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Shared Antigens between Bacteria and Guinea Pig Line 10 Hepatocarcinoma Cells[1]

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SUMMARY

This study was undertaken to investigate the possibility that *Listeria monocytogenes*, *Brucella abortus*, and *Salmonella typhimurium* share antigenic components with guinea pig line 10 hepatocarcinoma cells. Rabbits were immunized with sonicates of these bacteria or line 10 tumor cells. Other rabbits were immunized with line 1 cells, a tumor with antigenic characteristics different from those of line 10. The binding of antibodies to radiolabeled antigens prepared from extracts of bacteria and line 10 cells was studied by precipitation of radiolabeled antigen-antibody complexes with anti-rabbit immunoglobulin.

Antibodies in sera from rabbits immunized with these bacteria and line 10 cells bound both the labeled bacteria and line 10 antigens. Antibodies in sera from rabbits immunized with line 1 cells did not bind the bacterial antigens. Inhibition studies involving reactions between radiolabeled *Listeria* and line 10 antigens and antibodies to *Listeria* and line 10 cells confirmed that the binding reactions were specific and that line 10 cells shared antigens with *Listeria* cells. The possibility that *B. abortus* and *S. typhimurium* also shared antigens with line 10 cells was suggested. Whether antigens shared by these bacteria and line 10 cells are identical with tumor-specific antigens was not determined.

INTRODUCTION

BCG[3] and *Listeria monocytogenes* have been shown to suppress the growth of several tumors (1–3, 7). One of these tumors, the guinea pig line 10 hepatocarcinoma, has been reported to share antigenic components with BCG (4, 5, 15). Since previous studies have shown that BCG shares antigens with *L. monocytogenes* and a variety of other taxonomically unrelated microorganisms (13, 14), experiments were carried out to test the possibility that *L. monocytogenes* and other bacteria also share antigens with line 10 tumor cells. If this were the case it would raise the question as to whether other bacteria might also share antigens with other tumors that do not have antigenic similarities with BCG.

Soluble extracts were prepared from *Listeria*, *Brucella abortus*, *Salmonella typhimurium*, and line 10 hepatocarcinoma cells. Rabbits were immunized with these bacteria and with line 10 cells and the resulting antisera were tested for their capacity to bind radiolabeled components of the bacterial and tumor extracts. Both direct binding and inhibition studies were carried out to determine whether antigenic components could be detected that were shared by these bacteria and line 10 tumors.

MATERIALS AND METHODS

Antigens. *L. monocytogenes*, *B. abortus*, and *S. typhimurium* were obtained from the stock bacterial cultures maintained at the National Jewish Hospital and Research Center. The bacteria were grown, killed with heat, and washed as previously described (14). Some of the washed cells were used to absorb antisera and others were disrupted sonically for 5 min. A portion of the product of this sonic disruption was used to immunize rabbits and another portion was subjected to ultracentrifugation. The resulting supernatants are referred to as List-SS, Bruc-SS, and Salm-SS. Details of the preparation of these antigens have been described (13, 14).

Two groups of strain 2 guinea pigs that had been given i.p. injections of either line 10 or line 1 hepatoma cells, a tumor with antigenic characteristics different from those of line 10, were kindly provided by Dr. B. Zbar, National Cancer Institute, Bethesda, Md. Soluble extracts of line 10 and line 1 cells were prepared by extraction with hypertonic potassium chloride as previously described (10, 15) and are referred to as SA-10 and SA-1.

Tumor cells and extracts were handled under sterile conditions insofar as possible to avoid bacterial contaminaton. Dialysis was carried out using sterile pyrogen-free, commercially prepared 0.9% NaCl solution. When not in use, extracts were stored at −20°. Periodic bacterial cultures of cell suspensions and extracts were negative and endotoxin, as tested by the E-Toxate test (Limulus Amebocyte Lysate; Sigma Chemical Co., St. Louis, Mo.) (16), was also negative at less than 1 ng/ml.

The nitrogen content of antigens was determined by an automated micro-Kjeldahl method (8).

Radioactive Labeling. The bacterial antigens, line 10 ex-

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[3] The abbreviations used are: BCG, *Mycobacterium bovis;* SA-10, KCl extract of line 10 cells; SA-1, KCl extract of line 1 cells; ^{125}I-List, radiolabeled *Listeria* antigen; ^{125}I-Bruc, radiolabeled *Brucella* antigen; ^{125}I-Salm, radiolabeled *Salmonella* antigen; ^{125}I-SA-10, radiolabeled KCl extract of line 10 cells; ^{125}I-SA-1, radiolabeled KCl extract of line 1 cells; NRS, normal rat serum; SRBC, sheep RBC; GGG, goat γ-globulin.

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tracts, and line 1 KCl extracts were labeled with ^{125}I using a previously described modification of the chloramine T method (15) and are referred to as ^{125}I-List, ^{125}I-Bruc, ^{125}I-Salm, ^{125}I-SA-10, and ^{125}I-SA-1. They were diluted in 1:100 NRS in borate buffer so that their concentrations ranged from 0.005 to 0.06 μg nitrogen per test and so that the counts of labeled antigens added to each test serum were 10,000 to 15,000 cpm.

Antisera. Sonicates of the 3 bacteria were suspended in incomplete Freund's adjuvant and injected i.m. into New Zealand White rabbits. Each injection consisted of approximately 1 mg of nitrogenous material and was repeated 2 to 3 times at monthly intervals. Other groups of rabbits were similarly given injections of heterologous serum albumins (bovine, sheep, and human) SRBC, GGG, and unfractionated washed line 10 and line 1 cells. Each tumor cell injection consisted of approximately 10^8 cells. Sera that are referred to as anti-List, anti-Bruc, anti-Salm, antialbumin, anti-SRBC, anti-GGG, anti-line 10, and anti-line 1 were collected from all animals before immunization and 1 week after the final immunizing injection. Two rabbits received no injections and NRS was obtained from them at 2-month intervals. Details of the immunizing and bleeding procedures have been described (13-15).

Measurement of Antibodies. Sera from rabbits that were immunized with various antigens were tested for their capacity to bind the radiolabeled antigens. The primary interactions between labeled test antigens and antibodies in rabbit antisera were analyzed by precipitation of ^{125}I-labeled antigen:antibody complexes with anti-rabbit IgG prepared in goats. The amount of anti-rabbit IgG required to precipitate all the rabbit IgG in a given test sample was determined as previously described (12). In most experiments 0.1 ml of 1:5 dilutions of antisera was tested for its capacity to bind 0.1 ml of a radiolabeled test antigen. In some experiments 0.5 ml of a 1:5 dilution of antisera was reacted with 0.5 ml of antigen. Binding by serum from an immunized rabbit was usually compared with its matching preimmunization serum. The binding capacity of a given postimmunization antiserum to a radioactive antigen was expressed as the percentage increase of the cpm in the resulting precipitate to that obtained from its corresponding preimmunization serum. The percentage increase was determined as follows:

$$\left(\frac{\text{cpm postimmunization serum}}{\text{cpm preimmunization serum}} - 1\right) \times 100$$

The data were expressed in this manner because of the variability in amounts of radioactivity in precipitates of individual NRS with each labeled test antigen. For example, the cpm in precipitates from 30 normal rabbit sera that were tested with ^{125}I-List varied from 741 to 2611. This has been observed previously with antigens derived from other tumors and microorganisms and is believed to represent individual differences among animals in their past history of exposure to environmental immunogens or in their genetic backgrounds (14, 15).

The significance of these data was determined, unless otherwise stated, by comparing the cpm in the preimmunization sera precipitates with the cpm in the postimmunization sera precipitates. The Student's t-test was used for this analysis.

Inhibition Studies. Some postimmunization sera were preincubated with unlabeled test antigens in concentrations in excess of the labeled test antigens. Twenty-four hr later appropriate labeled test antigens were added and binding capacities of the antisera were determined. Details of these procedures are described elsewhere (11, 15).

Some antisera were absorbed with normal guinea pig spleen cells and line 10 cells. Before absorption, a given number of washed cells was centrifuged at 3000 × g for 10 min and the supernate was discarded. Serum was then added to the cell pellet and absorption was carried out at 37° for 1 hr and at 4° overnight, during which time the tubes containing sera and cells were continuously rotated. Following centrifugation at 3000 × g, the serum was separated and, in some cases, the absorption procedure was repeated with new cells. The total number of cells used to absorb a given volume of antiserum is indicated in each such experiment.

RESULTS

Binding of Radiolabeled Bacterial and SA-1 Antigens by Sera from Individual Rabbits That Were Immunized with Homologous Bacterial Antigens and Line 1 Cells.

The radiolabeled bacterial antigens ^{125}I-List, ^{125}I-Bruc, ^{125}I-Salm, and ^{125}I-SA-1 were reacted with antisera from groups of rabbits that had been immunized with homologous bacterial sonicates and with line 1 cells. The percentage increases in binding by sera from individual immunized animals as compared to binding by their matching sera obtained before immunization are shown in Table 1. As expected there were considerable increases in binding by all the postimmunization antisera.

Binding of Radiolabeled Bacterial Antigens by Sera from Individual Rabbits That Were Immunized with Line 10 and Line 1 Cells.

Sera from 7 of 9 animals immunized with line 10 tumor cells bound 25% or more ^{125}I-List after immunization than before (see Table 2). Sera from 5 of the 9 animals also bound increased amounts of ^{125}I-Bruc and 4 of these bound increased ^{125}I-Salm. Data from the 9 anti-line 10 animals differed with each antigen but, as a group, the anti-line 10 sera bound significantly increased amounts of all 3 bacterial antigens after the animals had been immunized with line 10 cells. By comparison, sera from rabbits immunized with line 1 tumor cells did not bind significantly to these antigens. Sera from control rabbits that were immunized with SRBC, albumins, and GGG and sera from unimmunized animals showed no significant increases. When the percentage increases of the anti-line 10 sera as a group were compared with those of anti-line 1, there was a marked difference for each labeled antigen ($p < 0.0025$); when compared to the control sera the difference was even more significant ($p < 0.0005$). Differences in the percentage changes between sera from the anti-line 1 rabbits and the control rabbits were not significant ($p < 0.2$).

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Table 1

Percentage increases in binding of radiolabeled bacterial and SA-1 antigens by sera from individual rabbits that were immunized with homologous bacterial antigens and line 1 cells

Percentage increases were determined as follows:

$$\left(\frac{\text{cpm of postimmunization serum}}{\text{cpm of preimmunization serum}} - 1\right) \times 100$$

Test system[a]			
^{125}I-List:anti-List	^{125}I-Bruc:anti-Bruc	^{125}I-Salm:anti-Salm	^{125}SA-1:anti-line 1
244	323	153	146
265	265	230	106
237	232	203	71
238	453	285	64
		164	144
			5
			134
			44

[a] p values represent differences in cpm of pre- and postimmunization sera and are <0.001 for each group.

Binding of ^{125}I-SA-10 Antigen to Anti-List, Anti-Bruc, and Anti-Salm

The capacity of some of the same pre- and postimmunization sera reported in Tables 1 and 2 to bind to ^{125}I-SA-10 was compared, and the results are indicated in Table 3. Sera from all animals that received line 10 cells bound increased amounts of this antigen. Sera from 5 of the 7 animals immunized with *Listeria*, however, also bound 25% or more ^{125}I-SA-10 after immunization than before ($p < 0.0005$). Sera from all 4 of the rabbits that had been immunized with the *Brucella* sonicate and from 3 of the 5 that had been immunized with the *Salmonella* sonicate bound increased amounts of ^{125}I-SA-10 ($p < 0.003$ and < 0.05). Control sera showed no significant increases ($p < 0.5$).

Inhibition and Absorption Studies

A series of experiments was carried out to evaluate the specificity of some of the above observations. Because the binding of ^{125}I-List by anti-line 10 was of greater statistical significance than that of the other bacterial antigens and because *L. monocytogenes* has already been reported to have antitumor effects (3), the reactions between ^{125}I-List and ^{125}I-SA-10 to anti-List and anti-line 10 were studied in greater detail.

Inhibition of Binding of ^{125}I-List and ^{125}I-SA-10 by Anti-List and Anti-line 10. The capacity of unlabeled List-SS and SA-10 to inhibit binding by the different antisera to their homologous and heterologous labeled test antigens was examined in a series of experiments that are summarized in Table 4. When anti-List and anti-line 10 were preincubated with unlabeled List-SS, their binding to ^{125}I-List-SS was inhibited (see Table 4, Experiments 1 and 3). There was no similar alteration of binding by these antisera to ^{125}I-SA-10 when preincubated with unlabeled List-SS in the concentrations used (see Table 4, Experiments 5 and 7). Unlabeled SA-10 inhibited the binding between ^{125}I-List and anti-line 10 (Table 4, Experiment 4) and between ^{125}I-SA-10 and both anti-line 10 (Table 4, Experiment 6) and anti-List (Table 4, Experiment 8). It did not, in these experiments, inhibit the binding between ^{125}I-List and anti-List (Table 4, Experiment 2).

Binding Capacity of Antisera to ^{125}I-List and ^{125}I-SA-10 after Absorption with Line 10 Cells, *Listeria* Cells, and Normal Guinea Pig Spleen Cells. In the following experiments, selected anti-List and anti-line 10 sera were absorbed with line 10 cells, *Listeria* cells, and normal strain 2 guinea pig spleen cells. Absorbed and unabsorbed sera were then tested for their capacity to bind ^{125}I-List and ^{125}I-SA-10. Results of these experiments are presented in Table 5.

When 1.0 ml of anti-List was absorbed with 4×10^8 line-10 cells and reacted with ^{125}I-List, there was a 17% reduction in its capacity to bind ^{125}I-List (see Table 5, Experiment 2). Absorption with 10^8 line 10 cells (Table 5, Experiment 1) resulted in a lesser change. In addition, these absorbed sera did not bind ^{125}I-SA-10 as effectively as did unabsorbed sera

Table 2

Percentage increases in binding of ^{125}I-List, ^{125}I-Bruc, and ^{125}I-Salm by sera from individual rabbits that were immunized with line 10 and line 1 cells

Percentage increases were determined as follows:

$$\left(\frac{\text{cpm of postimmunization serum}}{\text{cpm of preimmunization serum}} - 1\right) \times 100$$

Antisera	^{125}I-List	^{125}I-Bruc	^{125}I-Salm
Experimental			
Anti-line 10	60	53	50
	30	16	14
	57	113	81
	37	21	21
	12	22	9
	29	111	143
	40	68	56
	43	51	18
	15	18	3
	$p < 0.0005$[a]	$p < 0.005$	$p < 0.05$
Anti-line 1	21	57	24
	−12	−17	−15
	20	31	22
	18	30	25
	7	31	18
	−6	−14	7
	13	18	−14
	7	−1	18
	$p < 0.8$	$p < 0.6$	$p < 0.6$
Control			
Anti-SRBC	−13	−7	5
	−3	7	17
Anti-albumins	5	−11	11
	18	5	13
	1	10	−7
	−4	22	11
Anti-GGG	7	27	23
	−3	−9	2
NRS only	−4	2	9
	8	−3	1
	$p < 0.9$	$p < 0.8$	$p < 0.4$

[a] p values represent differences in cpm of pre- and postimmunization sera.

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Table 3

Percentage increases in binding of ^{125}I-SA-10 by sera from individual rabbits that were immunized with line 10 cells, bacterial sonicates, and control antigens

Percentage increases were determined as follows:

$$\left(\frac{\text{cpm of postimmunization serum}}{\text{cpm of preimmunization serum}} - 1\right) \times 100$$

Anti-line 10	Anti-List	Anti-Bruc	Anti-Salm.	Anti-SRBC	Anti-albumins	Anti-GGG	NRS only
303	18	34	38	0	2	15	−3
86	43	48	48	−3	4	3	0
210	58	41	15		2		
197	54	33	13		8		
	64		44				
	24						
	25						
$p < 0.003$[a]	$p < 0.0005$	$p < 0.003$	$p < 0.05$			$p < 0.5$	

[a] p values indicate differences in cpm of pre- and postimmunization sera.

Table 4

Inhibition of binding of ^{125}I-List and ^{125}I-SA-10 by preincubation of anti-List and anti-line 10 with unlabeled antigens

Test system	Experiment	Unlabeled inhibitors	cpm in precipitates	% decrease of cpm
^{125}I-List:anti-List	1	None	8,420	
		BSA[a] (240)[b]	8,655	0
		List-SS (240)	4,159	50.6
	2	None	12,047	
		SA-10 (520)	11,833	1.7
^{125}I-List:anti-line 10	3	None	2,857	
		BSA (240)	2,818	1.4
		List-SS (360)	1,865	34.7
	4	None	2,905	
		SA-10 (260)	1,892	35.0
		SA-10 (520)	1,636	43.6
^{125}I-SA-10:anti-line 10	5	None	5,073	
		BSA (240)	5.017	1.1
		List-SS (360)	4,885	3.7
	6	None	6,329	
		SA-10 (130)	3,193	50.0
		SA-10 (520)	2,343	63.0
^{125}I-SA-10:anti-List	7	None	3,190	
		BSA (240)	3,095	3.0
		List-SS (360)	3,120	2.2
	8	None	2,665	
		SA-10 (260)	1,406	47.2
		SA-10 (520)	1,047	60.7

[a] BSA, bovine serum albumin

[b] Numbers in parentheses, concentration in μg of nitrogen.

(Table 5, Experiments 7, 8, and 9). When anti-List was absorbed with 10^9 normal guinea pig spleen cells, however, there was no decrease (Table 5, Experiment 2), and when anti-List was absorbed with *Listeria* cells, there was an 83% reduction in its capacity to bind ^{125}I-List (Table 5, Experiment 2). When anti-line 10 sera were absorbed with 4×10^8 line 10 cells or with *Listeria* cells, their capacity to bind ^{125}I-List was similarly reduced (Table 5, Experiments 3 and 4). When anti-line 10 was absorbed with 10^7 line 10 cells, its binding to ^{125}I-SA-10 was only slighly reduced (Table 5, Experiment 5), but when the number of cells was increased to 4×10^8, the inhibition was much greater 72% (Table 5, Experiment 6). When anti-line 10 was absorbed with *Listeria* cells, the capacity of the serum to bind ^{125}I-SA-10 was reduced 31% (Table 5, Experiment 6). After absorption of anti-List with 10^9 normal guinea pig spleen cells, there was some reduction in its capacity to bind ^{125}I-SA-10 (Table 5, Experiment 9).

DISCUSSION

Antibodies in sera from rabbits immunized with sonicates of *L. monocytogenes*, *B. abortus*, and *S. typhimurium* bound radiolabeled antigens derived from both homologous bacterial and line 10 tumor cells. Antibodies in sera from rabbits immunized with line 10 cells, but not line 1

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cells, similarly bound significant amounts of radiolabeled antigens derived from both bacterial and line 10 cells. Because of the reported antitumor effects of *Listeria* (2, 3, 7), the reactions between radiolabeled *Listeria* and SA-10 antigens to anti-List and anti-line 10 were studied in greater detail.

Summary results of the inhibition (Table 4) and absorpton experiments (Table 5) are presented in Table 6. The binding of ^{125}I-List by anti-List (Table 5) was reduced after absorption of anti-List with 10^9 line 10 cells but not after preincubation with SA-10 (Table 4). This would suggest that there were greater numbers of cross-reacting determinants on the surfaces of 10^9 line 10 cells than in the KCl extract of line 10 that was made from only 1 to 2 $\times$ 10^8 cells. For the same reason there were greater reductions in binding by anti-line 10 and by anti-List to ^{125}I-SA-10 after these antisera had been absorbed with *Listeria* cells than after they had been preincubated with List-SS. Taken together, these results indicate a reduction in capacity to bind ^{125}I-List by both anti-List and anti-line 10 after inhibition with unlabeled List-SS and/or after absorption of antisera with *Listeria* on line 10 cells. Reduction in the capacity to bind ^{125}I-SA-10 by anti-List and anti-line 10 was similarly observed after preincubation with unlabled SA-10 and/or after absorption of the antisera with line 10 cells.

Although line 10 cells contain tumor-specific antigens, they also contain antigens unrelated to the neoplastic state. List-SS also consists of many components, only a few of which may be critical to the measurement of antibodies uniquely associated with tumor cell determinants. Such complexities probably explain the limited percentage of labeled antigens bound by antibodies in sera from the immunized animals and the sometimes limited reduction in binding noted in the inhibition and absorption experiments. There was a small reduction in binding by anti-List to ^{125}I-SA-10 after absorption of anti-List with 10^9 normal guinea

Table 5

Binding of ^{125}I-List and ^{125}I-SA-10 by anti-List and anti-line 10 after absorption of antisera with line 10 cells, listeria cells, and normal guinea pig spleen cells

Test system	Experiment	Absorbed with[a]	cpm in precipitates: Before absorption	cpm in precipitates: After absorption	% decrease of cpm
^{125}I-List:anti-List	1	10^8 line 10 cells	7375	6709	9
	2	4 × 10^8 line 10 cells	8653	7216	17
		10^9 line 10 cells	8653	7310	16
		Whole *Listeria* cells[b]	8653	1498	83
		10^9 normal guinea pig spleen cells	8653	8594	1
^{125}I-List:anti-line 10	3	4 × 10^8 line 10 cells	1976	1254	37
		4 × 10^8 normal guinea pig spleen cells	1976	1844	9
	4	4 × 10^8 line 10 cells	2454	1206	51
		Whole *Listeria* cells[b]	2454	1364	44
^{125}I-SA-10:anti-line 10	5	10^7 line 10 cells	3928	3440	13
	6	4 × 10^8 line 10 cells	5521	1520	72
		Whole *Listeria* cells[b]	5521	3790	31
^{125}I-SA-10:anti-List	7	10^8 line 10 cells	4091	3161	23
	8	4 × 10^8 line 10 cells	3944	1333	66
		Whole *Listeria* cells[b]	3944	1696	57
	9	4 × 10^8 line 10 cells	3403	1446	57
		10^9 normal guinea pig spleen cells	3403	2519	26
		Whole *Listeria*cells[b]	3403	1698	50

[a] Numbers of cells used to absorb equivalent of 1 ml of undiluted antiserum are indicated.
[b] One ml of antiserum was absorbed with 1 ml of packed washed *Listeria* cells.

Table 6

Summary of the inhibition and absorption experiments described in Tables 4 and 5

Test system	Reduction of cpm in precipitates that occurred after: Inhibition with unlabeled List-SS	Absorption with *Listeria* cells	Inhibition with unlabeled SA-10	Absorption with line 10 cells
^{125}I-List:anti-List	+	+	0	+
^{125}I-List:anti-line 10	+	+	+	+
^{125}I-SA-10:anti-line 10	0	+	+	+
^{125}I-SA-10:anti-List	0	+	+	+

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pig spleen cells (Table 5, Experiment 9). This reduction was much less than after the same antiserum was absorbed with 4×10^8 line 10 cells. In a previous study (15), it was found that absorption of anti-line 10 with 10^9 normal guinea pig liver and spleen cells did not alter the capacity of anti-line 10 to bind to ^{125}I-SA-10. The possibility that anti-line 10 had antibodies to antigens in normal guinea pig liver cells was also previously investigated (15). Neither anti-line 1 nor anti-line 10 had an increased capacity to bind to a radiolabeled KCl extract of normal guinea pig liver cells. The possibility, therefore, that cross-reactivity between line 10 and *Listeria* was due to normal tissue-associated antigens in line 10 cells, although not entirely excluded, would seem remote.

Further isolation and purification of specific antigens and antibodies for the reactions described above are needed before a more precise antigenic relationship between these bacteria and line 10 can be determined. For the present at least, the significant binding data (Tables 2 and 3) that were confirmed in the case of *L. monocytogenes* by the inhibition and absorption experiments (Tables 4 and 5) may be taken as evidence that line 10 cells share antigens with *Listeria* cells. The possibility that *B. abortus* and *S. typhimurium* share antigens with line 10 cells was suggested but not confirmed by inhibition studies. The question as to whether the line 10 components shared with *Listeria* constitute determinants concerned with the biological activity of this tumor or with the antitumor effects of *Listeria* was also not answered by this study.

The sharing of antigens between BCG and many unrelated microorganisms has previously been demonstrated (13, 14). Some microorganisms also have antigenic components expressed by certain mammalian cells (6, 9). Conceivably, as shown by the present study, shared antigens between tumors and microorganisms other than BCG and *Listeria* may exist and may be widespread. It is tempting, therefore, to speculate that microorganisms could be used in a specific way to guide immunotherapy or prophylactic programs for some tumors. With this in mind, studies are in progress to investigate the presence of shared antigens between some human tumors and a variety of microorganisms.

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REFERENCES

1. Bast, R. C., Jr., Zbar, B., Borsos, T., and Rapp, H. J. BCG and Cancer. New Engl. J. Med., *290:* 1413–1425, 1458–1469, 1974.
2. Bast, R. C., Jr., Zbar, B., Mackaness, G. B., and Rapp, H. J. Antitumor Activity of Bacterial Infection. I. Effect of *Listeria monocytogenes* on Growth of a Murine Fibrosarcoma. J. Natl. Cancer Inst., *54:* 749–755, 1975.
3. Bast, R. C., Jr., Zbar, B., Miller, T. E., Mackaness, G. B., and Rapp, H. J. Antitumor Activity of Bacterial Infection. II. Effect of *Listeria monocytogenes* on Growth of Guinea Pig Hepatoma. J. Natl. Cancer Inst., *54:* 757–761, 1975.
4. Borsos, T., and Rapp, H. J. Antigenic Relationship between *Mycobacterium bovis* (BCG) and a Guinea Pig Hepatoma. J. Natl. Cancer Inst., *51:* 1085–1086, 1973.
5. Bucana, C., and Hanna, M. G., Jr. Immunoelectronmicroscopic Analysis of Surface Antigens Common to *Mycobacterium bovis* (BCG) and Tumor Cells. J. Natl. Cancer Inst., *53:* 1313–1323, 1974.
6. Chase, R. M., Jr., and Rapaport, F. T. The Bacterial Induction of Homograft Sensitivity. I. Effects of Sensitization with Group A Streptococci. J. Exptl. Med., *122:* 721–732, 1965.
7. Dustoor, M. M., and Fulton, A. Effect of *Listeria monocytogenes* on Tumor Growth in Inbred Guinea Pigs. Federation Proc., *34:* 963, 1975.
8. Ferrari, A. Nitrogen Determinations by a Continuous Digestion and Analysis System. Ann. N. Y. Acad. Sci., *87:* 792, 1960.
9. Kaplan, M. H., and Suchy, M. L. Immunologic Relation of Streptococcal and Tissue Antigens. II. Cross-reactions of Antisera to Mammalian Heart Tissue with a Cell Wall Constituent of Certain Strains of Groups A Streptococci. J. Exptl. Med., *119:* 643–650, 1964.
10. Meltzer, M. S., Leonard, E. J., Rapp, H. J., and Borsos, T. Tumor-specific Antigen Solubilized by Hypertonic Potassium Chloride. J. Natl. Cancer Inst., *47:* 703–709, 1971.
11. Minden, P., and Farr, R. S. Binding between Components of the Tubercle Bacillus and Humoral Antibodies. J. Exptl. Med., *130:* 931–954, 1969.
12. Minden, P., Farr, R. S., and Trembath, J. Some Variable Controls for Accurate Heterologous Anti-immunoglobulin (HAI) Tests. Immunochemistry, *12:* 477–479, 1975.
13. Minden, P., McClatchy, J. K., Cooper, R., Bardana, E. J., Jr., and Farr, R. S. Shared Antigens between *Mycobacterium bovis* (BCG) and Other Bacterial Species. Science, *176:* 57–58, 1972.
14. Minden, P., McClatchy, J. K., and Farr, R. S. Shared Antigens between Heterologous Bacterial species. Infection Immun. *6:* 574–582, 1972.
15. Minden, P., McClatchy, J. K.. Wainberg, M., and Weiss, D. W. Shared Antigens between *Mycobacterium bovis* (BCG) and Neoplastic Cells. J. Natl. Cancer Inst., *53:* 1325–1331, 1974.
16. Rojas-Gorona, R. R., Skarnas, R., Tamakuma, S., and Fine, J. The Limulus Coagulation Test for Endotoxin. A Comparison with Other Assay Methods. Proc. Soc. Exptl. Biol. Med., *132:* 599–601, 1969.

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