Foodborne Gram-Negative Bacteria and Atherosclerosis: Is There a Connection?

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ABSTRACT

There is some evidence that endotoxin-containing bacteria may contribute to atherogenesis. The degree to which bacterial insults contribute to the total body burden of atherosclerotic lesions cannot be determined at this time. It is important to realize that there are other potential sources of injury to the vascular endothelium, mechanical, chemical, immunologic and biological, which may initiate formation of an atherosclerotic plaque. It must also be remembered that the process of atherogenesis is extremely complex and involves many factors other than the initial injury to endothelium. The suggested role for endotoxin, particularly endotoxin from degrading bacteria in macrophages, in concert with the inflammatory factors induced by endotoxin from endothelium and vascular smooth muscle cells, is an attractive hypothesis for several reasons. First, dampening of inflammatory responses by effects of N-3 polyunsaturated fatty acids (omega-3s) is explained, particularly their direct influence on monocyte functions. Second, the hypothesis provides a model system in which the first step in atherogenesis may be studied prospectively, while other factors may be varied to determine their influences on later stages in the process of plaque formation. Recombinant DNA techniques and sophisticated immunologic tools are available to study the entire process, as are animal models in which to conduct studies with relevance to the human. Although at present, the link between foodborne gram-negative bacterial pathogens and atherosclerosis is largely unproven, the possible role of such organisms warrants more research. Additionally, should the link be firmly established, it would further underscore the importance of food safety in the biological sense.

Numerous factors have been shown to contribute to atherosclerosis and coronary heart disease, including, but not limited to, cigarette smoking, high blood pressure, high serum cholesterol, genetic makeup, obesity, age, level of physical activity and behavior pattern. Ross and Glomset (18) recently reviewed the various hypotheses of atherogenesis. Two major questions have puzzled medical investigators: (a) What is the cause of, and nature of, the endothelial cell injury that permits the atherosclerotic lesion to form? (b) Can chronic hypercholesterolemia alone induce the changes in the endothelium that precede atherosclerotic plaque formation or is some other form of “injury” required?

It is obvious that atherosclerosis is a complex, multifactorial disease process, and is not attributable to any single cause. There may even be more than one source of endothelial cell injury that can lead to plaque formation. An accumulating body of evidence, however, suggests a role for gram-negative bacteria in the lesion induction process, so long as they can become blood-borne, either free or intracellular in macrophages/monocytes. Since many of the foodborne bacteria, pathogens and nonpathogens, are gram-negative bacteria, and since there is evidence that they can, and often do, gain access to lymphoid and/or general circulation, either free or intracellular, the possibility of a role for foodborne bacteria in atherosclerotic disease deserves consideration. This paper summarizes evidence linking early events in the process of atherogenesis with gram-negative bacteria, and further, discusses evidence suggesting that foodborne bacteria may gain access to lymphoid and/or general circulation with relatively frequent.

THE MACROPHAGE IN ATHEROGENESIS

A detailed review of the pathogenesis of atherosclerosis was recently published (18). Therefore only new information relevant to the potential role of bacteria will be discussed.

Davies (4), in a recent review, suggests three early events in the process of atherogenesis in which vascular
cell interactions play a key role. The events are as follows: (a) smooth muscle cells proliferate and synthesize extracellular connective tissue that thickens the intima of the vessel, (b) a substantial focal mononuclear leukocyte infiltration into the vessel wall occurs, and (c) smooth muscle cells and macrophages accumulate large intracellular deposits of cholesterol esters, thus becoming foam cells. The first interaction between macrophages and the vessel endothelium is adhesion of the monocytes to the endothelium. The chemotactic signal to attract the monocytes can be produced in vitro by endothelial cells, smooth muscle cells and macrophages. Thus the starting point for adhesion of the macrophage to the vessel endothelium has been in question until very recently (see below).

After adhesion, monocytes/macrophages are found beneath the endothelium where they can freely interact with smooth cells. In fact, monocyte infiltration of the vessel wall precedes the observed intimal smooth muscle cell hyperplasia (4). This has suggested to some investigators that the macrophage may induce smooth muscle cell proliferation via production of mitogenic monokines (4), although production of such factors by other cell types has not been ruled out. Endothelial cells produce inhibitor(s) of smooth muscle cell proliferation, one of which is heparin-like (4). In the process of atherogenesis, the balance may be tipped in favor of the mitogenic stimulus because of endothelial damage or the overwhelming of the negative feedback signal (heparin-like inhibitor) by accumulation of cells producing mitogen.

The cellular makeup of atherosclerotic lesions has mainly been studied by conventional microscopic techniques, although cellular morphology within plaques is quite altered from normal morphology. Gown et al. (9) recently studied three types of atherosclerotic lesions, namely, fibro-fatty lesions, fibrous plaques, and advanced plaques, by using monoclonal antibodies specific for smooth muscle cells and macrophages. These new monoclonals are very cell-specific but cross-react with the corresponding cell types in other species; this will facilitate use of animal models in studying atherogenesis. Application of these techniques verified the role(s) of macrophages and smooth muscle cells in the various stages of plaque formation, although the role(s) of the cells varies depending on the stage of plaque formation (9).

Using a rat model, Rogers et al. (17) recently studied the effects of dietary cholesterol on macrophages. General augmentation of macrophage adherence to both endothelium and vascular smooth muscle cells, an enhancement of production of factors mitogenic for smooth muscle cells, and increased vascular smooth muscle cell migration were observed during periods of dietary hypercholesteremia.

Accumulation of lipids in macrophages and smooth muscle cells at various stages of plaque formation involves a complex interaction among the cell types, metabolic alteration and altered transport of plasma lipoproteins, which is beyond the scope of the present report. A recent view of this complex chemistry was presented by Davies (4). Two aspects that must be mentioned in the context of this report are the role of low density lipoproteins (LDL) and the possible protective role of N-3 polyunsaturated fatty acids (the omega-3 fatty acids); the latter will be discussed below. High plasma levels of LDL have long been associated with atherogenesis. Several theories as to how LDL may participate in plaque formation by injuring endothelial cells have been suggested (reviewed by Ross in ref. 19). One hypothesis suggests a role for LDL in production of platelet-derived, growth factor-like substances from endothelial cells and macrophages that are potent mitogens for vascular smooth muscle cells (19).

### ENDOTOXINS AS A SOURCE OF ENDOTHELIAL CELL DAMAGE AND AS MEDIATORS OF ATHEROGENESIS

Endotoxins, the lipopolysaccharide (LPS) components of bacterial cell walls, have been shown to be toxic for endothelial cells of some species in vitro (16). In a recent study, Morel et al. (16) showed that human endothelial cells are not directly susceptible to endotoxin-induced toxicity. Oxidized LDL binds to LPS and facilitates its entry into human endothelial cells; once internalized, its damaging effects can be exerted. Whether cell death always results is uncertain; in studies of species other than humans, LDL facilitated transport of LPS across the endothelial cell membrane and resulted in production of chemotactic factors (11).

Libby et al. (14) have demonstrated the ability of endotoxin (Escherichia coli 055:B5-derived) to induce interleukin-1 (IL1) gene expression, as monitored by IL1 mRNA increase, and to increase production of biologically active IL1 by human vascular endothelial cells. It was previously thought that IL1 was exclusively a product of macrophages. IL1 can cause endothelial cells to express procoagulant synthesis and increased leukocyte adherence. Vascular endothelial cells may not produce IL1, but respond to IL1 as well. Thus, endothelial cells exposed to endotoxin (LPS) may produce IL1 that induces increased adherence of monocytes/macrophages, which may then produce more IL1 and other mediators. This type of cycle is referred to as an amplification loop. Libby et al. (14) suggest that endotoxin may thus initiate an amplification loop through interaction with vascular endothelium, and that the resultant accumulation of inflammatory cells (monocytes/macrophages, granulocytes) may lead to atherosclerotic plaque formation. The authors speculate that the source of endotoxin could be blood-borne bacteria during the hematogenous stage(s) of infection.

In a second study, Libby et al. (15) showed that human vascular smooth muscle cells also produce IL1 in response to endotoxin in vitro. Thus, the possibility of another amplification loop initiated by endotoxin, involving the vascular smooth muscle cell, the major cell type in the vessel wall, may also play a role in atherogenesis.
This observation awaits in vivo confirmation.

Libby et al. (14) envisioned exposure of the endothelium to endotoxin during traversing of the endothelium by bacteria during tissue invasive infections. Another possibility is that bacteria may be carried through the blood stream by macrophages and induce injury from this intracellular location. Duncan et al. (6) have demonstrated that macrophage-processed E. coli endotoxin is 10-100-fold more potent in its ability to induce IL1. Thus a host defense mechanism may contribute to an enhanced inflammatory response that is potentially damaging. Whether other gram-negative bacterium-derived endotoxins are also potentiated by macrophage processing awaits study. Previous studies showed that although bacterial products are rapidly exocytosed from macrophages during the killing process, endotoxins are slowly exocytosed. Thus bacteria would not need to be viable to participate in atherogenesis via potentiated endotoxin release from circulating monocyte/macrophages with resultant IL1 production by vascular endothelial or smooth muscle cells. The macrophages that contain LPS could also be producing IL1. It is important to recall that two previous reports suggested a role for LDL in transport of endotoxin into cells that may participate in an amplification loop (11,16). Activated macrophages within the vessel wall may provide a constant source of endotoxin and inflammatory mediator production/induction. Additionally, Altieri and Mannucci (1) recently demonstrated that activated macrophages, in addition to promoting clots, may produce thromboxane via the cyclooxygenase pathway; this thromboxane exerts a potent platelet aggregating effect.

In summary, evidence is accumulating that endotoxin, either in native form or processed/potentiated by monocytes/macrophages, may initiate a complex sequence of cellular responses and interactions within the blood vessel wall that result in plaque formation.

FOODBORNE PATHOGENS: ARE THEY BLOODBORNE?

It is well known that certain of the gram-negative foodborne pathogens are invasive (reviewed in ref. 7). The shigellae, salmonellae, invasive E. coli, certain vibrios, Campylobacter jejuni, Aeromonas hydrophila and Yersinia enterocolitica are among those known to invade the intestinal epithelium. Some may become bloodborne directly; others may enter monocytes/macrophages either during the initial invasion process (i.e., from damaged intestinal epithelium), or during the bloodborne phase of infection.

Determining what proportion of gastrointestinal pathogens may become bloodborne is an extremely difficult task. The ability of organisms such as Campylobacter, for example, to cause extraintestinal infections in some persons underscores the complexity of the issue when both host factors (e.g., age, health status) and microbial factors (e.g., cell wall chemistry) are involved (3). It is possible that during many gastrointestinal infections, there is a transient bloodborne phase that is dealt with easily by a healthy individual. Alternatively, it is also possible that intracellular bacteria (i.e., in monocytes/macrophages), either killed or viable, may circulate for an unknown time. Kiehlbauch et al. (13) recently reported the intracellular survival of C. jejuni in human peripheral blood monocytes for 6 to 7 d in vitro and suggested that phagocytosis may actually promote survival of C. jejuni. The fact that most extraintestinal infections with invasive foodborne pathogens arise in debilitated persons does not necessarily indicate that the bacteria do not become bloodborne in normal, healthy persons. Many microbes have evolved mechanisms to thwart intracellular killing by macrophages (8).

Many gram-negative bacteria cause systemic infections in debilitated persons even though they are not invasive in the ordinary sense. That is to say, there are bacteria that do not cause overt acute disease except in certain persons, yet their becoming bloodborne in normal persons cannot be discounted. For example, A. hydrophila, E. coli, Klebsiella pneumoniae and Pseudomonas aeruginosa are frequent killers of persons undergoing chemotherapy for malignancy. Malignancy itself, without chemotherapy, predisposes to lethal systemic infections from Salmonella typhimurium, A. hydrophila, Campylobacter fetus and Acinetobacter calcoaceticus (2). Again, there is little knowledge as to whether these bacteria, which may or may not cause acute, symptomatic disease, may be transiently bloodborne in a normal person. Researchers who study classical foodborne pathogens generally investigate those factors responsible for the acute symptoms, whereas those who study the bacteria responsible for bacteremias generally investigate bacterial surface factor(s) that allow the organism to survive in the blood or intracellularly. Both groups of pathogens can be present in, and transmitted by, food.

Transient bacteremia in the normal person would be extremely difficult to detect, as the bacteria may be present in low numbers, be present for a limited time, reside intracellularly while bloodborne, be damaged by leukocytes or be non-viable. Not much effort has been directed at detecting exocytosed LPS from circulating normal human macrophages following gastrointestinal infection or at any other time.

HOW MICROBES MAY USE NORMAL BODY DEFENSES TO GAIN ACCESS TO THE BLOOD

As previously mentioned, invasive enteric pathogens may gain access to general circulation, but acute gastrointestinal infections usually accompany the invasive occurrence. The larger question involves the ability of noninvasive pathogens to gain access to circulation and the manner in which this is accomplished.

Sneller and Strober (20) recently reviewed the role of the M cell in host defense. The M cell is the specialized
epithelial cell that overlies the lymphoid follicles of the respiratory and gastrointestinal tracts; it has been thought of as the immune system's sampling port. M cells, in addition to sampling proteins in the G.I. tract, may also actively engulf and transport microbes to the lymphoid follicles. Recent studies have shown that certain microbes bind preferentially to M cells, while others bind equally well to M cells and the surrounding absorptive epithelium (20). Other microbes, most notably the normal flora, do not bind to either. The total lack of binding of normal flora constituents is chiefly due to specific antibodies produced by the mucosal immune system. It was also recently shown that even though amicrobe may be preferentially bound to M cells, it will not necessarily be taken up (20). Inman et al. (12), using recombinant techniques, were able to generate an RDEC-1 E. coli that expressed the Shigella flexneri somatic antigen. This recombinant strain was less virulent than another recombinant strain that expressed the original O15 somatic antigen. Both adhered equally well to the M cell, but the less virulent strain was taken up into the lymphoid follicle (where an immune response could be generated), whereas the virulent strain was not taken up. This suggests the possibility that the ability to adhere to M cells without being taken up, for some organisms, permits a focus of colonization to be established without elicitation of an immune response; that focus of colonization may subsequently spread to adjacent absorptive epithelium. The factors that facilitate binding to M cells are likely to be dissimilar from those that permit attachment to absorptive epithelium.

Invasive organisms, however, such as S. typhimurium, may be given an advantage by being transported into the lymphoid follicles, as they possess attributes that make their destruction by components of the immune system less likely. Uptake through the M cell, for example, has been shown to be the first step in Salmonella infection (10).

The M cell is an important component of the mucosal immune system. Certain pathogens, however, have evolved means to turn the host defense role of the M cell (binding) to their own advantage. Even in situations in which an organism binds and is taken up, the end point need not be to the advantage of the host. Once in the lymphoid follicles, the organism comes into contact with macrophages that may in turn migrate from the follicles. Organisms that may reside, and even multiply, in macrophages thus may gain initial entry through the M cell without the need for invasive factors. As previously stated, even nonviable organisms in macrophages may gain access to circulation via the M cell; endotoxins may be slowly exocytosed from these macrophages. Not many bacteria have been studied with regard to their interaction(s) with the M cell. It is tempting to speculate, however, that strains of bacteria responsible for bacteremias in certain populations, but lacking invasive potential, may gain access to the body proper via the M cell.

In summary, the degree to which gram-negative bacteria gain access to general circulation, either free or intracellular in macrophages, cannot be stated with certainty. It seems clear, however, that such exposure is probably not an uncommon event, even in the normal person.

REFERENCES


