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Impaired Function of Immune Reactivity to *Listeria monocytogenes* in Diet-Fed Mice

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Mice fed a diet high in cholesterol, lard, and sucrose were shown to exhibit an impairment of specific immunity to *Listeria monocytogenes*. Whereas titers of *L. monocytogenes* in livers of normal mice decreased rapidly after 6 days of infection, *L. monocytogenes* persisted in livers of diet-fed mice. Adoptive transfer experiments indicated that *L. monocytogenes*-immune spleen cells are generated in diet-fed mice. However, the function of immune spleen cells from donors of either nutritional status was impaired in diet-fed recipients. The results indicate that the site(s) of impairment of specific immunity to *L. monocytogenes* in diet-fed mice occurs at a stage beyond the generation of immune T-cells.

We have previously reported that C57BL/6 mice fed a diet high in cholesterol, lard, and sucrose have exhibited decreased resistance to infectious agents and transplantable tumors (1, 6, 7). These results, coupled with the inability of macrophage stimulants such as *Corynebacterium parvum* to increase resistance, suggested that decreased resistance in hypercholesterolemic hosts could be attributed to a nonspecific defect in macrophage function (2). In the current studies, using *Listeria monocytogenes* as a model, we present data which suggest that an impairment in specific immune mediated effector function exists in diet-fed mice.

C57BL/6 mice from Jackson Laboratories, Bar Harbor, Maine, were maintained on Purina Lab Chow, and 50% of the mice were started on a diet containing 52% sucrose, 18.5% casein, 18% lard, 1% cholesterol, 0.5% cholic acid, and vitamin supplements at 7 weeks of age as previously described (6). All experiments were conducted with animals fed the diet for 6 months. Plasma cholesterol levels were consistent in diet-fed animals in all experiments. Growth of *L. monocytogenes* in livers was analyzed in groups of 5 to 10 diet-fed and control mice at various intervals after intravenous inoculation with 1.3×10^3 CFU (50% lethal dose for diet-fed mice) of *L. monocytogenes*. Livers were removed aseptically and homogenized, and the number of viable *L. monocytogenes* cells was determined by colony formation on brain heart infusion agar after incubation overnight at 37°C.

In chow-fed mice, titers in the liver increased through day 2 after infection and then plateaued through day 6 post infection (Fig. 1). A drop in titer was seen at day 7, presumably due to the generation of specifically sensitized T-lymphocytes in spleens (3, 8, 11). Titers in livers of diet-fed mice paralleled those in the controls for the first 6 days after infection but continued to remain high throughout the 10-day observation period. Even at 14 and 21 days after infection, viable *L. monocytogenes* cells were present in livers of diet-fed animals (data not shown), a situation similar to that observed in nude mice (4). To establish whether sensitized spleen cells were being produced in these animals, an adoptive transfer of immunity experiment was performed, adapting previously described methods (5, 9, 11).

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Animals to be used as spleen cell donors received either 10^3 CFU of *L. monocytogenes* or 10^8 sheep erythrocytes (specificity control) intravenously 6 days before spleen cell harvest. To reduce the number of contaminating *L. monocytogenes* cells in the spleen cell suspension, donors were inoculated subcutaneously with 10,000 U of penicillin G 24 h before spleen cell harvest, and 500 μg of ampicillin per ml was added to their drinking water (9). Spleen cell suspensions were prepared in RPMI 1640 medium by pressing the spleens through a stainless steel mesh, aspirating twice through a 21-gauge needle, and washing by centrifugation for 10 min at $300 \times g$. Cells were suspended in 25 ml of RPMI 1640 medium, and adherent cells were removed by incubation at 37°C for 1 h. Nonadherent cells were washed twice more by centrifugation, and a viable count was obtained using trypan blue. Recipients were injected intravenously with 10^8 spleen cells in 0.2 ml followed by 10^4 CFU of *L. monocytogenes* in 0.2 ml. After 48 h, recipients were sacrificed. The spleens were removed, their volumes were recorded, and the spleens were homogenized. The number of viable *L. monocytogenes* cells per spleen was determined by plating on brain heart infusion agar. Titers in the spleens of animals receiving spleen cells from *L. monocytogenes*-infected (immune) donors were compared with titers of those that received spleen cells from donors that had been injected with sheep erythrocytes (nonimmune donors). The difference is expressed as the $\log_{10}$ protection.

In the first experiment (Table 1) spleen cells from chow-fed donors were transferred to recipients that had been fed the diet for 6 months and to age-matched, chow-fed controls. A significant reduction in the titer of *L. monocytogenes* in the spleens of the recipients of cells from the immune donors occurred in both cases, but the magnitude of the reduction was greater in the chow recipients (2.10 versus 0.94 log protection). It appeared, therefore, that sensitized spleen cells from chow-fed donors functioned less efficiently in the diet-fed recipients. When the experiment was repeated using spleen cells from diet-fed donors, significant protection was also observed in chow-fed and diet-fed recipients of spleen cells from diet-fed immune donors. This suggested that functional immune lymphocytes were produced in the diet-fed animals. This experiment was repeated using the total splenic population from *L. monocytogenes*-immune diet-fed mice to ascertain whether a subpopulation of adherent cells

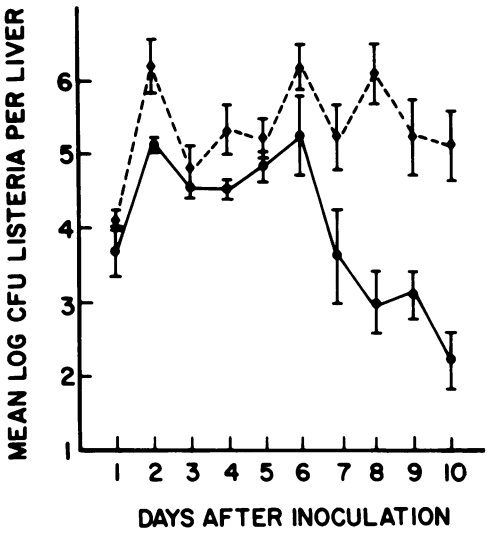

FIG. 1. Growth of *L. monocytogenes* in livers of diet-fed (- - -) mice compared with age-matched, chow-fed (——) controls. Brackets denote standard error, and n = 5 to 10 mice per point.

with some suppressor activity might be present in diet-fed animals, with evaluation of the spleen cells in diet-fed and control mice.

Protection was conferred by total spleen cells from immune diet-fed donors to both diet-fed and chow-fed recipients. In addition, total spleen cells from diet-fed immune donors conferred a higher degree of protection to chow-fed recipients (3.43 versus 1.92 log protection). This again suggested an impaired protective function of sensitized spleen cells in the diet-fed recipients.

The plateau effect of log CFU of *L. monocytogenes* per liver (Fig. 1) in diet-fed mice is consistent with results previously reported for nude mice (4) and thus consistent with a lack of T-cell function. However, spleen cells capable of transferring resistance to *L. monocytogenes* to normal mice could be isolated from diet-fed mice, indicating that immune T-cells are generated. The expression of immunity of transferred spleen cells from donors of either nutritional status was significantly lower in diet-fed recipients than in control mice. This latter observation suggests a qualitative defect in the ability of liver macrophages from diet-fed mice to respond to T-cell-mediated activation. Such a defect could be due to a quantitative or qualitative defect or both in the functional capacity of liver macrophages from diet-fed mice or alternatively to some influence of the physiological environment of the diet-fed host on production or activity of lymphokines.

Previous studies on diet-fed mice have indicated liver involvement. Kupffer cells from hypercholesterolemic mice were suggested to be hypofunctional with respect to viral clearance (2). The inflammatory response to *C. parvum* within the livers of hypercholesterolemic mice was impaired despite a normal inflammatory response in the peritoneal cavity and footpads of similar animals. With respect to lymphocyte and macrophage function in diet-fed mice, previous studies have shown a diminished splenic antibody response to sheep erythrocytes and a normal in vitro B-cell response to lipopolysaccharide and T-cell response to phytohemagglutinin. In addition, the appearance of a macrophage surface antigen associated with activation, as well as macrophage-mediated tumoricidal activity, was normal in peritoneal macrophages from diet-fed mice treated with *C. par-*

TABLE 1. Adoptive transfer of immunity to *L. monocytogenes* with spleen cells

Expt	Donor nutritional status	Donor immune status	Recipient nutritional status	n	Mean of log CFU of *L. monocytogenes* per spleen[a]	Log protection[b]	P
1	Chow	Immune	Chow	4	3.62	2.10	
	Chow	Nonimmune	Chow	5	5.70		<0.01
	Chow	Immune	Diet	5	4.81	0.94	
	Chow	Nonimmune	Diet	4	5.74		
2	Diet	Immune	Chow	6	3.96	1.68	
	Diet	Nonimmune	Chow	3	5.64		<0.01
	Diet	Immune	Diet	4	5.05	1.10	
	Diet	Nonimmune	Diet	6	6.15		
3	Diet	Immune	Diet	5	4.27	1.92	
	Diet	Nonimmune	Diet	5	6.19		NS[c]
	Diet[d]	Immune	Diet	4	3.96	1.92	
	Diet[d]	Nonimmune	Diet	5	5.88		<0.01
	Diet[d]	Immune	Chow	5	2.36	3.43	
	Diet[d]	Nonimmune	Chow	5	5.79		

[a] Immune donors conferred significant protection in all cases ($P < 0.01$).

[b] Difference between mean of log CFU of *L. monocytogenes* per spleen of recipients of spleen cells from immune versus nonimmune donors. Comparisons were made using analysis of variance and Duncan's multiple-range test (10).

[c] NS, Not significant.

[d] Total spleen cells were used in these cases; all others received only nonadherent spleen cells.

vum, despite a deficient response in the livers of these animals (6).

Further studies need to be performed before the exact nature of the impairment of specific immunity to *L. monocytogenes* in diet-fed hosts can be elucidated.

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