Cell death is found in human and animal atherosclerotic plaques.1–4 There is now extensive evidence for both apoptosis and necrosis, the latter being either a primary process or secondary to apoptosis (secondary necrosis). There is also extensive evidence that other processes or structural changes in the plaque accompany cell death, such as inflammation, thrombosis, calcification, and changes in size of both the fibrous cap and necrotic core (reviewed in5). Plaque cell death is also associated with a systemic proinflammatory state, with changes in multiple serum cytokines.6–8 However, the most difficult problem has been determining just what the cause and effect relationship is between cell death and these processes. Fortunately, studies performed over recent years have begun to directly address this problem.

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Apoptosis is detectable in atherosclerosis, with increasing frequencies as the plaque develops.4 Thus, the consequences of apoptosis are likely to be more important in advanced rather than early plaques. However, even this simple assumption may not be correct. The first problem relates to detection and measurement of apoptosis in plaques. Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) or cleaved caspase-3 antibodies are most frequently used to detect apoptotic cells. However, it is not clear how much of the apoptotic process is detectable using these systems: clearly, if cleaved caspase-3 antigen is only detectable for part of the time the cell is undergoing apoptosis, measurement of the cleaved enzyme alone will underestimate the frequency. Similarly, the presence of TUNEL-positive bodies long after apoptosis has finished may grossly overestimate the frequency. Note that what is measured are frequencies and not rates. We do not know how long apoptosis takes in vivo, and most studies have not examined 2 or more time points to calculate rates. We also do not know how long apoptotic body clearance takes in vivo, as previous studies identifying “delayed clearance” have again only studied 1 time point. It is also extremely difficult to determine whether an apoptotic body is inside or outside a cell in a histological section given the multiple planes of view.

The second problem relates to the cell type undergoing apoptosis in an atherosclerotic plaque. It is probable that all cell types within a lesion can and do undergo apoptosis. However, detection of cell type relies either on geographical location within the lesion, a notoriously poor method, or some form of lineage labeling. However, vascular smooth muscle cells (VSMCs) lose many lineage markers by dedifferentiation as the plaque develops, and many cells lose antigenicity to lineage markers when the cell dies. This may account for the very high percentage of unlabeled dead cells seen in plaques.4

The third problem is determining cause and effect and not association. While many processes accompany apoptosis, only direct manipulation of apoptosis alone in a single cell type can be reliably assumed to determine a direct consequence of apoptosis of that cell type. It is hard to interpret many studies that show changes in plaque composition and apoptosis with drugs or genetic manipulations that have multiple effects other than apoptosis, or affect multiple cell types.

It is this area that the study in this issue of Arteriosclerosis, Thrombosis, and Vascular Biology by Thorp et al sheds considerable light on the role of macrophage apoptosis in atherosclerosis. Macrophage apoptosis has been implicated in numerous events during atherogenesis, most frequently the accumulation of macrophage-derived apoptotic bodies late in atherosclerosis which contributes to core formation and expansion (see for review). It is also postulated that secondary necrosis of macrophages after inefficient phagocytosis and clearance in vivo may promote inflammation. However, the evidence underlying these assumptions has been controversial.

Direct induction of macrophage apoptosis has been achieved in vivo via a variety of approaches. First, administration of a proapoptotic stimulus can preferentially induce macrophage apoptosis in plaques in vivo when the other plaque cells (for example ECs and VSMCs) are resistant. Administration of TRAIL to diabetic ApoE knockout mice induces apoptosis in established plaques, with most dying cells being macrophages.10 Interestingly, plaques got smaller with fewer macrophages and less inflammation in lesions.10 This study argues that macrophage apoptosis is beneficial in atherosclerosis, even in advanced lesions, if apoptosis is augmented as the plaque develops. Similarly, we have used CD11b-diphtheria toxin receptor transgenic mice to examine the effect of monocyte/macrophage death in atherogenesis, and both acutely and chronically in established plaques.11 Monocyte/macrophage apoptosis reduced atherogenesis. However, despite increasing macrophage apoptosis 3-fold and reducing total macrophages by 27% acutely in estab-
Figure. Schematic diagram of the consequences of macrophage apoptosis. Macrophage apoptosis alone can promote expansion of the necrotic core. However, the article by Thorp et al suggests that binding and phagocytosis of apoptotic macrophages (even in the milieu of the plaque) is efficient enough to prevent additional consequences such as local or systemic inflammation, fibrous cap thinning, changes in collagen content, or increased plaque size. If there is an additional defect in phagocytosis, over and above any defect related to atherosclerosis alone, secondary necrosis of macrophages could occur, which may result in local and systemic inflammation, and may increase plaque size.

lished lesions, long-term monocyte/macrophage apoptosis over 10 weeks (from 12 to 22w of fat feeding) in ApoE knockout mice had no discernable effect on plaque size or composition of advanced plaques. This argued that, unlike VSMCs, apoptotic macrophages were easily replaced by recruitment of circulating cells, and long-term apoptotic body accumulation did not occur. In contrast, 2 recent studies using macrophages lacking the phagocytic receptor MerTK (which have a defect in clearance) show accumulation of apoptotic bodies, an increase in necrotic core size, a variable effect on plaque size (either increased or unchanged), and (in 1 study) both local plaque inflammation (assessed by increased macrophages and T cells) and proinflammatory cytokine production by splenocytes. A similar study in mice with macrophages deficient in the phagocytic protein lactadherin showed a proinflammatory alteration in splenocyte and plaque cytokines, and accelerated atherosclerosis.

Against this background, the study by Thorp et al provides important insights into the limited effects of macrophage apoptosis alone. To induce macrophage apoptosis these workers excised the antiapoptotic protein Bcl-2 using the LysM Cre-lox system. Although this manipulation is moderately specific for macrophages, LysM cre-induced deletion also occurs in granulocytes and to a lesser extent dendritic cells. Similarly, although Bcl-2 is antiapoptotic in multiple cell types, Bcl-2-deficient macrophages exhibit increased caspase-1 processing and interleukin (IL)-1β production, indicating that macrophage Bcl-2 deletion is proinflammatory irrespective of its effects on apoptosis. Accepting these caveats, Bcl-2 was absent from plaque macrophages in transgenic mice, macrophages from these mice underwent apoptosis more readily in vitro, and increased apoptosis frequencies were seen in vivo, at least at later time points. However, despite the clear effect of this manipulation, macrophage apoptosis had no discernable effect on plaques after 4 weeks of feeding. After 10 weeks of feeding, lack of macrophage Bcl-2 increased apoptotic frequencies by 40% to 45%, but there was a more modest increase in necrotic cores of 25%, this was observed only in female mice, and there was no effect on other structural features that determine plaque stability, including fibrous cap thickness or collagen content. In addition, there was no effect at any time point on plaque size, and no effect on the serum proinflammatory cytokines IL-6 and tumor necrosis factor (TNF)-α.

This study further supports the literature (see references within Thorp et al) that macrophage apoptosis contributes to formation of the acellular necrotic core in plaques, in itself an important contributor to plaque instability. Macrophage apoptosis appears to be more important in advanced rather than early lesions, but the relatively modest effect on necrotic core size in the current study limited to female mice, despite a significant increase in macrophage apoptosis in plaques, may suggest that macrophage apoptosis is only one of many determinants of core size. In particular, acellular cores are formed by multiple processes, including accumulation of both intracellular and extracellular lipid and death of a number of cell types. Indeed, VSMC apoptosis can very effectively increase necrotic core sizes (by more than 200% in some studies), in the absence of changes in macrophage apoptosis. In addition, macrophage apoptosis does not appear to promote cap thinning or induce systemic inflammation.

So what can we conclude from the findings from these different studies? (Figure) Macrophage apoptosis appears to be both more frequent and more detrimental to plaque composition in more advanced plaques. Although likely, it is not proven whether more advanced lesions have increased rates of apoptosis, or just greater detection of apoptosis, possibly attributable to reduced clearance of corpses. Macrophage apoptosis, particularly when monocyte numbers are also depleted, can reduce atherogenesis. Macrophage apoptosis contributes to acellular core formation in more advanced lesions, but it is not the only process, and may not be the most important process. Manipulations that are predicted to delay macrophage apoptotic body clearance by macrophages in vivo are associated with systemic inflammation; however, macrophage apoptosis alone did not promote systemic or local inflammation and it is not clear whether the systemic proinflammatory cytokine profile associated with atherosclerosis is attributable in part to defective clearance. Macrophage apoptosis alone can promote expansion of the necrotic core. However, the article by Thorp et al suggests that binding and phagocytosis of apoptotic macrophages (even in the milieu of the plaque) is efficient enough to prevent additional consequences such as local or systemic inflammation, fibrous cap thinning, changes in collagen content, or increased plaque size. If there is an additional defect in phagocytosis, over and above any defect related to atherosclerosis alone, secondary necrosis of macrophages could occur, which may result in local and systemic inflammation, and may increase plaque size.
phage apoptosis combined with defective clearance may have greater effects than the limited effects of macrophage apoptosis alone. For example, the earlier studies in which there are predicted defects in clearance in vivo show higher augmentation of necrotic core percentages than the current study. However, until studies examine clearance using the kinetics of change in apoptotic body number between 2 or more time points, the absolute slowing of rates of clearance cannot be elucidated. Finally, changes in plaque composition may not automatically translate into changes in plaque progression. Whereas apoptosis of VSMCs accelerates atherogenesis and progression of established plaques,8 macrophage apoptosis alone, even in advanced lesions, may not increase absolute plaque size.

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