INFECTION AND IMMUNITY, Jan. 1979, p. 34-40
0019-9567/79/01-0034/07$02.00/0

Vol. 23, No. 1

Correlation Between In Vivo and In Vitro Functional Tests for Activated Macrophages

JOAN M. RHODES,* J. BENNEDSEN, S. OLESEN LARSEN, S. RIISGAARD, AND J. V. SPÄRCK

International Escherichia and Klebsiella Centre (WHO), the Tuberculosis, Biostatistical, and Bloodtyping Departments, Statens Seruminstitut, DK-2300 Copenhagen S, Denmark

Received for publication 20 October 1978

Mice undergoing graft-versus-host reaction, skin grafting, and inoculation with tumor cells were tested for nonspecific resistance by intravenous challenge with *Listeria monocytogenes*. Peritoneal exudate macrophages from mice treated in a similar manner were tested in vitro for increased degradation of [1-^{14}C]glucose, ability to degrade antigen/antibody complexes, ability to inhibit intracellular growth of listeria, and staining for β-galactosidase. There was good correlation between in vivo resistance towards *L. monocytogenes* and in vitro inhibition of intracellular growth. There was also good correlation between increase in β-galactosidase and in vivo resistance in mice undergoing a graft-versus-host reaction.

Mice immunized with BCG are resistant not only to a subsequent challenge with BCG but also to unrelated bacteria such as listeria (7). The experimental conditions required for this BCG-induced nonspecific resistance to listeriosis have recently been studied (1). Nonspecific resistance can, however, be induced by agents other than bacteria. Thus, it has been shown that mice undergoing graft-versus-host (GVH) reaction (3) and tumor-bearing mice (8) exhibit nonspecific resistance towards facultative intracellular bacteria in vivo.

It has also been shown that peritoneal exudate (PE) macrophages obtained from mice which exhibit resistance in vivo have the following in vitro characteristics: increase in microbicidal activity, number of lysosomes, content of lysosomal enzymes, spreading of macrophages on glass, phagocytosis of particles, and oxidation of glucose through the hexose-monophosphate shunt (5).

We have demonstrated that both proteose-peptone-stimulated and BCG-activated PE cells showed an increased [1-^{14}C]glucose oxidation (11), whereas the former cells had a higher digestive capacity than the latter (10). BCG-activated PE macrophages degraded complexes to the same extent as normal cells. The determination of the intracellular multiplication of listeria was the only assay that could distinguish between stimulated and activated PE macrophages (2). Thus, we define macrophages as activated when they show an increased ability to inhibit multiplication of facultative intracellular bacteria when compared with normal macrophages; stimulated macrophages do not necessarily exhibit an enhanced capacity to kill these bacteria.

In view of these findings, it was of interest to test PE cells from mice subjected to GVH, skin transplantation, and tumor cell implantation, using the three in vitro functional tests mentioned above to ascertain whether the same type of results would be obtained as those found for PE cells from mice immunized with BCG. In addition, PE cells from mice treated as above were stained for β-galactosidase, because it has been suggested that increase in this enzyme might be an expression of immunological activation (4).

We have also attempted to correlate the results from these in vitro tests with in vivo resistance towards listeria as assessed by survival times and bacterial multiplication in the spleen after challenge with listeria.

MATERIALS AND METHODS

Mice. Male and female 3-month-old C3H/Ssc/1 and female 3-month-old C3H/C57Bl-F_1 hybrid mice were both bred at Statens Seruminstitut, and female 3-month-old C57Bl/gJ/Bom mice were purchased from Bomholdtgaard, Denmark.

Bacteria. BCG vaccine was derived from a concentrated experimental batch prepared in the BCG Production Department, Statens Seruminstitut. *Listeria monocytogenes* Ssc 1423 was obtained from H. Lautrop, Statens Seruminstitut. For further details, see Bennedsen et al. (2).

Radiolabel. Carrier-free ^{125}I was purchased from the Radiochemical Centre, Bucks, England.

HSA. Human serum albumin (HSA) was obtained from the Blood Fractionation Department, Statens Seruminstitut and was labeled at a specific activity of about 0.5 μg/μg according to Hunter and Greenwood (6).

Anti-HSA serum. Anti-HSA serum (batch 82) was prepared as described previously (10).

Latex suspension and acridine orange. Latex suspension and solution of acridine orange were prepared as described previously (10).

Treatment of mice. (i) BCG. BCG (1.5×10^7 viable units) was injected intravenously (i.v.) in 0.2 ml of Sauton medium. Control groups received Sauton medium only.

(ii) GVH reaction. Spleen cells from C3H mice (10^8) were injected i.v. into each of 10 C3H/C57Bl-F_1 hybrids. Controls were either untreated hybrids or hybrids injected with 10^8 spleen cells from hybrids. The spleen weights of hybrids injected with parental cells were doubled, indicating that they were undergoing a GVH reaction, whereas untreated hybrids or hybrids injected with cells from hybrids showed no increase in spleen weight.

(iii) Skin transplantation. Full-thickness skin from C57Bl mice was grafted into the backs of C3H mice by a suturing technique. Controls were either untreated C3H mice or C3H mice grafted with C3H skin.

(iv) Tumor transplantation. Primary mammary tumors arisen spontaneously in C3H mice were removed, cut into small pieces, and pressed through a sieve into Tc medium. The cells were washed in Tc medium, counted, and adjusted to 2.5×10^7 viable cells per ml (trypan blue test). A 0.1-ml volume of the cell suspension was injected subcutaneously (s.c.) into syngenic recipients (12).

Challenge with *L. monocytogenes* in vivo. (i) Survival assay. All mice were challenged i.v. with about 2×10^5 *L. monocytogenes* (about 20× the 50% lethal dose) on the following days after treatment: GVH, 18 days; skin transplantation, 7 and 11 days; tumor cell transplantation, 3 and 8 weeks. The day of death was recorded for each mouse, and resistance was expressed as survival in days. In the groups where small differences were expected (skin, tumor), recordings were made three times daily, but resistance was still expressed as survival in days. Significance was evaluated by a conditional rank sum test as described previously (1).

(ii) Spleen multiplication assay. Other groups of mice treated in the same way were challenged i.v. with about 2×10^3 *L. monocytogenes*. The number of bacteria was determined in the spleens 24 h after challenge by a plating technique (1). Significance was evaluated by the Wilcoxon two-sample test.

In vitro functional tests. PE cells were harvested from other groups of mice treated as described above and investigated as follows.

(i) [1-^{14}C]glucose oxidation. This was determined by a modified Warburg technique in macrophage monolayers, in both resting cells and cells that had phagocytosed latex, as described previously (11).

(ii) Degradation of ^{125}I-labeled HSA-antibody complexes. Suspensions of PE cells were incubated with ^{125}I-labeled HSA-antibody complexes as described elsewhere (10). Trichloroacetic acid was added to the mixtures after incubation; these were then centrifuged, and the radioactivity in the supernatants was measured. Evaluation of the breakdown of ^{125}I-labeled HSA-antibody complexes is based on the non-protein-bound radioactivity in the supernatant, calculated as a percentage of the original activity added. Degradation was expressed as the percentage of degradation per 10^6 macrophages.

(iii) Inhibition of growth of *L. monocytogenes*. Infection of macrophage monolayers has been described in detail elsewhere (2). Briefly, cells and bacteria were in contact for 30 min in an atmosphere of CO_2. After removal of the supernatants and washing, fresh medium containing penicillin (0.75 μg/ml) was added, and the tubes were replaced in the incubator. After a further incubation for 0.5 and 4 h, the supernatants were removed, and the cells were lysed by adding distilled water. The number of bacteria in the cell lysate was determined by means of a plating technique, and the slopes of the growth curves were evaluated and compared as before (2). The growth pattern was also expressed as generation time in minutes, assuming an exponential growth. A negative generation time corresponds to a negative slope. The corresponding positive value could justifiably be called a half-life.

(iv) Identification of β-galactosidase. This was carried out according to Pearson et al. (9) using 5-bromo-4-chloro-3-indolyl-β-D-galactoside (Sigma) as the substrate. Smears prepared by cytocentrifugation were incubated with or without substrate or with sodium fluoride (1.0 M). The intensity of the reaction was arbitrarily graded as follows: ± = weakly positive; + and ++ = positive; +++ and ++++ = intense reaction, the majority of the cells being stained. Macrophages were assessed by morphological criteria, i.e., size and ratio of nucleus to cytoplasm. Macrophages were arbitrarily divided into medium-sized and large macrophages (over 20 μm), and the percentage of these cells that were stained was recorded.

(v) Determination of the percentage of macrophages in PE cells. This was estimated as described previously (10): (i) morphologically after staining with Wright stain; (ii) after ingestion of latex; and (iii) after ingestion of acridine orange. A total of 1,000 cells was counted for each preparation.

RESULTS

Primary resistance to *L. monocytogenes* in vivo. Table 1 illustrates the survival times and bacterial counts in the spleen of mice challenged i.v. with *L. monocytogenes* after being treated in various ways.

Survival times were increased 7 days after skin transplantation in both allografts and syngenic grafts, but only in the allograft 11 days after grafting. Spleen multiplication was inhibited in the allograft (7 days) and in the syngenic graft (11 days).

Injection of tumor cells did not increase resistance; on the contrary, 21 days after inoculation there was a decrease in resistance to listeria, as assessed by survival times, and multiplication in the spleen. The greatest increase in resistance to listeria, as measured by survival times or multiplication, was seen in the GVH reaction.

TABLE 1. *Death rates of mice (10 mice per group) and bacterial counts in spleen after challenge with L. monocytogenes*

Treatment of mice	Challenged on day	No. of mice dead on day						2P values	Median bacterial counts in spleen 24 h after challenge	2P values
		2	3	4	5	6	>10			
GVH reaction										
Control (hybrids)			10						4.81×10^4	
Hybrid cells i.v. → hybrids	18		1	7				<0.001[a]	2.08×10^4	<0.01[b]
C3H cells i.v. → hybrids	18						10	<0.001[a]	3.99×10^2	<0.001[b]
Skin transplantation										
Control (C3H mice)		5	5						5.26×10^4	
C3H → C3H	7		6		2		1	<0.01	4.20×10^4	
C57Bl → C3H	7	1	2		1	1	2	<0.05	2.12×10^4	<0.05
Control (C3H mice)		7	3						3.72×10^4	
C3H → C3H	11	6			3		1		2.10×10^4	
C57Bl → C3H	11		6		2		1	<0.01	3.54×10^4	
Tumor implantation										
Control (C3H mice)			6	4					2.12×10^4	
Tumor cells s.c. → C3H mice	21		10					<0.05	7.50×10^4	<0.01
Control (C3H mice)			4	4		1			4.26×10^4	
Tumor cells s.c. → C3H mice	56		4	2			3		3.18×10^4	

[a] Difference in survival times after GVH and syngenic cell transfer: $2P < 0.001$.
[b] Difference in bacterial multiplication: $2P < 0.01$.

Syngenic cells also caused an increased resistance, but this did not reach the same magnitude as in the GVH (difference in survival times after GVH and syngenic cell transfer, $2P < 0.001$; in bacterial multiplication, $2P < 0.01$).

Morphology of cells. PE macrophages harvested from mice after skin transplantation, injection of tumor cells, GVH reaction, and i.v. immunization with BCG had certain features in common. Many of the cells were larger than normal, attaining the size of giant cells but containing only one nucleus. The macrophages contained many more lysosomes than normal, as assessed by the uptake of acridine orange.

There was no obvious morphological difference in the macrophages harvested from mice receiving syngenic or parental cells (GVH), between cells from mice receiving syngenic or allogenic skin grafts, or between macrophages from mice harvested on different days after immunization with BCG i.v.

Percentage of macrophages in PE cells. The percentage of PE macrophages, estimated by uptake of acridine orange or latex or after staining with Wright stain, was consistent for the three methods. The percentage increased after treatment with BCG i.v. (16 days), skin transplantation (11 days) both syngenic and allogenic, and after injection of tumor cells (3 weeks). Otherwise the percentage of macrophages in the treated mice resembled that of the untreated mice.

In vitro results. (i) [1-^{14}C]glucose oxidation. The oxidation of adherent cells from C3H mice after skin transplantation, injection of tumor cells, and GVH reaction was compared with the oxidation of cells from untreated control animals (Fig. 1) (unless otherwise stated, all differences mentioned throughout this section were significant at the 0.1% level). Oxidation was increased in phagocytosing cells from mice 7 days after skin transplantation (allograft), but not in the syngenic graft. At 11 days after skin transplantation, both resting and phagocytosing cells from mice that received an allograft showed increased oxidation when compared with cells from mice receiving a syngenic graft. PE cells from mice injected with tumor cells only showed an increase in oxidation in both resting and phagocytosing cells 8 weeks after implantation. The most dramatic increase in oxidation occurred in the cells obtained from hybrids injected either with syngenic or parental cells (GVH) when compared with the hybrid control cells. There was no difference in the metabolic activity of PE cells (resting and phagocytosing) from mice that received either syngenic or parental cells.

Degradation of ^{125}I-labeled HSA-antibody complexes by PE cells. PE cells obtained from mice 7 or 11 days after skin transplantation showed no indication of a greater digestive capacity than PE cells from untreated mice (Fig. 2). Figure 2 also shows that 10^6 PE

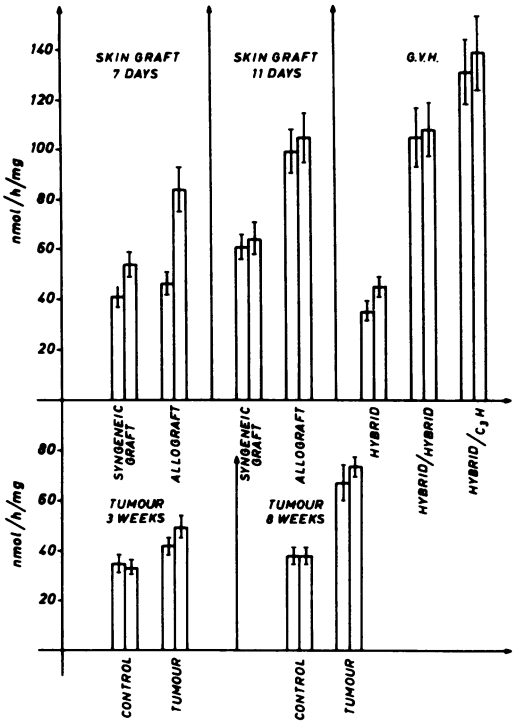

FIG. 1. *Oxidation of [1-^{14}C]glucose by PE macrophages from C3H mice after skin transplantation, GVH reaction, and injection of tumor cells. Each set of columns represents resting cells (left) and cells that phagocytose latex (right), expressed in nanomoles per hour per milligram of macrophage protein. The mean of 10 cultures is shown with ±2 standard error.*

cells from mice injected with tumor cells (3 weeks) degraded the complexes to a greater extent than control cells, whereas 8 weeks after tumor cell implantation the PE cells were less capable of degrading complexes than control cells. A GVH reaction caused an increased capacity to degrade complexes, which was significantly above that of control values. The syngenic graft had no effect on the digestive capacity of PE cells.

Intracellular multiplication of *L. monocytogenes*. Table 2 shows the type of results obtained after treating C3H mice in various ways. A GVH reaction gave rise to PE cells which were unequivocally capable of inhibiting the growth of listeria in vitro. There was no conclusive result with the skin transplantation. This particular experiment was designed to elucidate the difference between syngenic and allogenic grafting. No difference was seen after 7 days, but there was a weakly significant increase (5% level) in the generation time in the syngenic graft after 11 days. The injection of tumor cells had no effect on the capacity of PE cells to kill *L. monocytogenes*.

Identification of β-galactosidase. β-Galactosidase was visualized as a green granular reaction in the cytoplasm of the macrophages in the presence of its substrate. The reaction observed in the PE cells is shown in Table 3. An intense reaction was seen in the PE cells immunized i.v. with BCG at 16 and 19 days after injection, but there was also a strong reaction after 13 days. PE cells from mice injected with tumor cells or after skin transplantation (11 days, syngenic) showed the same degree of staining, which was slightly more intense than that obtained with normal cells. A strong reaction was observed after skin allograft and syngenic graft (7 days) and allograft (11 days). PE cells from mice undergoing a GVH reaction stained as intensely as those from mice immunized with BCG (16 days). The transfer of syngenic cells also caused an increase in the enzyme in the PE cells, but the staining was not as intense. The

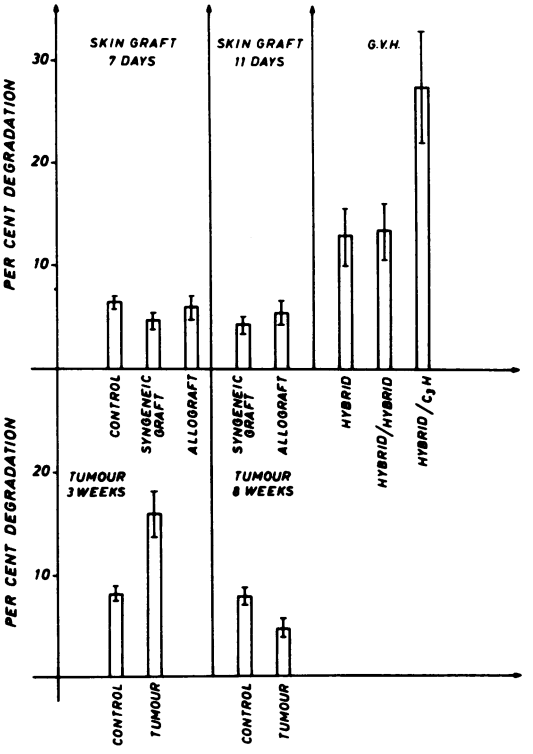

FIG. 2. *Degradation of ^{125}I-labeled HSA-antibody complexes by PE macrophages from mice after skin transplantation, GVH reaction, and injection of tumor cells. Degradation is expressed as percentage of degradation by 10^6 macrophages. Each column represents the mean of at least five simultaneous determinations with ±2 standard error.*

TABLE 2. *Intracellular generation time of L. monocytogenes in monolayer cultures of PE macrophages*

Treatment of mice	PE cells harvested on day	Generation time (min)	Slope × 10^3
GVH reaction			
Control (hybrids)	18	138.4	2.18
Hybrid cells i.v. → hybrids	18	153	1.96
C3H cells i.v. → hybrids	18	−2,888	−0.104[a]
Skin transplantation			
C3H → C3H	7	176	1.71
C57Bl → C3H	7	138	2.18
C3H → C3H	11	414	0.727[b]
C57Bl → C3H	11	210	1.43
Tumor implantation			
Control (C3H mice)		125	2.40
Tumor cells s.c. → C3H mice	21	170	1.76
Control (C3H mice)		136	2.2
Tumor cells s.c. → C3H mice	56	193	1.55

[a] $2P < 0.005$.
[b] $2P < 0.05$.

majority of the cells stained were large macrophages, and these usually showed intense staining, although some of the medium-sized cells were also intensely stained. Cells from mice with a GVH reaction were quite distinctive because 95% were stained, 60% being large macrophages. Approximately the same number of macrophages were stained after transfer of syngenic spleen cells, but the reaction was not as intense. Twice as many PE macrophages were stained after immunization with BCG i.v. (13, 16, and 19 days) when compared with normal cells, over 30% being large macrophages. Only a few PE cells were stained after allograft skin transfer (7 days) and allograft and syngenic graft (11 days).

DISCUSSION

Acquired resistance in C3H mice towards in vivo challenge with *L. monocytogenes* is achieved after immunization with BCG i.v., as assessed by survival times in days and increase in listeria in the spleen (1).

It has also been shown that PE cells from mice immunized with BCG i.v. were capable of enhanced [1-^{14}C]glucose oxidation and inhibition of growth of listeria, but were not capable of degrading antigen-antibody complexes (2, 10, 11). The present experiment shows that PE mac-

TABLE 3. *Presence of β-galactosidase in PE macrophages from C3H mice*

Treatment of mice	PE cells harvested on day	Intensity of reaction[a]	Percent macrophages stained		
			Total	Large	Medium
Immunization					
Control (C3H mice)		±	26.3	8.0	18.3
BCG i.v. (1.5×10^7 viable units)	12	±	28.2	18.4	9.8
	13	+++	54.3	31.5	22.8
	16	++++	42.1	30.0	12.1
	19	++++	46.3	36.5	9.8
Tumor implantation					
Tumor cells s.c. → C3H mice	21	+	4.6	2.6	2.0
	56	+-++	19.3	10.3	9.0
GVH reaction					
Control (hybrids)		±	29.7	8.5	21.2
Hybrid cells i.v. → hybrids	18	++	80.1	49.8	30.3
C3H cells i.v. → hybrids	18	++++	95.1	60.6	34.5
Skin transplantation					
C3H → C3H	7	+++	38.7	20.2	18.5
C57Bl → C3H	7	++-+++	14.5	7.3	7.2
C3H → C3H	11	+	4.0	2.0	2.0
C57Bl → C3H	11	+++	13.5	7.4	6.1

[a] ±, Weakly positive; + and ++, positive; +++ and ++++, intense reaction.

rophages from mice immunized with BCG i.v. have an increased content of β-galactosidase.

Dannenberg et al. (4) observed an increase in this enzyme in the macrophages locally stimulated in a dermal BCG lesion in rabbits, an increase associated with the destruction of tubercle bacilli. These authors considered this activation to be a local phenomenon. However, our results indicate that BCG injected i.v. exerts a systemic effect, since β-galactosidase was increased in PE macrophages.

Dannenberg et al. (4) pointed out that not all enzymes might be increased to the same extent when macrophages are activated, and this has been confirmed by David (5). This observation would explain why we found increased β-galactosidase in PE macrophages after i.v. injection of BCG but no increased degradation of antigen-antibody complexes by these cells (10).

Treatment of mice for immunological activation other than direct infection yielded variable results. Thus, injection of tumor cells gave virtually negative results, and there was no indication that resistance towards *L. monocytogenes* was achieved (Table 1). On the contrary, after 3 weeks there was a decrease in the resistance. This has also been observed by North et al. (8), who demonstrated a factor, present in the serum of tumor-bearing mice, which could suppress the antibacterial function of macrophages. On the other hand, North et al. (8) also demonstrated that this tumor-induced state of suppressed antibacterial resistance was short lived and that it was soon replaced by a contrasting state of greatly enhanced antibacterial resistance. We have not been able to confirm this result, but this may be due to differences in time schedules for the assays and tumor progression.

PE cells from tumor-bearing mice did degrade antigen-antibody complexes after 3 weeks, the metabolic rate was increased after 8 weeks, and there was a slight increase in the content of β-galactosidase at 8 weeks. Thus, PE macrophages from tumor-bearing mice exhibit some of the features of stimulated cells (10), but these cells are not activated under our experimental conditions.

Skin grafting (allograft) also resulted in resistance to *L. monocytogenes* in vivo and in vitro, oxidation of [1-^{14}C]glucose, and increase in β-galactosidase (Table 1), although only the allograft (11 days) showed both in vivo and in vitro resistance to *L. monocytogenes*. The syngenic graft (7 and 11 days) also gave rise to resistance in vivo but was not positive in both methods used for assessment. PE cells from all the skin grafts showed a positive reaction in the oxidation of [1-^{14}C]glucose and increase in β-galactosidase, whereas they could not degrade antigen-antibody complexes. PE cells from the syngenic graft (7 days) and allograft (11 days) had an intense reaction for β-galactosidase, similar to that found after infection with BCG. Thus, there is no clear-cut evidence that skin grafting always activates macrophages to produce acquired resistance. Furthermore, the results found with the syngenic graft imply that all the positive findings could be due to a nonspecific mechanism, e.g. postsurgical inflammation.

Mice undergoing a GVH reaction or injected i.v. with BCG showed the same type of results for resistance in vivo (1) and for functional tests for PE macrophages in vitro (2) with one exception; i.e., PE cells from mice with a GVH reaction were capable of degrading antigen-antibody complexes. The injection of syngenic cells (F_1) into hybrids had some effect on the PE macrophages, since there was resistance to listeria as assessed by survival times, increase in metabolic rate, and a slight increase in β-galactosidase despite the fact that the main criterion for the existence of an immunological reaction was missing, i.e., increase in spleen weight.

However, statistical evaluation showed that although the syngenic graft afforded resistance towards listeria, the parental graft (GVH) gave rise to a resistance of a higher magnitude. In addition, the determination of in vitro inhibition of multiplication of listeria after injection of syngenic cells was negative, implying that activation of PE macrophages had not taken place. This does not exclude the possibility that syngenic cells could stimulate the cells of recipient mice to a greater metabolic activity or increase in enzyme than normally seen. However, according to our criteria for immunological activation, the β-galactosidase reaction should be intense, which was not the case here. Our conclusion is that syngenic cells do not cause activation of macrophages under our experimental conditions, but they can stimulate certain functional aspects.

Blanden (3) observed that mice undergoing GVH reaction were resistant to listeria infection, as manifested by inhibition of growth of organisms in their spleens. Injection of isogenic or allogenic spleen cells did not influence the course of the infection. In addition, the peritoneal macrophages of parental cell recipients inactivated *Salmonella typhimurium* at a greatly increased rate when compared with cells from untreated mice. These results agree with ours, if we take the above-mentioned results into consideration.

The results shown in Table 3 suggest that β-

galactosidase might well be a marker for macrophages which inhibit the growth of facultative intracellular bacteria such as listeria (in vitro), provided that an intense reaction (+++ to ++++) is recorded (see BCG and GVH), since this also correlates with resistance in vivo.

However, PE macrophages from mice receiving skin allograft (11 days) and syngenic skin graft (7 days) also gave an intense reaction (+++) (Table 3), which corresponds to in vivo resistance but not inhibition of growth of listeria in vitro. Therefore, it is probably not justifiable, until the enzyme reaction has been investigated in more detail, to use the increase in β-galactosidase as an unequivocal criterion for the activation of the bactericidal capacity of PE macrophages.

The variability in macrophage function following various immunological treatments suggests that the difference discussed above may depend on the method of activation. Wing et al. (14) used three types of infection to activate macrophages, and they found that PE macrophages characterized as activated by one criterion did not satisfy other criteria of activation. This is in agreement with our results after treatment of mice in various ways involving an immunological reaction to activate macrophages.

It is known that different subpopulations of macrophages exist having different functional capacities (13); e.g., as pointed out by Wing et al. (14), macrophages which inhibit tumor cell DNA synthesis may be distinct from those which inhibit intracellular multiplication. In terms of our experiments this would mean that macrophages which can, for example, degrade antigen-antibody complexes are distinct from those which inhibit the growth of listeria, or that the same cell requires two different signals for activation.

ACKNOWLEDGMENTS

We thank Ulla Bjørn Rasmussen and Bodil Melander Nielsen for excellent technical assistance.

LITERATURE CITED

1. **Bennedsen, J., and S. Olesen Larsen.** 1975. The BCG-induced resistance to listeriosis. Acta Pathol. Microbiol. Scand. Sect. C **83**:377–382.
2. **Bennedsen, J., S. Riisgaard, J. M. Rhodes, and S. Olesen Larsen.** 1977. In vitro studies on normal, stimulated and immunologically activated mouse macrophages. III. Intracellular multiplication of *Listeria monocytogenes*. Acta Pathol. Microbiol. Scand. Sect. C **85**:246–252.
3. **Blanden, R. V.** 1969. Increased antibacterial resistance and immunodepression during graft-versus-host reactions in mice. Transplantation **7**:484–497.
4. **Dannenberg, A. M., O. T. Meyer, J. R. Esterly, and T. Kambara.** 1968. The local nature of immunity in tuberculosis, illustrated histochemically in dermal BCG lesions. J. Immunol. **100**:931–941.
5. **David, J. R.** 1975. Macrophage activation by lymphocyte mediators. Fed. Proc. Fed. Am. Soc. Exp. Biol. **34**:1730–1736.
6. **Hunter, W. M., and F. C. Greenwood.** 1962. Preparation of iodine-131 labeled human growth hormone of high specific activity. Nature (London) **194**:495–496.
7. **Mackaness, G.** 1970. Cellular immunity, p. 461–475. *In* R. van Furth (ed.), Mononuclear phagocytes. Blackwell Scientific Publications, Oxford.
8. **North, R. J., D. P. Kirstein, and R. L. Tuttle.** 1976. Subversion of host defense mechanisms by murine tumours. II. Counter-influence of concomitant antitumour immunity. J. Exp. Med. **143**:574–584.
9. **Pearson, B., P. L. Wolf, and J. Vazquez.** 1963. A comparative study of a series of new indolyl compounds to localize β-galactosidase in tissues. Lab. Invest. **12**:1249–1259.
10. **Rhodes, J. M., G. Nielsen, S. Olesen Larsen, J. Bennedsen, and S. Riisgaard.** 1977. In vitro studies on normal, stimulated and immunologically activated mouse macrophages. II. Degradation of radioactive antigen-antibody complexes. Acta Pathol. Microbiol. Scand. Sect. C **85**:239–245.
11. **Riisgaard, S., J. Bennedsen, and J. M. Rhodes.** 1977. In vitro studies on normal, stimulated and immunologically activated mouse macrophages. I. Oxidation of 1-^{14}C glucose by macrophages in monolayer cultures. Acta Pathol. Microbiol. Scand. Sect. C **85**:233–238.
12. **Spärck, J. V.** 1969. The function of the immune response in tumour growth and its genetical regulation. A new hypothesis. Acta Pathol. Microbiol. Scand. **77**:1–23.
13. **Walker, W.** 1976. Functional heterogeneity of macrophages, p. 91–110. *In* D. S. Nelson (ed.), Immunobiology of the macrophage. Academic Press Inc., New York.
14. **Wing, E. J., I. D. Gardner, F. W. Ryning, and J. S. Remington.** 1977. Dissociation of effector functions in populations of activated macrophages. Nature (London) **268**:642–644.