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VEGF production is induced by ligation of CD40

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Keywords

CD40 ligand, fibroblasts, rheumatoid arthritis, VEGF

Context

The primary site of inflammation in RA is the synovium, which undergoes infiltration by monocytes and T cells, with concomitant synovial cell proliferation. Characteristically there is an alteration in the density of blood vessels in the synovium. It is thought that the synovial neovascularisation perpetuates the inflammatory and destructive conditions in the developing lesion. VEGF plays a key role in both normal and pathological angiogenesis, and is involved in the pathogenesis of RA. CD40L, a member of the tumour necrosis factor (TNF) family, is expressed on activated T cells. Stimulation with CD40L-expressing cells or purified recombinant CD40L induces the secretion of pro-inflammatory cytokines, adhesion molecules and metalloproteinases. The effect of CD40L on the production of VEGF by cells present in RA synovium has not been addressed. To investigate whether CD40 ligation induces VEGF production from rheumatoid synovial cells and to analyse the mechanism of VEGF production in response to CD40 ligation.

Significant findings

Although unstimulated FLSs constitutively produce VEGF, the levels of VEGF were significantly increased by the addition of $CD40L^{+}$ L cells when compared with $CD40L^{-}$ L cells. Anti-CD40 monoclonal antibody completely abrogated the production of VEGF. To investigate the effect of CD40-CD40L interactions on VEGF under physiological conditions, synovial fluid T cells from three patients with RA were used. Stimulation of synovial fluid T cells with PMA and ionomycin strongly increased CD40L expression on the cells. When the stimulated T cells were incubated with FLSs, VEGF production was significantly increased in a dose-dependent manner. Moreover, anti-CD40 monoclonal antibody significantly inhibited the ability of synovial fluid T cells to produce VEGF. Inhibition studies using antibodies to interleukin (IL)-1, TNF-a and transforming growth factor (TGF)-? demonstrated no significant effect on the production of VEGF by CD40L. IL-1, TGF-? and TNF alone increased VEGF production, and had an additive effect on VEGF production driven by CD40L. To determine whether the protein levels of VEGF were reflected at the RNA level, expression of VEGF mRNA in FLSs was

examined. Unstimulated FLSs or FLSs stimulated with membranes of $CD40L^-$ L cells showed a very low constitutive expression of VEGF mRNA, whereas stimulation of FLS with membranes from $CD40L^+$L cells resulted in high levels of VEGF mRNA. Finally, pyrrolidine dithiocarbamate and dexamethasone inhibited VEGF production.

Comments

Blood vessels in arthritic synovium play a central role in maintaining and augmenting proliferation of synovial cells and mononuclear cell infiltration, which occur in rheumatoid arthritis (RA). Anti-angiogenic approaches have been successful in animal models of arthritis, suggesting that an increased understanding of angiogenesis in RA could help in the design of better therapeutic approaches. This paper is the first to demonstrate that cell-cell interactions (in this case, between T cell and fibroblasts) can upregulate expression of the key angiogenic factor vascular endothelial growth factor (VEGF). The involvement of the CD40:CD40L (CD40 ligand) interaction is of relevance in the context of RA. Previously it was thought that intra-articular hypoxia was the primary stimulus for VEGF expression in RA, but now CD40 ligation on fibroblasts must also be considered. This observation is a significant contribution to our understanding of VEGF production in arthritic synovium.

Methods

Fibroblast-like synovial cells (FLSs) were prepared by enzymatic digestion of synovial tissue from RA patients. FLSs were stimulated in the absence or presence of mouse fibroblastic L cells transfected with human CD40L ($CD40L^+$), or untransfected ($CD40L^-$). VEGF production was measured by ELISA or northern blot analysis. Synovial fluid mononuclear cells from patients with RA were separated by density gradient centrifugation. T cells were either incubated or stimulated with phorbol 12-myristate 13-acetate (PMA) and ionomycin. Fixed T cells were then cultured with FLSs.

References

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