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Correspondence

To the Editor-in-Chief:

Atherosclerosis is the leading cause of death in the developed societies, probably related in part to relatively high-fat diets. The leading risk factor for the disease, however, is aging. A study of 19 different ethnic population groups demonstrated approximately linear rates of increases (although with different slopes) in the extent to which the intimal surfaces of aging aortas and coronary arteries develop raised, fibrotic, lipid-containing atheromas.[1] The great majority of deaths from atherosclerosis (typically via myocardial infarctions) occur after the age of 45. Quantitative studies[2–4] have demonstrated that genetic alleles responsible for phenotypes that are expressed beyond this age essentially fail to contribute to the gene pool of successive generations, thus escaping the force of natural selection. These alleles, however, may have been under strong selective pressure during earlier phases of the life course. This phenomenon has been referred to as "antagonistic pleiotropy" or "negative pleiotropy."[5,6]

I suggest that atherosclerosis may fall into this category of gene action and that its remarkably high prevalence in our species is a result of strong selective pressures for the retention of genes that enhance reproductive fitness early in the life course despite their deleterious effects on the vascular system postreproductively. It is likely that others have entertained or published such notions. One purpose of this correspondence is to ask my colleagues to inform me about the scientific history of this idea, which, it seems to me, is of seminal importance to the incipient discipline of evolutionary medicine.[7]

The second purpose of this communication is to point out recent experimental support for this proposition[8] that was not discussed in the context of evolutionary biology. The study was motivated by a theory of atherogenesis that invokes a major role for arterial wall damage mediated by posttranslationally modified (particularly oxidized) low density lipoproteins.[9–11] These molecules are picked up by macrophages, the body's scavengers, via particular classes of promiscuous receptors ("molecular flypaper"),[12] the macrophage scavenger receptors. These macrophages are thought to be the major sources of the lipid-laden foam cells that appear in the early "fatty streak" stage of atherogenesis.

Although rodents are notoriously resistant to spontaneous atherosclerosis, mice homozygous for null mutations at the apolipoprotein E locus develop marked hyperlipidemia and a form of progressive atherosclerosis; lipoprotein oxidation appears to play a role in the pathogenesis.[13] Suzuki et al[8] crossed such mice with mice bearing targeted lesions in a macrophage scavenger receptor gene. Such doubly deficient mice were found to be significantly more resistant to atherosclerosis. Also of great interest was the finding that mice deficient in macrophage scavenger receptor function were highly susceptible to infection by a gram-positive bacterium, *Listeria monocytogenes*, and by the type 1 human herpesvirus. The relevant macrophage receptor had previously been shown to bind to a wide range of gram-positive bacteria, including streptococci, staphylococci, and enterococci[14] as well as to a form of bacterial endotoxin.[15] There is less information concerning the potential role of macrophage scavenger receptors in the defense against viral agents, but a role for nonparenchymal liver cells in the clearance of plasmids[16] and the binding of certain classes of polynucleotides to macrophage scavenger receptors[17] is consistent with such a role.

The genomes of today's populations of *Homo sapiens* have been substantially shaped by the selective resistance to infectious disease of remote ancestors.[18] Gene action at the macrophage scavenger receptor locus on chromosome 8[19] has undoubtedly played an important role in this respect. Domains of that gene have ancient evolutionary origins[20] and potential roles in the defenses of multicellular organisms against microbial pathogens and their toxins. Given our present diets and our relatively long life spans (which, of course, have also been molded by ancient selective forces),[2] we now appear to be paying a price for such reproductive phases of our life histories. This hypothesis predicts that mutations and polymorphisms at the macrophage scavenger locus on chromosome 8 will modulate individual susceptibility to atherogenesis.

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To the Editor-in-Chief:

We read with interest the article by Kockx et al.[1] They cited our article[2] saying that up to 60% of the cells in atherosclerotic plaques are reported to be apoptotic. We believe that there was a misinterpretation of our results. The percentages of apoptosis reported in our article are mean percentages in four transversal sections taken from the most stenotic region of the plaque. Our aim was not to determine the rate of apoptosis in human atherosclerosis but to analyze the relationship between apoptosis and cellular expression of caspases. Therefore, because we did not analyze the whole plaque, the percentages of apoptosis in our study reflect only the occurrence of the phenomenon in selected areas.

We are aware that the TUNEL method lacks specificity but we wish to point out that in our hands a percentage of apoptosis as low as that reported by Kockx et al (2% apoptosis) is not unexpected when the whole plaque is taken into consideration. The distribution of apoptosis is heterogeneous within the plaque and we believe that as far as plaque instability is concerned, it is more important to emphasize this heterogeneity rather than reporting a mean percentage in the whole plaque.

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Authors' Reply:

We completely agree with Mallat and Tedgui that atherosclerotic plaques are heterogeneous and that estimates of apoptotic cell death are strongly dependent on the stage of atherosclerosis.[1–3] Mallat et al counted the percentage of TUNEL-labeled nuclei in 10 random fields of the plaque.[1] Their letter states that they have done the analysis in selected areas. Even if counting in selected areas, the values they report are very high, which may imply that these plaque areas are close to a state of disintegration as remarked by Newby.[4] We have demonstrated that a large fraction of the TUNEL-positive cells in these regions of the atherosclerotic plaques are capable of RNA transcription and splicing, indicating that the cells are not in the execution phase of apoptosis.[5] Even in the early stages of execution caspase 3 (CPP-32) will cleave the 70-kd protein of the splicing factor UlsnRNP, resulting in a loss of RNA splicing.[6] Classically it was stated that the TUNEL technique recognizes only oligo-nucleosomal-sized DNA fragments that occur during the final phase of the apoptotic cascade and possibly also as a secondary early event in necrosis.[7] Others have also noted that the TUNEL technique may lack specificity due to proteinase K pretreatment of differences in fixation and prefixation time. In agreement with Hegyi,[8] we also found that the technique is very sensitive and therefore requires careful titration of proteolytic pretreatment and terminal deoxynucleotidyl transferase concentration. Otherwise a high fraction of nonapoptotic nuclei will be labeled. A strong relationship exists[5] between the synthetic state of the cells and unspecific labeling by the TUNEL technique. This indicates that cells with high RNA synthesis will be unspecifically labeled, whereas regions without obvious RNA synthesis are not labeled. Interestingly, several groups have used a modification of the nick translation method for DNA labeling for the detection of sites of active gene transcription.[9]