6/10	(60%)*	8.3*	7/10	(70%)*	6.5	ND	
	ABPP	200	I.P.	-1	3/10	(30%)*	> 14.0*	10/10	(100%)	3.3	ND	
	ABPP	100	I.P.	-1	1/10	(10%)*	> 14.0*	ND			ND	
	ABPP	200	P.OS	-1	2/10	(20%)*	> 14.0*	ND			ND	
	ABPP	100	P.OS	-1	7/10	(70%)	7.1	ND			ND	
	ABPP	200	I.P.	-2, -1, -1/3	ND			10/10	(100%)	2.5	ND	
	ABPP	200	P.OS	-2, -1, -1/3	ND			9/10	(90%)	5.6	ND	
	ABPP	200	I.P.	-3, -2, -1	ND			ND			9/10 (90%)	11.1
	ABPP	200	P.OS	-3, -2, -1	ND			ND			3/9 (33%)*	16.8*
	ABPP	100	I.F.	-3, -2, -1	ND			ND			4/10 (40%)*	17.1*
	ABPP	100	P.OS	-3, -2, -1	ND			ND			5/10 (50%)	15.0

* $p < 0.05$ as compared with placebo control, using the Chi Square test for mortality data and Mann Whitney U test for survival analysis.

TABLE 2

Protective Effects of Bacterial Cell Wall Types of Immunomodulators Against Infections in Normal CD-1 Mice

Drug	Treatment			EMC			<i>Listeria monocytogenes</i>		
				Mortality		Survival	Mortality		Survival
				Dead/ Total	(%)	Median Day	Dead/ Total	(%)	Median Day
CMC	1.0%	I.P.	-1 or -7	14/15	(93%)	4.1	15/15	(100%)	3.5
MVE-2	50	I.P.	-1	0/10	(0%)*	10.0*	ND		
<u>C. parvum</u>	35	I.P.	-7	ND			1/10	(10%)*	9.3*
MTP-PE	1	I.P.	-7	9/10	(90%)	4.7	10/10	(100%)	4.0
MTP-PE	1	P.OS	-7	10/10	(100%)	4.2	8/10	(80%)	5.6
MTP-PE	1	I.P.	-1	9/10	(90%)	5.0	ND		
MTP-PE	1	P.OS	-1	10/10	(100%)	4.4	ND		
MTP-PE	1	I.P.	+2	ND			8/10	(80%)	5.2
MTP-PE	1	P.OS	+2	ND			7/10	(70%)	6.3
NaCl	0.85%	I.P.	-1,0,+1,+2	13/15	(87%)	5.6	ND		
MDP	4	I.P.	-1,0,+1,+2	4/10	(40%)*	8.6	ND		
MDP	1	I.P.	-1,0,+1,+2	9/10	(90%)	5.0	ND		
MDP-GDP	4	I.P.	-1,0,+1,+2	9/10	(90%)	4.7	ND		
MDP-GDP	1	I.P.	-1,0,+1,+2	5/9	(56%)	7.6	ND		
CMC	1.0%	I.P.	-1,0,+1,+2	5/10	(50%)	8.1	ND		
MTP-PE	1	I.P.	-1,0,+1,+2	10/10	(100%)*	3.9*	ND		
CMC	1.0%	P.OS	-1,0,+1,+2	10/11	(91%)	4.9	ND		
MTP-PE	1	P.OS	-1,0,+1,+2	9/10	(90%)	5.0	ND		
Squalene	2%	I.P.	-7	10/10	(100%)	3.7	8/10	(80%)	8.2
Detox LPS	4	I.P.	-7	7/10	(70%)	6.6	0/10	(0%)*	>12.0*
TDM	4	I.P.	-7	7/10	(70%)*	6.4	0/10	(0%)*	>12.0*
Detox LPS +TDM	4+4	I.P.	-7	5/10	(50%)*	8.3*	0/10	(0%)*	>12.0*

* $p < 0.05$ as compared with placebo control, using the Chi Square test for mortality data and Mann Whitney U test for survival analysis.

TABLE 4

Effect of ^{89}Sr on APD-I activity index^a

	B6C3F1 Mice		CD-1 Mice	
	Exp. 1 ^b	Exp. 2 ^c	Exp. 1	Exp. 2
Control	10.0	10.0	29.2	33.7
^{89}Sr	3.9 ^b	3.0 ^c	5.9 ^b	5.56 ^c

^aRatio of the APD specific activity (SA) between untreated resident MØ and the APD S.A. of MØ activated with C. parvum.

^bAPD-I activity index in mice injected with C. parvum 3-4 days after ^{89}Sr .

^cAPD-I activity index in mice injected with C. parvum 7-8 days after ^{89}Sr .

TABLE 5

Effect of ^{89}Sr on MØ CFU in the Peritoneal Population

	^{89}Sr SA (m Ci/gr Sr)	MØ CFU / 10^3 MØ plated					
		Res	+ ^{89}Sr	<u>C. parvum</u>	+ ^{89}Sr	TG	+ ^{89}Sr
Exp 1	6,000,000	3.2	4.0	16.6	35.0	12.6	14.3
Exp 2	100	5.1	1.5	75.8	49.1	ND	102.2

Peritoneal cells were plated in liquid medium containing 10% FCS, 10% HoS, 10% LCM in a MEM and incubated for 2 weeks (Exp 1) or 3 weeks (Exp 2). Cultures were stained with Dif-Quik, and colonies containing more than 50 cells were counted.

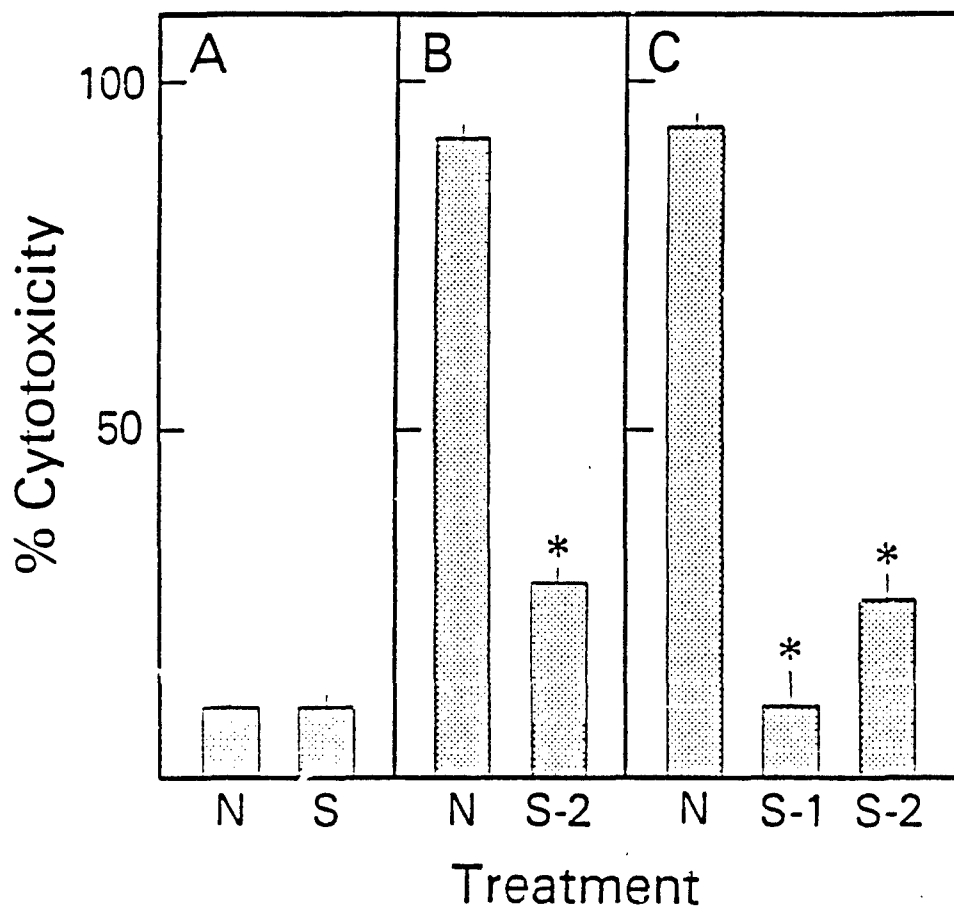


Figure 1 : Effect of ^{89}Sr on the AT activity against B16F10 melanoma target cells of resident (A) and activated MO four (B) and seven (C) days after P.acnes administration. The MO:target ratio is 10:1. Data are expressed as the mean (SEM) from four different experiments with 5-8 animals per treatment group processed individually.

N = Untreated (A) or P.acnes activated (B,C) MO from control mice.

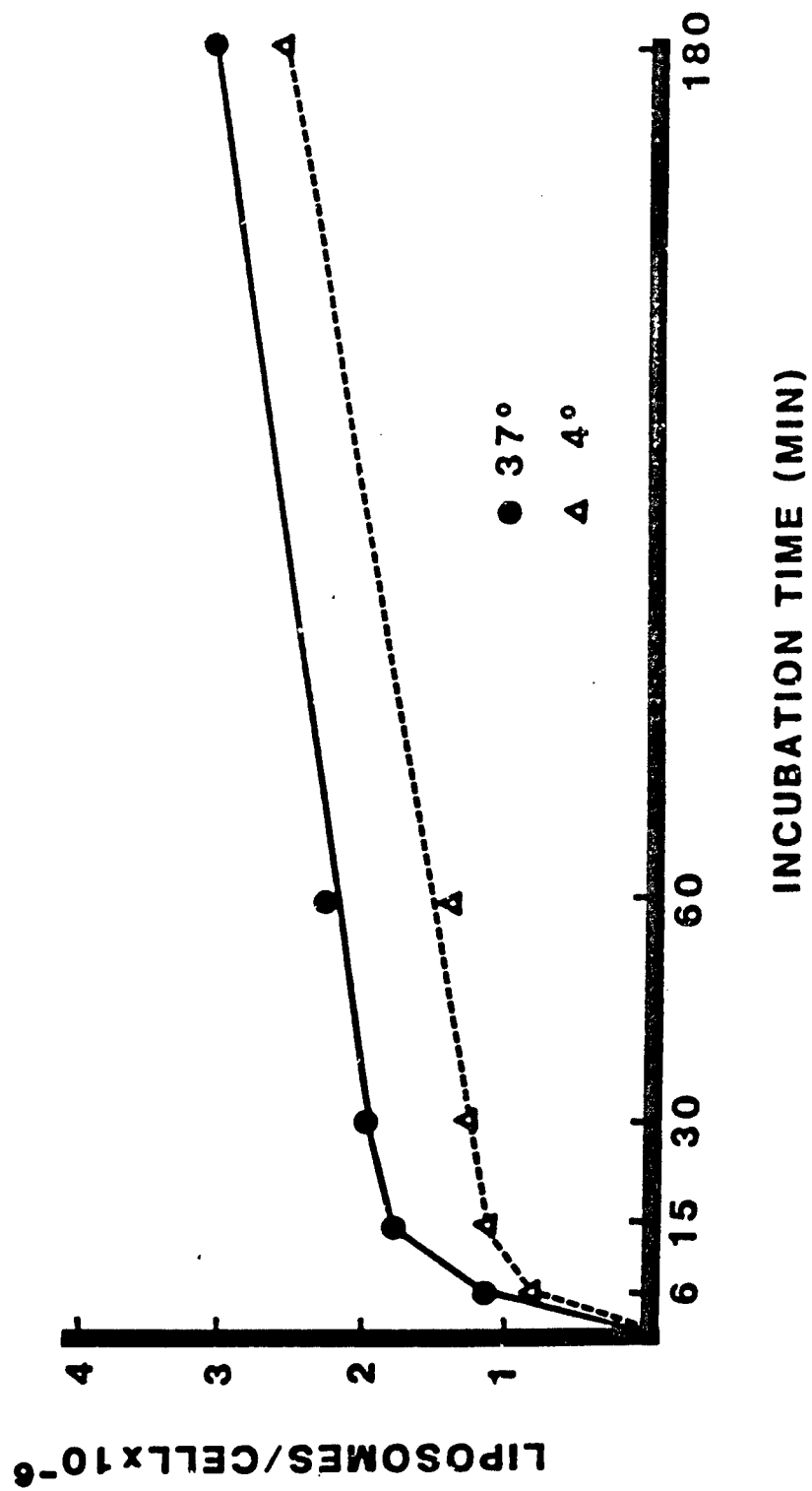
S = Resident MO from mice treated with ^{89}Sr .

S-1 = P.acnes injected 3 or 4 days after ^{89}Sr treatment.

S-2 = P.acnes injected 7 or 8 days after ^{89}Sr treatment.

* = $P < 0.05$ as compared with P.acnes activated MO from naive control mice.

CELL ASSOCIATED RADIOACTIVITY (CPM)
THE EFFECT OF TEMPERATURE



END

FILMED

9-85

DTIC