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The role of CD27-CD70-mediated T cell co-stimulation in vasculogenesis, arteriogenesis and angiogenesis

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ABSTRACT

Background: T cells have a distinctive role in neovascularization, which consists of arteriogenesis and angiogenesis under pathological conditions and vasculogenesis under physiological conditions. However, the role of co-stimulation in T cell activation in neovascularization has yet to be established. The aim of this study was to investigate the role T cell co-stimulation and inhibition in angiogenesis, arteriogenesis and vasculogenesis.
Methods and results: Hind limb ischemia was induced by double ligation of the left femoral artery in mice and blood flow recovery was measured with Laser Doppler Perfusion Imaging in control, CD70$^{-/-}$, CD80/86$^{-/-}$, CD70/80/86$^{-/-}$ and CTLA4$^{+/-}$ mice. Blood flow recovery was significantly impaired in mice lacking CD70 compared to control mice, but was similar in CD80/86$^{-/-}$, CTLA4$^{+/-}$ and control mice. Mice lacking CD70 showed impaired vasculogenesis, since the number of pre-existing collaterals was reduced as observed in the pia mater compared to control mice. In vitro an impaired capability of vascular smooth muscle cells (VSMC) to activate T cells was observed in VSMC lacking CD70. Furthermore, CD70$^{-/-}$, CD80/86$^{-/-}$ and CD70/80/86$^{-/-}$ mice showed reduced angiogenesis in the soleus muscle 10 days after ligation. Arteriogenesis was also decreased in CD70$^{-/-}$ compared to control mice 10 and 28 days after surgery.
Conclusions: The present study is the first to describe an important role for T cell activation via co-stimulation in angiogenesis, arteriogenesis and vasculogenesis, where the CD27-CD70 T cell co-stimulation pathway appears to be the most important co-stimulation pathway in pre-existing collateral formation and post-ischemic blood flow recovery, by arteriogenesis and angiogenesis.

1. Introduction

Peripheral arterial disease (PAD) is characterized by the formation of atherosclerotic plaques in lower extremities and is a major cause of morbidity and mortality [1,2]. The body can restore the blood flow to ischemic tissues by initiating neovascularization, which is a similar mechanism that occurs in patients after myocardial infarction. Neovascularization consists of angiogenesis and arteriogenesis under pathological conditions, such as PAD or myocardial infarction, and vasculogenesis under physiological conditions. Angiogenesis is the process of sprouting of new capillaries from pre-existing microvasculature, which is due to hypoxia and occurs mainly far distal to the occlusion [3]. Arteriogenesis initiates by inflammation, shear stress and circumferential stretch on the vascular wall, which causes inactive pre-existing arterioles, formed by vasculogenesis, to mature into functional collateral arteries, which occurs mainly nearby the occlusion [4–6]. Vasculogenesis is the formation of new blood vessels during embryogenesis through differentiation of angioblasts into endothelial cells followed by the recruitment of vascular smooth muscle cells (VSMC), which can shape new blood vessels [7]. In PAD patients, for collateral artery formation a proper vascular bed of pre-existing arterioles is essential. These pre-exiting arterioles are formed by vasculogenesis. Therefore, this is an important process in PAD. The maturation of pre-existing collateral arteries by arteriogenesis, together with the angiogenetic sprouting of new capillaries, can restore blood flow towards ischemic tissues [8,9].

We and others have shown a specific role of CD4+ T cells in arteriogenesis by using a hind limb ischemia (HLI) model [10,11]. CD4+ T cells have the capacity to attract macrophages and monocytes to the site of occlusion, which in turn triggers arteriogenesis through the release of inflammatory cytokines. Various studies showed increased release of VEGF by hypoxic cells triggered through inflammatory cytokines, indicating a possible role of CD4+ T cells in angiogenesis as well

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[12,13]. CD8+ T cells also contribute to the early phase of arteriogenesis and recruit CD4+ mononuclear cells through the expression of IL-16 [14]. Others also suggest a role for CD8+ T cells in angiogenesis [15]. A previous study further showed that T cells play an important role in vasculogenesis [16]. However, it is still unknown what the activation mechanism of T cells in vasculogenesis, angiogenesis and arteriogenesis is.

For T cell activation, different T cell co-stimulation and inhibitory pathways are described. Co-stimulatory molecules of the B7 family, such as CD80 and CD86, were the first described and are the most well-known and studied molecules [17]. CD28 is a co-stimulation receptor expressed constitutively on the cell-surface of T cells, which interacts with both CD80/CD86 proteins present on antigen presenting cells (APC) [18] and promotes T cell activation and proliferation [19]. As a counteracting system, CTLA4 is an inhibitory receptor on T cells, which down regulates the immune response by binding to CD80/86 with a higher affinity than CD28 [17]. CD27 is a second co-stimulation receptor located constitutively on the surface of T cells, which interacts with CD70 proteins on APC to activate T cell [20]. In contrast to their receptors, co-stimulatory ligands CD80, CD86, and CD70 are transiently up regulated upon activation. The signalling pathway of CD27 in T cells is different compared to CD28 and CD27 promotes T cell survival via up regulation of anti-apoptotic factors [21,22].

The CD28-CD80/86 T cell co-stimulation pathway and CD28-CTLA4 T cell inhibitory pathway were shown to regulate the development of both native atherosclerosis [23,24] as well as post-interventional accelerated atherosclerosis [25]. But this pathway was also shown to be involved in other vascular diseases [26], graft arterial disease [27–29] and inflammatory diseases such as rheumatoid arthritis [30]. The CD27-CD70 T cell co-stimulation pathway is less investigated, however, immune activation via the CD27-CD70 T cell co-stimulation pathway showed to protect against atherosclerosis [31]. In the current study we aimed to elucidate the role of the CD27-CD70 and CD28-CD80/86 T cell co-stimulation pathway, and CD28-CTLA4 T cell inhibitory pathway in post-ischemic neovascularization and vasculogenesis. By visualising pre-existing collaterals in the pia mater of CD70$^{-/-}$, CD80/86$^{-/-}$ and CD70/80/86$^{-/-}$ mice we observed a particular effect of CD27-CD70-mediated T cell co-stimulation on vasculogenesis. Furthermore, CD27-CD70-mediated T cell co-stimulation was also important for optimal blood flow recovery, angiogenesis and arteriogenesis.

2. Materials and methods

Materials and expanded methods are presented in the Online Data Supplement.

3. Results

3.1. Differential impact of co-stimulation pathways on T cell activation in lymphoid organs and blood

Initially, we determined if co-stimulation has a differential impact in lymphoid organs and blood, by analysing the T cell activation levels in blood, bone marrow, lymph node and spleen of control CD80/86$^{-/-}$, CD70$^{-/-}$, CD70/80/86$^{-/-}$ and CTLA4$^{+/-}$ mice. The phenotypical markers KLRG1+ CD62L− were used to determine the percentage of activated CD4+ or CD8+ T cells in each compartment. CD4+ and CD8+ T cell activation in bone marrow, lymph node and spleen of CD80/86$^{-/-}$ and CD70/80/86$^{-/-}$ mice was significantly decreased. In CD70$^{-/-}$ mice, CD4+ T cells in the bone marrow and CD8+ T cells in the lymph nodes and spleen showed significantly decreased T cell activation compared to control (Figs. S1 and S2), indicating a more important role of the CD28-CD80/86 co-stimulation pathway compared to the CD27-CD70 co-stimulation pathway in lymphoid organs. This was also demonstrated by a trend towards increased T cell activation in lymphoid organs, blood and bone marrow in both CD4+ and CD8+ T cells of CTLA4$^{+/-}$ mice compared to control mice. However, CD80/86$^{-/-}$, CD70$^{-/-}$ and CD70/80/86$^{-/-}$, all showed no difference on T cell activation in blood. Together, these results demonstrate a differential effect of co-stimulatory pathways on T cell activation in bone marrow and lymphoid organs compared to the blood circulation.

3.2. Impact of CD27-CD70-mediated T cell co-stimulation on post-ischemic blood flow recovery

The above described results confirm that co-stimulatory pathways have distinct effects in lymphoid organs and blood, but whether such differential effects also occur in peripheral (non-lymphoid) tissues such as blood vessels or the formation thereof remains to be elucidated. Here we aimed to address the (differential) role of co-stimulation in neovascularization. First, we studied post-ischemic blood flow recovery by analysing paw perfusion in control, CD80/86$^{-/-}$, CD70$^{-/-}$, CD70/80/86$^{-/-}$ and CTLA-4$^{+/-}$ mice before ligation of the femoral artery and serially after surgery until sacrifice of the mice after 28 days. Paw perfusion was decreased directly after surgery and control mice showed 74% blood flow recovery in 28 days after surgery with a small drop in recovery between 7 and 13 days (Fig. 1a). CD80/86$^{-/-}$ and CTLA4$^{+/-}$

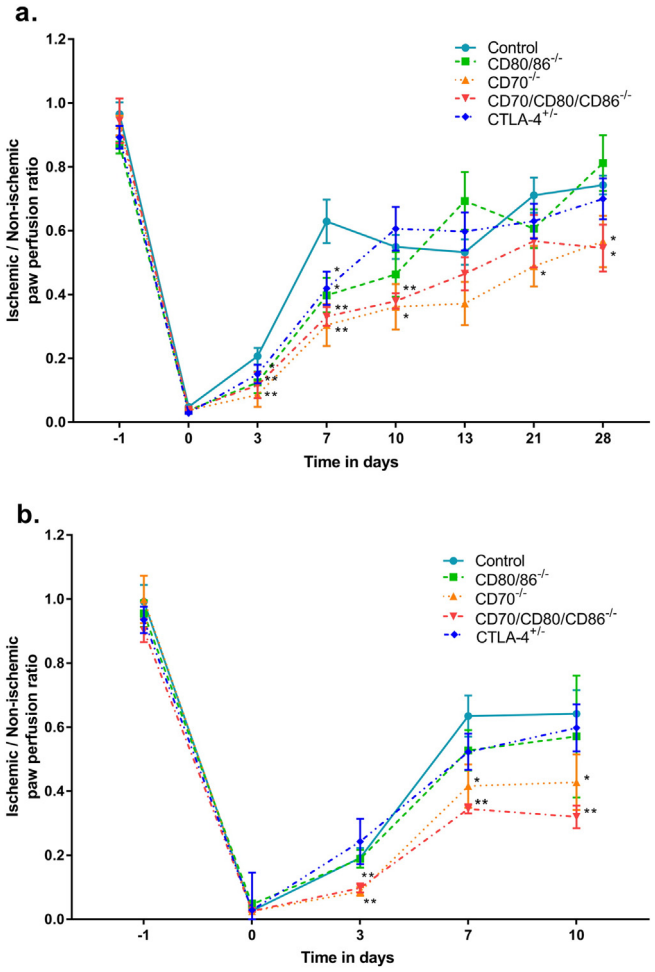

Fig. 1. Blood flow recovery after induction of hind limb ischemia. a. Paw perfusion was measured before and after surgery and 3, 7, 10, 13, 21 and 28 days after surgery. Control mice (n = 11, light blue), CD80/86$^{-/-}$ mice (n = 11, green), CD70$^{-/-}$ mice (n = 7, orange), CD70/80/86$^{-/-}$ mice (n = 8, red) and in CTLA-4$^{+/-}$ mice (n = 11, dark blue) were sacrificed after 28 days. b. Paw perfusion was measured before and after surgery and 3, 7 and 10 days after surgery. Control mice (n = 9), CD80/86$^{-/-}$ mice (n = 10), CD70$^{-/-}$ mice (n = 11), CD70/80/86$^{-/-}$ mice (n = 10) and in CTLA-4$^{+/-}$ mice (n = 6) were sacrificed after 10 days. Paw perfusion is expressed as a ratio of left (ischemic) to right (non-ischemic) paw perfusion. Data is presented as mean SEM; *p < 0.05; **p < 0.01.