INFECTION AND IMMUNITY, June 1978, p. 685-691
0019-9567/78/0020-0685$02.00/0

Vol. 20, No. 3
Printed in U.S.A.

Relationship of Macrophages to Cell-Mediated Immunity in Experimental *Nocardia asteroides* Infection

T. SUNDARARAJ AND S. C. AGARWAL*

Department of Microbiology, Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry-605006, India

Received for publication 15 November 1977

Marked in vivo intracellular killing of *Nocardia asteroides* occurred in the peritoneal macrophages obtained 72 h after an intraperitoneal challenge with *N. asteroides*, in guinea pigs either actively immunized with ribonucleic acid protein or passively immunized by immune spleen cell transfer from actively immunized donor guinea pigs. This specific killing of *N. asteroides* in immune macrophages persisted for at least up to 60 days. Administration of antimacrophage sera before intravenous challenge with *N. asteroides* in the immune guinea pigs produced an early death of the animals, and the total tissue counts of *N. asteroides* in the liver, spleen, lungs, and heart remained the same in them as in unimmunized controls.

We reported earlier on the development of cell-mediated immunity (CMI) during experimental infection with *Nocardia asteroides* in guinea pigs (5) and after immunization with its ribonucleic acid protein (P-RNA) fraction (6). CMI was protective as shown by the survival of guinea pigs passively immunized by spleen cell transfer and by a decrease in *Nocardia* tissue counts in the different organs. We have now investigated the role of macrophages in affording such protection (1) by measuring in vivo multiplication of *Nocardia* organisms in peritoneal macrophages after an intraperitoneal challenge with *N. asteroides* organisms in normal and immunized guinea pigs and (ii) by observing the effect of antimacrophage sera (AMS) on the survival of immunized guinea pigs challenged with *N. asteroides* and the multiplication of *Nocardia* organisms in different tissues of these animals.

MATERIALS AND METHODS

Animals. Healthy outbred guinea pigs of both sexes, weighing 350 to 400 g and bred in our central animal house, were used. They were given a diet of Bengal gram and vegetables. Young adult white rabbits, weighing 1.5 to 2 kg and also bred in our central animal house, were used for preparing AMS.

Bacteria. *N. asteroides* suspensions were used for challenge, and *Listeria monocytogenes* was used for the assay of microbicidal activity in control and immune macrophages as described previously (4, 5).

Immunogens, immunization procedures, and CMI measurements. Techniques similar to those described earlier were used (5, 6). Suspensions of live *N. asteroides* and P-RNA fraction were used as immunogens. Guinea pigs were actively immunized with live *N. asteroides* or P-RNA. Passive immunization was carried out by immune spleen cell transfer from these actively immunized animals. CMI was measured by using the macrophage migration inhibition (MMI) test and by macrophage microbicidal activity. The latter was assayed by the technique described by Simon and Sheagren (4). MMI was tested using the technique of David et al. (1). *Nocardia* polypeptide, purified protein derivative, and P-RNA fraction were added as antigens for stimulating immune macrophages while measuring the percentage of MMI or microbicidal activity.

The following groups of guinea pigs were used: (i) normal healthy guinea pigs or those in which normal spleen cells had been passively transferred—these animals served as controls; (ii) guinea pigs actively immunized with live *N. asteroides*—in these animals, 0.2 ml of a suspension of *N. asteroides* (1.3×10^8 organisms per ml) in incomplete Freund adjuvant was injected subcutaneously, and lesions were allowed to develop and heal for 60 days; (iii) guinea pigs actively immunized by the P-RNA fraction of *N. asteroides* in incomplete Freund adjuvant—a dose of 0.2 ml which contained 6 μg of RNA and 5 μg of protein, as determined earlier, was inoculated intradermally, 0.1 ml each, at two different sites, a second injection of the same dose was repeated after 1 week, and 30 days after the second injection, these animals were challenged intraperitoneally with *N. asteroides*; (iv) passively immunized guinea pigs obtained by immune spleen cell transfer from actively immunized donor guinea pigs of group iii—the spleen cells were injected intravenously into normal guinea pigs in doses ranging from 1×10^8 to 3×10^9 cells.

Intraperitoneal challenge and viable counts in macrophages. Actively or passively immunized guinea pigs were challenged intraperitoneally with *N. asteroides*. The viable count of *Nocardia* was done in peritoneal macrophages at 0 and 72 h. Accumulation

of peritoneal exudate cells (PEC) in the peritoneal cavity was induced by intraperitoneal injection of 20 ml of brain heart infusion broth containing 3% peptone (Difco Laboratories) into guinea pigs. Forty-eight hours after induction, live *N. asteroides* suspension in saline (8.2×10^7 organisms in 5 ml) was inoculated intraperitoneally, and the abdomen was gently kneaded for thorough mixing of the fluid inside the peritoneal cavity. Phagocytosis was allowed to occur for 2 h. At the end of this period, 20 ml of cold Hanks balanced salt solution (BSS) containing heparin (5 U/ml) was injected intraperitoneally, and 5 ml of fluid was aspirated. The fluid was centrifuged at 1,000 rpm for 10 min to deposit PEC. These cells were washed thrice in Hanks BSS and resuspended in it. The number of cells was counted in a hemocytometer. PEC (10^6) were lysed with distilled water for 10 min, and serial dilutions were made in 0.85% saline. From each dilution, 0.2 ml was inoculated into the blood agar plates. The plates were incubated at 37°C for 3 to 5 days, and the number of viable organisms was counted. The same procedure was repeated 72 h after the intraperitoneal injection of *N. asteroides*, and the number of viable intracellular bacteria was counted.

Intravenous challenge and viable count in tissues. Different groups of guinea pigs (as described above) were treated with AMS and later challenged intravenously with *N. asteroides* suspensions. The effect of prior administration of AMS on their survival time and on the total *Nocardia* tissue count in various organs was recorded. For intravenous challenge, a suspension of live *N. asteroides* in 0.1 ml of saline containing 2×10^8 cells was injected intracardially into guinea pigs. They were sacrificed at definite time intervals, and viable counts were done in different tissues for *N. asteroides*. Lungs, heart, spleen, liver, and kidneys were homogenized. Serial dilutions of homogenates were made in 0.16 M saline. From different dilutions, 0.2 ml was inoculated into blood agar plates. The plates were incubated at 37°C for 3 to 5 days, and the number of colonies was counted.

Preparation of AMS. AMS was prepared in rabbits to determine the effect of prior administration of AMS (i.e., before immunization in the above groups of actively and passively immunized animals) on their survival and on tissue counts in lungs, liver, spleen, heart, and kidneys. The following procedure, as described by Pearson and Osebold (2), was used. First, peritoneal macrophages were induced and harvested as follows. A 20-ml amount of brain heart infusion broth, containing 3% peptone (Difco Laboratories), was injected intraperitoneally into normal guinea pigs. PEC were collected 2 days later in Hanks BSS containing heparin (5 U/ml). They were washed in Hanks BSS and suspended in enriched Eagle minimal essential medium. PEC were then cultured in 50-ml conical flasks incubated at 37°C in the presence of 5% CO_2. The medium was changed after 1, 24, and 48 h, removing the nonadherent cells. After 4 days, the remaining adherent cells were collected by scraping with a rubber policeman. They were washed and suspended in minimal essential medium containing no serum. The viability was checked by the trypan blue dye exclusion method, and the viable macrophages were counted. More than 95% of the cells were viable. These macrophages were injected intravenously into the rabbits in doses ranging from 10^7 to 3×10^7 cells. A second injection was given 14 days later. Seven days after the last injection, the sera were collected, inactivated at 56°C for 30 min, and stored at −70°C. The macrophage antisera were tested for their cytotoxicity and hemagglutinating activity.

Macrophage cytotoxicity assay of AMS. The macrophage cytotoxicity assay was carried out using the method described by Pearson and Osebold (2). PEC obtained from normal guinea pigs were washed, suspended in Hanks BSS, and adjusted to a concentration of 10^6 cells per ml. Twofold serial dilutions of AMS were made in Hanks BSS. To 0.5 ml of different dilutions of AMS 0.5 ml of macrophage suspension (PEC) was added, and the mixture was incubated at 37°C for 30 min. After incubation, 0.1 ml of pooled fresh guinea pig complement was added, and the mixture was reincubated at 37°C for 30 min. At the end of this time, 0.2 ml of 0.4% trypan blue in Hanks BSS was added, mixed, and kept for 5 to 10 min. It was filled in a Neubar counting chamber and the number of dead cells was counted under a light Olympus microscope using ×400 magnification and bright-field optics. The reaction was judged to be cytotoxic when 50% or more of the cells were stained by trypan blue. The log of the reciprocal of the highest dilution of AMS which caused cytotoxicity was designated as the titer. The AMS contained high titers of cytotoxic antibodies to macrophages. The cytotoxic titer for macrophages was 2^7. It contained little cytotoxic activity against lymphocytes; the titer was only 2^3.

Hemagglutination test. The hemagglutinating activity of AMS was tested according to the procedure described by Pearson and Osebold (3). Freshly collected guinea pig blood was washed three times in a 0.85% NaCl solution containing heparin (5 U/ml). Twofold serial dilutions of AMS were made in minimal essential medium containing 0.1% bovine serum albumin, using microtiter plates. A 0.05-ml amount of 1% guinea pig erythrocytes was added to an equal volume of diluted (vol/vol) serum in each well. After mixing, the plates were incubated at 37°C for 30 min. The highest dilution of serum which caused 50% of the cells to agglutinate was recorded as the titer. AMS also possessed hemagglutinating activity up to a titer of 2^4.

RESULTS

The effect of immunization on in vivo intracellular killing of *N. asteroides* by peritoneal macrophages in different groups of guinea pigs after intraperitoneal challenge with 10^7 *Nocardia* organisms was as follows.

(i) Guinea pigs immunized with P-RNA and challenged intraperitoneally with live *N. asteroides* at different time intervals after determining the CMI. *N. asteroides* counts were done in the peritoneal macrophages collected 2 and 72 h after challenge (Table 1). Twenty-five days postimmunization, the guinea pigs (no. 210, 211, and 212) possessed high CMI, as indicated by increased MMI and microbicidal

TABLE 1. *In vivo intracellular killing of N. asteroides by peritoneal macrophages obtained from P-RNA-immunized guinea pigs*

Guinea pig no.	CMI at time of challenge					Challenge i.p.[a] (days post-immunization)	No. of intracellular viable *Nocardia* organisms in 10^6 PEC		
	MMI, % (P-RNA)	No. of viable *Listeria* ($\times 10^3$)					2 h	72 h[d]	*P* value (*t* test)
		Control[b]		+ P-RNA[c]					
		1.5 h	4 h	1.5 h	4 h				
210	27	2,350	4,500	1,000	400	25	6,600	215	
211	35	50	100	150	20		8,000	225	<0.001
212	40	2,100	16,500	1,500	105		7,050	345	
213	19	11,800	20,000	500	40	30	3,200	40	
214	26	15,200	18,750	6,850	3,400		3,650	1,000	<0.001
215	34	3,200	4,000	1,700	450		4,450	45	
216	28	2,500	3,000	3,500	325	40	7,500	60	
217	51	3,100	10,500	8,500	355		10,000	5	<0.001
218	31	3,350	16,000	11,500	410		2,000	45	
192	−4[e]	250,000	185,000	55,000	250	60	6,600	2,450	
193	24	45,500	14,000	25,500	500		5,750	2,600	<0.001
207	2	40,500	10,000	850	95		5,950	485	

[a] i.p., Intraperitoneal.
[b] Controls show increase in 4-h bacterial count in macrophages or intracellular growth.
[c] Immune macrophages stimulated with P-RNA show decrease in 4-h *Listeria* count or intracellular killing.
[d] Marked decrease in 72-h *Nocardia* count showing intracellular killing.
[e] Enhancement of MMI (percentage).

activity. When these guinea pigs with high CMI were challenged intraperitoneally with *N. asteroides*, the peritoneal macrophages exhibited specific in vivo killing of *N. asteroides*, as shown by a decrease in the 72-h intracellular count in comparison to the 2-h count. There was a 20- to 30-fold decrease ($P < 0.001$). Similarly, 30 days postimmunization, guinea pigs no. 213, 214, and 215 showed a marked in vivo intracellular killing of *N. asteroides* by macrophages. The 2-h *Nocardia* count in macrophages decreased from 3,200–4,450 to 40–1,000 organisms in 72 h, indicating a decrease of 3.6- to 98-fold ($P < 0.001$). At 40 and 60 days postimmunization, guinea pigs no. 216, 217, and 218 and guinea pigs no. 192, 193, and 207, respectively, showed a similar in vivo killing. In contrast, control unimmunized guinea pigs (no. 208, 231, and 232) or animals which received spleen cells from normal guinea pigs (no. 204, 205, and 206) showed increased intracellular growth of *N. asteroides* in macrophages after intraperitoneal challenge with *N. asteroides* (Table 2). There was a 1.8- to 8-fold increase in the number of bacteria in these macrophages.

(ii) Guinea pigs passively immunized by transfer of immune spleen cells from P-RNA-immunized animals (Table 2). To eliminate the effect of humoral immunity, immune spleen cells were passively transferred from P-RNA-immunized donor guinea pigs (no. 190 and 191) and the recipients (no. 194 to 199) were challenged intraperitoneally with *N. asteroides*. In these recipients, there was considerable intracellular killing of *N. asteroides* in macrophages. The 2- and 72-h intracellular counts varied from 3,950 to 7,750 and from 455 to 1,500, respectively. The decrease in the 72-h count ranged from 3.9- to 17-fold ($P < 0.001$).

(iii) Guinea pigs passively immunized by transfer of immune spleen cells from donors which had been actively immunized with live *N. asteroides* (Table 2). CMI was transferred from immune donor guinea pigs (no. 153, 154, and 155) actively immunized with live *N. asteroides* to normal recipients (no. 182 to 189). The donor animals possessed high CMI because MMI (percentage) and microbicidal activities were enhanced after stimulation of their macrophages with different *N. asteroides* antigens (purified protein derivative, polypeptide, and P-RNA). Immune spleen cells were transferred into recipients from the guinea pigs possessing high CMI. The intracellular viable count of *N. asteroides* in the recipients (no. 182 to 189) challenged intraperitoneally with *N. asteroides* varied at 2 h from 2,150 to 8,350. Considerable intracellular killing of *N. asteroides* was present as shown by a marked decrease in the intracellular count at 72 h. It showed an average decrease of 123-fold ($P < 0.001$).

Effect of antimacrophage sera on *Nocardia* tissue counts in normal and P-RNA-immunized guinea pigs after *N. asteroides*

TABLE 2. *Intracellular killing of N. asteroides by peritoneal macrophages from guinea pigs passively immunized with immune spleen cells from donors actively immunized with live N. asteroides and P-RNA*

Cells	Donor						Recipient		
	CMI at time of transfer						Intracellular viable *Nocardia* in 10^6 PEC		
	Guinea pig no.	MMI, % (P-RNA)	Microbicidal activity (no. of viable *Listeria* $\times 10^3$)				Guinea pig no.		
			No. of antigens added[a]		P-RNA[b]				
			1.5 h	4 h	1.5 h	4 h		2 h	72 h[c]
Immune spleen cells from live *N. asteroides*-immunized guinea pigs	153	43	5,500	1,600	2,400	70	182	2,350	0
							183	8,350	0
							184	5,420	41
	154	26	1,750	6,000	2,180	1,950	185	3,100	0
							186	3,250	30
							187	2,400	75
	155	42	1,750	6,250	51,000	275	188	2,150	35
							189	7,550	110
Immune spleen cells from P-RNA-immunized guinea pigs	190	64	4,000	107,500	7,900	890	194	5,450	460
							195	6,750	505
							196	5,900	1,500
	191	35	4,350	5,500	48,000	185	197	5,450	750
							198	7,750	455
							199	3,950	755
Unimmunized control guinea pigs									
Spleen cells not received	208	−54[d]	2,100	2,000	1,800	2,500		4,500	8,250
	231	14	3,400	10,500	21,000	88,500		1,050	8,450
	232	0	21,000	11,500	30,000	33,500		1,100	4,000
Normal spleen cells from nonimmune guinea pigs	203	−73[d]	50,000	80,000	26,500	33,300	204	4,250	10,000
							205	3,650	7,000
							206	1,235	5,000

$P < 0.001$ (t test) (rows 154–155)

$P < 0.001$ (t test) (rows 190–191)

[a] Controls show increase in 4-h bacterial count in macrophages or intracellular growth.
[b] Immune macrophages stimulated with P-RNA show decrease in 4-h *Listeria* count or intracellular killing.
[c] Marked decrease in 72-h *Nocardia* count showing intracellular killing.
[d] Enhancement of MMI (percentage).

challenge (Tables 3 and 4). Normal unimmunized guinea pigs, not treated with AMS but challenged with *N. asteroides* (no. 165, 166, and 167), became sick in 7 to 10 days and died in 15 days. Normal unimmunized guinea pigs treated with AMS and challenged with *N. asteroides* (no. 242, 243, and 256 to 259) became very sick and died in 5 days. Three days after challenge, there was an increase in the number of *N. asteroides* in lungs, heart, kidneys, liver, and spleen. Total tissue counts in different organs in 3 or 5 days (just before death) after *N. asteroides* challenge are shown in Table 3. When animals immunized with P-RNA were challenged after AMS treatment (no. 223 to 230), they became sick in 3 days and died in 5 days. The various organs showed a large number of *Nocardia* organisms. There was no significant difference in the number of *Nocardia* organisms in different organs in AMS-treated, control immunized animals sacrificed in 3 days: lungs, $P = 0.2$; heart, $P = 0.2$; kidneys, $P = 0.2$; liver, $P = 0.2$; spleen, $P = 0.2$. In animals dying in 5 days, the P values for *Nocardia* tissue counts in lungs, heart, liver, kidneys, and spleen were >0.5.

Effect of AMS on *Nocardia* tissue counts after *N. asteroides* challenge in guinea pigs passively immunized by transfer of spleen cells from donors actively immunized with P-RNA (Table 3). Recipient guinea pigs containing immune cells passively transferred from spleens of P-RNA-immunized guinea pigs and challenged after AMS treatment (no. 244 to 249 and 251 to 254) became sick in 3 days and died in 5 to 6 days. In 3 days, however, there was an initial reduction in the number of *Nocardia* in lungs ($P = 0.001$), liver ($P = 0.001$), and spleen ($P = 0.001$). There was no significant difference in the number of bacteria in heart ($P = 0.2$) and kidneys ($P > 0.5$) when compared with AMS-

TABLE 3. *Effect of AMS on total tissue count of N. asteroides in different organs in guinea pigs actively immunized with P-RNA and passively immunized by immune spleen cell transfer from donors actively immunized with P-RNA*

Immunization[a]	Guinea pig no.	Day of sacrifice[b]	Gross lesions in:					No. of viable *Nocardia* in various organs × 10^{2}[c]									
			Lungs	Heart	Kidneys	Liver	Spleen	Lungs		Heart		Kidneys		Liver		Spleen	
Active	223	3	+	+	+	+	+	5,250	(0.2)	7,500	(0.2)	4,000	(0.2)	65,000	(0.2)	2,300	(0.2)
	225		+	+	+	+	+	2,500		17,000		29,500		20,000		1,500	
	227		+	+	+	+	−	4,500		45,500		5,000		1,175		200	
	224	5	+	+	+	+	+	1,300	(0.5)	300,000	(>0.5)	600	(>0.5)	50	(>0.5)	500	(>0.5)
	226		+	+	+	+	+	81,500		90,000		500		2,200			
	228		+	+	+	+	−	280,000				3,750		1,875		415	
	230		+	+	+	+	−	2,500		34,000		265		250		750	
										200							
Passive	244	3	+	−	+	−	−	450	(0.001)	1,500	(0.2)	500	(0.5)	750	(0.001)	500	(0.001)
	247		−	+	+	−	+	850		200		6,500		250		1,000	
	251		+	+	+	+	−	700		1,500		1,650		1,500		35	
	253		+	+	+	+	+	650		1,500		2,050		750		100	
	254		−	+	+	+	+	5		2,000		500		250		100	
	245	5	+	+	+	+	+	5,000	(>0.5)	3,550	(>0.5)	8,000	(>0.5)	7,500	(0.02)	45	(0.2)
	246		+	+	+	+	+	1,050		13,750		575		1,500		150	
	248		+	+	+	+	+	6,000		30,000		4,500		7,500		2,800	
	249		+	+	+	+	+	3,750		1,350		5,500		7,250		6,000	
	252		+	+	+	+	+	1,250		6,500		1,250		3,500		2,500	

[a] Challenged with *N. asteroides*.

[b] The animals were sacrificed when they were very sick or moribund.

[c] Numbers in parentheses are *P* values (*t* test).

TABLE 4. *Effect of AMS on the total viable count in different organs in guinea pigs adoptively immunized by immune spleen cell transfer from donors actively immunized with live N. asteroides*

Guinea pigs[a]	No.	Day of sacrifice[b]	Gross lesions in:				No. of viable *Nocardia* in various organs × 10²[c]						
			Lungs	Heart	Kidneys	Liver	Spleen	Lungs	Heart	Kidneys	Liver	Spleen	
(i) Adoptively immunized, treated with AMS	235	3–5	+	+	+	+	+	2,000	14,500	70,500	1,500	450	
	236		−	+	+	+	+	5,500	400	200	500	200	
	237		−	−	−	+	+	3,000	2,150	5,000	42,500	5,500	$P > 0.5$
	238		+	−	+	+	+	750	450	600	500	150	
	239		−	+	+	+	+	6,000	3,150	5,200	6,500	1,500	
								$P > 0.5$	$P > 0.5$	$P > 0.5$	$P > 0.5$		
(ii) Nonimmune, treated with AMS	242	3	−	−	+	+	+	5,000	250	200	175	450	
	243		+	−	+	+	+	7,500	2,900	600	7,500	2,000	
	256		+	+	+	+	+	2,000	2,500	2,750	2,500	1,500	
	257	5	+	+	+	+	+	200	12,500	125	1,000	1,050	
	258		+	+	+	+	+	2,400	3,000	6,500	750	60	
	259		+	+	+	+	−	5,000	8,000	500	250	200	
(iii) Nonimmune, not treated with AMS	167	6	+	−	+	−	−	15,000	400	4,750	150	750	
	165	15	+	+	+	−	−	1,500	2,000	1,000	50	3	
	166	15	+	+	+	−	−	10,000	2,000	5,000	150	30	

[a] All groups were challenged with *N. asteroides*.
[b] The animals were sacrificed when they were very sick or moribund.
[c] *P* values (*t* test) of group i in comparison with group ii; no significant difference is seen.

treated and challenged controls. The latter were either normal guinea pigs or those in which normal spleen cells were passively transferred. In 5 days, the animals showed an apparent increase in the number of bacteria in various organs, but there was no significant difference when compared with AMS-treated controls: lungs, $P > 0.5$; heart, $P > 0.5$; kidneys, $P > 0.5$; and spleen, $P > 0.2$; except in liver, $P = 0.02$. There was no protection in these animals.

Effect of AMS in guinea pigs passively immunized with immune spleen cells obtained from donors actively immunized with live *N. asteroides* (Table 4). The donors were guinea pigs actively immunized with live *N. asteroides*, in whom subcutaneous lesions were allowed to develop and heal for 60 days. The immune spleen cells were transferred from them into recipients (no. 235 to 239). The donors possessed high CMI, and they showed a high percentage of MMI. MMI varied from 46 to 65% after stimulation of macrophages with purified protein derivative, from 35 to 60% with polypeptide, and from 18 to 73% with P-RNA antigens. The recipient guinea pigs possessing high CMI, transferred from donors as above, were administered AMS and then challenged with *N. asteroides*. They became very sick in 3 days and showed a large number of viable organisms in different organs. The viable count was not significantly different from that of control unimmunized guinea pigs.

DISCUSSION

The exact mechanism by which cellular immunity protects guinea pigs in experimental *Nocardia* infection is not known. Our results show that this cellular immunity is mediated by a marked increase in the intracellular resistance of immune macrophages and consequent elimination of *Nocardia*. This is clearly demonstrated by a marked in vivo decrease in the intracellular viable count of *N. asteroides* in peritoneal macrophages obtained from guinea pigs actively immunized with *N. asteroides* P-RNA. Such activity was observed at least up to 60 days postimmunization. Humoral activity has little or no role in such protection because an increased intracellular in vivo decrease of *Nocardia* organisms in peritoneal macrophages is seen in recipients passively immunized by immune spleen cell transfer from donors actively immunized with either live *N. asteroides* or P-RNA.

The significant role of macrophages in protection against *N. asteroides* infection is further corroborated by the nullifying effect of AMS on such protection. It was shown earlier (5) that normal unimmunized guinea pigs challenged in-

tracardially became sick in 5 to 7 days and died in 15 days, with gross lesions in lungs, heart, and kidneys. When, however, the guinea pigs were actively or passively immunized and later challenged with *N. asteroides*, they survived for at least up to 60 days. In such animals, the different tissues, i.e., liver, lungs, heart, spleen, and kidneys, showed little or no growth of *N. asteroides* on sacrifice at different time intervals. We have now found that these *Nocardia* are eliminated specifically by immune macrophages in immunized animals. Furthermore, it is seen that administration of AMS before challenge with *N. asteroides* in actively and passively immunized animals nullified the protective effect of immunization. After administration of AMS and later intravenous *N. asteroides* challenge, the actively or passively immunized animals became sick in 3 days and died in 5 days. The total *Nocardia* count in different tissues such as heart, liver, spleen, and kidneys showed no significant differences in immunized and AMS-treated control animals. After administration of AMS, *N. asteroides* multiplied as freely in immunized animals as in unimmunized controls.

It should also be stated that some investigators (2, 3) have failed to obtain significant alterations in immune responses by AMS in in vivo experiments. The enhanced mortality after AMS injection in our study could also be partly the effect of antilymphocyte antibodies in AMS or T-cell loss. It is felt that our data only establish that a resistance mechanism is operating and that it can be transferred with spleen cells. It could be that the microbial counts performed on cells washed from the peritoneal cavity do not necessarily establish that death occurs intracellularly, even though AMS was shown to interfere with host resistance.

LITERATURE CITED

1. **David, I. R., S. Al-Askari, M. S. Lawrence, and L. Thomas.** 1964. Delayed hypersensitivity in vitro. I. Specificity of inhibition of cell migration by antigens. J. Immunol. **93**:264-273.
2. **Pearson, L. D., and J. W. Osebold.** 1973. Effects of antithymocyte and antimacrophage sera on the survival of mice infected with *Listeria monocytogenes*. Infect. Immun. **7**:479-486.
3. **Pearson, L. D., and J. W. Osebold.** 1974. Effect of antithymocyte sera and antimacrophage sera on cell-mediated immune reactions in *Listeria*-infected mice. Infect. Immun. **9**:127-133.
4. **Simon, H. B., and J. N. Sheagren.** 1971. Cellular immunity in vitro. I. Immunologically mediated enhancement of macrophage bactericidal capacity. J. Exp. Med. **133**:1377-1389.
5. **Sundararaj, T., and S. C. Agarwal.** 1977. Cell-mediated immunity in experimental *Nocardia asteroides* infection. Infect. Immun. **15**:370-375.
6. **Sundararaj, T., and S. C. Agarwal.** 1977. Cell-mediated immunity to *Nocardia asteroides* induced by its ribonucleic acid protein fraction. Infect. Immun. **18**:253-256.