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TRANSLATIONAL MEDICINE: BENCH TO BEDSIDE

Lymphangiogenesis in Inflammatory Bowel Disease; A New Therapeutic Target?

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In their study, D' Alessio *et al.*[1] investigated the results of stimulating lymphatic function and adaptive immune response via vascular endothelial growth factor (VEGF)-C/VEGFR3 signaling on various parameters including intestinal inflammation, lymphatic drainage, bacterial antigen clearance, and macrophage (MΦs) activation during gut inflammation. The mechanism through which this pathway acts in disease progression was also evaluated.

Initial investigation focused on an examination on lymphangiogenesis in human inflammatory bowel disease (IBD), with an immunohistologic characterization of colonic tissues from IBD patients and controls that were stained with antibodies recognizing podoplanin. The total number of lymphatic vessels (LVs) per field in the lamina propria and submucosa of IBD specimens was significantly increased compared with that of the controls. Moreover, the VEGF-C expression was found to be upregulated in the mucosal extracts of IBD patients (mainly those with ulcerative colitis) and increased VEGFR3+ vessel density in inflamed tissue was noticed. An overexpression of VEGFR3 in IBD human intestinal lymphatic endothelial cells (LECs) was also observed using immunofluorescence.

The second part of the study was an investigation of the functional role of the VEGF-C/VEGFR3 pathway by systemic inhibition of VEGFR3 or by delivery of the human lymphangiogenic factor VEGF-C in two models of chronic colitis (dextran sodium sulfate and interleukin-10—knockout) using either a blocking Ab or adenoviral transfer. VEGFR3 protein levels were significantly increased in colonic tissue lysates (mainly in the DDS model) during both acute and chronic intestinal inflammation. The systemic administration of Ad-hVEGF-C improved the chronic intestinal inflammation (as was measured by percentage of body weight, endoscopy and disease activity index) in both animal models of colitis. On the other hand, the anti-VEGFR3 antibody (mF431C1) worsened the clinical course of experimental colitis.

Increased VEGFR3+ vessel density in inflamed tissues and increased VEGF-C expression protein levels in extracts from the colons of colitic mice compared to controls were also demonstrated. Treatment with the anti-VEGFR3 Ab mF431C1 was found to reduce the number of LVs per area compared with that in the wild-type control group in both animal models of colitis.

LV decrease with anti-VEGFR3 Ab was found to inversely correlate with increased disease activity and increased weight loss, suggesting that lymphangiogenesis *per se* might be important for the resolution of inflammation. Exacerbation of experimental colitis by VEGFR3 blockade with resultant exacerbation of experimental colitis was associated with decreased afferent lymph flow and inflammatory cell migration to draining nodes.

The antigen clearance from the inflamed colon was accelerated by systemic delivery of VEGF-C through MΦ mobilization. MΦ depletion was associated with less protection in VEGF-C-treated mice during chronic experimental colitis. MΦ plasticity and activation was found to be regulated both *in vivo* and *in vitro* by the VEGF-C/VEGFR3. Finally, the VEGF-C/VEGFR3 pathway was demonstrated to modulate activation of signal transducer and activator of transcription 6 during experimental colitis and in cultured bone marrow – derived MΦs.

Commentary

Angiogenesis and lymphangiogenesis have been identified as hallmark features of chronic gut inflammation, and expansion of both vascular populations has been hypothesized to play a pathogenic role in IBD. However, the data on the role of lymphangiogenesis in IBD are limited. The complexity of this feature of chronic inflammation is highlighted by the fact that lymphangiogenesis may be either protective or pathogenic.[2] The functions of lymphatics include the control of tissue edema by eliminating the accumulation of interstitial fluid and inflammatory cells as well as the clearance of bacterial antigens and inflammatory chemokines. The obstruction and dysfunction of the LVs are well-known features of IBD.[3,4] Moreover, some experimental data suggest that LVs may be important for the resolution of intestinal inflammation.[5] Research has recently focused

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on the function of LECs showing that they promote leukocyte migration, enhance immune responses, and attenuate T-cell-mediated immune responses by mediating tolerance. However, mechanisms of CD4 T-cell tolerance and possible roles of various inhibitory molecules on LECs are still under investigation.[6]

In normal conditions there is a lymphangiogenic balance between prolymphangiogenic and antilymphangiogenic factors, which regulates LV homeostasis. In inflammation lymphangiogenesis is associated with increased prolymphangiogenic factors and/or decreased antilymphangiogenic factors. Thus, interventions to restore the balance in LV homeostasis should ideally target either a reduction in pro-lymphangiogenic factors and/or a reduction in the antilymphangiogenic factors.

The most important finding of the study of D'Alessio *et al.*[1] was the demonstration that systemic inhibition of VEGFR3 blocks lymphangiogenesis, which was associated with a significant increase in inflammatory edema formation and an inhibition of disease resolution. On the other hand, systemic delivery of VEGF-C enhanced lymphatic drainage, with resulting improvement of intestinal inflammation. The results of this study raise the question "could modulation of the VEGF-C/VEGFR3 pathway be used with safety and efficacy in the treatment of human IBD?". Based on the results of D'Alessio *et al.*, the modulation of lymphangiogenesis appears to represent an attractive and novel IBD therapeutic strategy warranting further exploration.

Activation of LV function by delivery of VEGF-C has shown anti-inflammatory effects in several models of cutaneous and joint inflammation. In experimental chronic skin inflammation VEGF-C gene transfer was found to promote significant reduction of ear swelling and ear weight in a mouse model of the chronic contact hypersensitivity reaction.[7] Moreover, blockade of VEGFR3 expression showed a significant delay in the animals' recovery from the skin inflammation. The use of human recombinant VEGF-C in the presence of a monoclonal anti-VEGFR-3 antibody in animal lymphedema models showed amelioration of lymphatic function and alleviation of the signs of lymphedema.[5] Viral delivery of VEGF-C demonstrated increase of lymphangiogenesis and lymphatic flow with resultant reduction of the severity of joint lesions in a model of chronic inflammatory arthritis.[8] However, there are still many unanswered questions with regard to possible use of LV as a therapeutic target in human inflammatory disorders.[9]

Future investigation on the role of LECs, LVs, and lymphangiogenesis in IBD is expected to shed light on our understanding of disease mechanisms and may lead to the development of new therapies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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