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Killer cells in atherosclerosis

Tin Kyaw[a,b,*], Peter Tipping[b], Ban-Hock Toh[b], Alex Bobik[a,c]

[a] Baker Heart and Diabetes Institute, Melbourne, Australia
[b] Centre for Inflammatory Diseases, Department of Medicine, Monash University, Melbourne, Australia
[c] Department of Immunology, Monash University, Melbourne, Australia

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ABSTRACT

Cytotoxic lymphocytes (killer cells) play a critical role in host defence mechanisms, protecting against infections and in tumour surveillance. They can also exert detrimental effects in chronic inflammatory disorders and in autoimmune diseases. Tissue cell death and necrosis are prominent features of advanced atherosclerotic lesions including vulnerable/unstable lesions which are largely responsible for most heart attacks and strokes. Evidence for accumulation of killer cells in both human and mouse lesions together with their cytotoxic potential strongly suggest that these cells contribute to cell death and necrosis in lesions leading to vulnerable plaque development and potentially plaque rupture. Killer cells can be divided into two groups, adaptive and innate immune cells depending on whether they require antigen presentation for activation. Activated killer cells detect damaged or stressed cells and kill by cytotoxic mechanisms that include perforin, granzymes, TRAIL or FasL and in some cases TNF-α. In this review, we examine current knowledge on killer cells in atherosclerosis, including CD8 T cells, CD28- CD4 T cells, natural killer cells and γδ-T cells, mechanisms responsible for their activation, their migration to developing lesions and effector functions. We also discuss pharmacological strategies to prevent their deleterious vascular effects by preventing/limiting their cytotoxic effects within atherosclerotic lesions as well as potential immunomodulatory therapies that might better target lesion-resident killer cells, to minimise any compromise of the immune system, which could result in increased susceptibility to infections and reductions in tumour surveillance.

1. Introduction

Atherosclerosis is a cholesterol-initiated inflammatory disease of large- and medium-sized arteries. It takes decades to establish unstable or rupture-prone plaques, but once established, ruptured plaques initiate thrombotic arterial occlusion that can abruptly block blood supply to the heart or brain resulting in sudden onset of heart attacks and strokes that are the current leading cause of global deaths. Haemorrhagic strokes constitute only ~ 10% of total strokes (Andersen et al., 2009). Together with the fact that most heart attacks are ischemic in origin, atherosclerosis is the most important and major contributing factor to heart attacks and strokes. Despite extensive use of cholesterol-lowering statins, atherosclerosis-related deaths increased to 13.43 million in 2012 from 12.53 million in 2002 (World Health Organization, 2014) and are expected to continue to rise to 15.32 million by 2030 (World Health Organization, 2016), suggesting that targeting cholesterol alone is not sufficient enough to prevent heart attacks and strokes. In vulnerable/unstable plaques, apoptosis followed by caspase 3-mediated secondary necrosis (Rogers et al., 2017) are largely responsible for lesion cell death (Kockx and Herman, 2000; Martinet et al., 2011) that then initiates a sterile inflammatory response (Chen and Nunez, 2010). A large necrotic core area is one of the important characteristics of unstable/rupture-prone plaques (Finn et al., 2010). A study using human coronary plaques collected from patients with sudden death showed that coronary plaques have large necrotic core area with ruptured thin fibrosis cap containing very few smooth muscle cells and large infiltrations of lymphocytes (Burke et al., 1997), suggesting important roles for killer lymphocytes that can induce apoptosis of lesion cells. Evidence of cytolytic CD8 T cells in both mouse and human atherosclerotic lesions has strengthened this suggestion (Gewaltig et al., 2008; Kolbus et al., 2010). We have shown that CD8 T cell-induced apoptosis and secondary necrosis are responsible for generation of vulnerable atherosclerotic lesions and identified lesion macrophages, endothelial cells and vascular smooth muscle cells (VSMC) as cellular targets of killer CD8 T cells (Kyaw et al., 2013b). Despite two major pathways to induce apoptotic target cell death by lysis via cytolytic granules and/or via Fas-FasL interaction (Henkart and Sitkovsky, 1994), our understanding as to how killer cells are

* Correspondence to: Vascular Biology and Atherosclerosis Laboratory, Baker IDI Heart and Diabetes Institute, 75 Commercial Road, Melbourne, Victoria, Australia.
E-mail address: tinsoe.kyaw@baker.edu.au (T. Kyaw).

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activated, migrate to atherosclerotic lesions and kill targeted cells remains poor. Also pharmacological strategies to limit killer cells' effector function have not been fully investigated in atherosclerosis, particularly with regard to vulnerable/unstable plaques.

2. Killer cells

Killer cells are lymphocytes that kill cells which are either infected or severely damaged using cytotoxin-dependent or death ligand-dependent mechanisms. Major killer cells are CD8 T cells (Konjar et al., 2010), Natural Killer T (NKT) cells (Exley and Koziel, 2004), NK cells (Konjar et al., 2010) and γδ-T cells (Holderness et al., 2013). To some extent, CD4 T cells (Marshall and Swain, 2011) including regulatory T cells (Cao et al., 2007) and B cells (Hagn et al., 2012; Xia et al., 2016) have also been reported to be able to produce cytotoxic granules; perforin and granzyme B, but cytolytic action of these cells are yet to be fully elucidated in the context of atherosclerosis. In this review, we will focus on mainly CD8 T cells, CD4 T cells, NK cells and γδ-T cells and discuss potential therapeutic strategies to limit their cytolytic activity. NKT cells in atherosclerosis is reviewed by Dr. G. van Puijvelde in this special issue.

Killer cells can be categorised into adaptive killer cells and innate killer cells based on requirement of prior-activation for their effector function.

2.1. Adaptive killers cells

T cell receptor (TCR) recognition of antigens is an important step in the adaptive immune system. Killer cells expressing TCRs can be considered as adaptive immune cells, namely CD8 T cells and CD4 T cells (Fang et al., 2012; Hombach et al., 2006) except for TCRγδ-expressing γδ-T cells. CD8 T cells, traditionally known as cytolytic T cells are the most dominant subset of killer cells. Their main effector function is to eliminate pathogen-infected cells, stressed cells and cancer cells by utilising cytolytic granules. The evidence that CD4 T cells can also produce cytolytic granules has been documented in viral immunity (Jacobson et al., 1984; Soghoian and Streeck, 2010). Both CD8 T cells and CD4 T cells require MHC-dependent antigens presented by antigen presenting cells (APC). All adaptive killer cells express FasL and TRAIL (Mirandola et al., 2004; Soghoian and Streeck, 2010; Stalder et al., 1994; Trapani and Smyth, 2002).

2.2. Innate killer cells

NK cells, as effector cells of the innate immune system do not require prior-priming. They modulate the immune responses, both in acute responses such as microbiological infections and in chronic inflammation associated with cancer and autoimmunity. NK cells can detect infected cells and damaged cells through stressed/danger signals. In contrast to TCRαβ-expressing cells, γδ-T cells are a small subset of T cells expressing γδ-TCR and mainly reside in the gut mucosa. Despite the presence of TCR, γδ-T cells may not need antigen presentation for their activation; also they have characteristics of antigen presenting cells. They can process, present and cross-present antigens utilising MHC molecules to αβ T cells (Himoudi et al., 2012). Activated NK and γδ-T cells produce cytolytic granules (Koizumi et al., 1991; Topham and Hewitt, 2009) as well as FasL/TRAIL (Li et al., 2013; Wallin et al., 2003). Besides being potent innate effector cells, NK cells and γδ-T cells can have immunological memory for a prompt and effective responses in repeated viral infections, allergic reactions and cancers (Cerwenka and Lanier, 2016; O'Sullivan et al., 2015; Ramirez-Valle et al., 2015).

3. Killer cells and cytotoxic mechanisms

Cytolytic granule components such as perforin and granzyme B are the hallmark effector molecules of killer cells. Upon recognition of antigens, adaptive killer cells are activated, proliferate and bind to target cells to form an immunological synapse (Griffiths et al., 2010) through which release of cytolytic granules eventually results lysis of target cells. In contrast, innate killer cells can recognise stressed cells and/or infected cells without requiring antigen presentation. Their activation is regulated by NK killer activation receptors (KARs). This includes natural cytotoxic receptor (NCR) comprising of NKp46, NKp44 and NKp30 receptors (Pegram et al., 2011). NK cell activation is also regulated by both activating natural killer group 2 (NKG2) receptors such as NKG2D and NKG2C/CD94 complex and non-inhibiting killer inhibition receptors (KIRs), such as KIR2DS1 (Farag et al., 2002). Other activating receptors include FcγRIII (CD16) and DNAM-1 (Watzl, 2014). NK cells activated by surface receptor recognition of stressed signals, produce cytotoxic granules that directly kill targeted cells (Farag et al., 2002), similar to the mechanism by which adaptive killer cells kill target cells. γδ-T cells comprise CD27+ and CD27- subsets where the former produces IFNγ and the latter IL-17 (Pang et al., 2012). Activated γδ-T cells express NK activating receptors such as NK proteins, NKG2D, DNAM-1 and FcγRIII and produce cytotoxic granules (Koizumi et al., 1991) that induce cytotoxicity to myeloma cells (von Lilienfeld-Toal et al., 2006). In addition to cytotoxic granules, both adaptive and innate killer cells have an ability to produce death recognition ligands such as FasL and TNF-related apoptosis-inducing ligand (TRAIL) (Herbeuval et al., 2005; Li et al., 2013; Mirandola et al., 2004; Wallin et al., 2003) that facilitate target cell apoptosis. FasL- and TRAIL-mediated mechanisms are well accepted mechanisms that killer cells utilise to induce apoptosis to targeted cells. Killer cells can induce cell death via FasL- and TRAIL-mediated pathway independently of cytotoxins (Wallin et al., 2003). To some extent, the proinflammatory cytokine, tumour necrosis factorα (TNFα) produced by activated killer cells can induce apoptosis and necroptosis.

It is important to note that one killer cell can target more than one cell. CD8 T cells seem to exhibit most potent cytolytic efficiency at ~10 target cells/cell (Ganusov and De Boer, 2008) compared to NK cells at ~4 target cells/cell (Bhat and Watzl, 2007). Serial killing is associated with gradual loss of cytotoxins; IL-2 and IL-15 upregulate the cytosolic stocks and restore cytoxicity (Bhat and Watzl, 2007).

4. Killer cells in atherosclerosis

CD8 T cells, CD4 T cells, NK cells and γδ-T cells have been implicated in both human and mouse atherosclerosis (Tables 1 and 2). These cells are abundant in vulnerable/unstable plaques suggesting that their killer function is critically important in plaque rupture as well as in initial development of atherosclerosis. Current research suggests that killer cells are responsible for lesion cell death by inducing target cell apoptosis and necrosis via cytotoxin-, FasL/TRAIL- and/or cytokine-dependent mechanisms (Table 3).

4.1. CD8 T cells

CD8 T cells were initially thought to have no effect (Elhage et al., 2004) or be protective (Fyfe et al., 1994) in atherosclerosis. Recently antibody-mediated CD8 T cell depletion reduced atherosclerosis (Cochain et al., 2015; Kyaw et al., 2013b) suggesting a pathogenic role of CD8 T cells in atherosclerosis. Utilising adoptive transfer of CD8 T cells purified from various donor mice to lymphocyte-deficient ApoE-/- mice, we found that perforin-, granzyme B- and TNFα-deficient CD8 T cells failed to increase atherosclerosis, lesion apoptosis, necrosis, lesion inflammation compared to wild type and IFN-γ-deficient CD8 T cells, suggesting that CD8 T cells promote lesion apoptosis through cytotoxins and proinflammatory cytokine TNFα that is then followed by secondary necrosis (Kyaw et al., 2013b). While we found no role for IFN-γ produced by CD8 T cells in atherosclerosis (Kyaw et al., 2013a),