A novel pro-lymphangiogenic function for Th17/IL-17

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Th17 cells, in addition to their proinflammatory functions, have been recognized as potent inducers of angiogenesis in autoimmune diseases and malignancies. In the present study, we demonstrate distinct mechanisms by which IL-17 induces lymphangiogenesis. Using the mouse cornea micropocket and cell culture assays, our data demonstrate that IL-17 directly promotes growth of lymphatic vessels by inducing increased expression of prolymphangiogenic VEGF-D and proliferation of lymphatic endothelial cells. However, IL-17–induced growth of blood vessels is primarily mediated through IL-1β secretion by IL-17–responsive cells. Furthermore, in vivo blockade of IL-17 in a preclinical model of Th17-dominant autoimmune ocular disease demonstrates a significant reduction in the corneal lymphangiogenesis and in the progression of clinical disease. Taken together, our findings demonstrate a novel prolymphangiogenic function for Th17/IL-17, indicating that IL-17 can promote the progression and amplification of immunity in part through its induction of lymphangiogenesis. (Blood. 2011;118(17):4630-4634)
CD3 antibody (0.5 μg/mL; eBioscience) to activate Th17 cells to secrete IL-17, and 1 × 10⁶ primary corneal epithelial cells to secrete different VEGFs in response to IL-17–IL-17 receptor interaction. Primary LECs (5 × 10⁴) were added in the lower well on Matrigel (Geltrex; Invitrogen). In some wells of Th17–LEC coculture, soluble IL-17 receptor-Fc or soluble-VEGFR3-Fc (R&D Systems) was added in media.

**Immunohistochemistry**

Corneal mounts were immunostained with FITC-conjugated CD31 (Santa Cruz Biotechnology) and LYVE-1 (Abcam), and rhodamine-conjugated secondary antibody to LYVE-1 for epifluorescent microscopy. The area covered by blood (CD31hi/LYVE1**+(periphery of the cornea), which grows toward the central cornea in vessel-free character. Vessels are restricted only to the limbal area by its accessible location, transparent nature, and blood and lymphatic vessels.

**Results and discussion**

**Human VEGF array**

Human VEGF-A, VEGF-C, VEGF-D, and GAPDH (Applied Biosystems). In addition, IL-17 pellet with concurrent VEGFR3 blockade induced expression of VEGF-A and VEGF-C, but not of VEGF-D (even in the presence of IL-17, which was further enhanced in the presence of IL-1β blockade (P < .05; Figure 1E). Moreover, IL-17–mediated LEC proliferation was significantly inhibited in the presence of sVEGFR-3 (P < .05), suggesting that IL-17 mediates LEC proliferation indirectly via the VEGFR-3 pathway. Corroborating our findings on the suppressive effects of IL-1β on VEGF-D, a previous report has shown that IL-1β down-regulates VEGF-D expression in cardiac microvascular endothelial cells via the involvement of ERK1/2, JNKs, and PKCα/β pathways.

Next, to establish a direct link between Th17 cells, IL-17, and lymphangiogenesis, we conducted an ex vivo experiment coculturing Th17 cells and LECs, and measured LEC tube formation (Figure 1F). LECs cocultured with Th17 cells showed significantly higher numbers of tube formations, which were comparable with positive control. However, LEC tube formation was significantly inhibited when either IL-17 or VEGFR3 was blocked in the Th17-LEC cocultures (P < .025). These findings clearly demonstrate and confirm that Th17-secreted IL-17 induces lymphangiogenesis via a VEGFD/C–VEGFR3 signaling pathway, corroborating the data of previous experiments showing increased expression of VEGF-D by IL-17–responsive cells (Figure 1D).

Lymphangiogenesis plays a critical role in tumor metastasis and in the inflammatory diseases by facilitating immune cell trafficking. A recent study on lung cancer showed an association between intratumoral IL-17–positive cell frequency and lymphatic density, suggesting that IL-17 may play a role in the metastasis of lung cancer by promoting lymphangiogenesis. In addition, in a preclinical model of Th17-dominant autoimmune DED, we have recently reported the occurrence of corneal lymphangiogenesis and significantly elevated homing of MHC II**+CD11b**+ antigen-presenting cells to the lymphoid tissues where they induce autoreactive T-cell responses. We therefore next tested the relevance of IL-17–mediated lymphangiogenesis using a mouse model of autoimmune DED by investigating whether in vivo IL-17 blockade could inhibit corneal lymphangiogenesis and progression of the disease. Our results demonstrate that IL-17 blockade significantly reduced the lymphangiogenesis compared with untreated and isotype Ab-treated DED corneas (P < .0016; Figure 2A-B). Similarly, harvested corneas showed significantly low expression of prolymphangiogenic VEGF-D and VEGF-C in anti–IL-17 Ab-treated DED corneas compared with untreated and isotype Ab-treated DED corneas (P < .05; Figure 2C). Moreover, corneal disease scores showed significant progression in the reduction of clinical disease in the anti–IL-17 Ab-treated group compared with untreated and isotype Ab-treated DED groups (P < .004; Figure 2D). Taken together, these data suggest that, in addition to causing corneal damage, Th17 cell-secreted IL-17 may promote the growth of corneal lymphatic vessels in autoimmune disease. Although it is generally recognized that innate immunity plays a crucial role in the induction of lymphangiogenesis, our data indicate a new adaptive immune Th17/IL-17–mediated mechanism in inducing lymphangiogenesis.

In conclusion, the present study provides compelling novel evidence that Th17/IL-17 has a prolymphangiogenic function that can further promote the progression and amplification of immunity, in part through its induction of lymphangiogenesis. Because Th17 cell dysfunctions and lymphangiogenesis are involved in a diverse array of immunoinflammatory and malignancy disorders, our...
**Figure 1. IL-17 promotes lymphangiogenesis by inducing VEGF-D secretion, and proliferation of and tube formation by LECs.** (A) Pellets containing 100 ng of IL-1β, IL-17, or IL-17 along with systemic blockade of IL-1β or VEGFR3, were placed in corneal micropockets (n = 6 mice/group) to induce angiogenesis. After 7 days, corneas were evaluated biomicroscopically and then harvested for immunostaining with CD31 (green) and Lyve1 (red). Digital micrographs using epifluorescence microscopy were captured, and ImageJ 1.34s software was used to quantify the growth of (B) blood (CD31^hiLyve1^) and (C) lymphatic (CD31^loLyve1^) vessels. (D) Primary human corneal epithelial cells were cultured with 10 ng/mL concentration of IL-1β, IL-17, and IL-17 with IL-1β-blocking antibodies for 24 hours, and expression levels of VEGF-A, VEGF-C, and VEGF-D mRNA in cells, and protein in culture supernatants, were measured by real-time PCR and ELISA, respectively. (E) Primary human LECs were cultured with 10 ng/mL concentrations of IL-1β, IL-17, and IL-17 with blockade of IL-1β or VEGFR3 for 24 hours, and then proliferation was measured using BrdU incorporation assay. (F) In a transwell Matrigel assay, in vitro polarized 2 × 10^5 Th17 cells (in transwell) were cocultured with 5 × 10^4 LECs (on Matrigel) in basal MEM with reduced serum (2% FBS). Positive controls consisted of LEC cultures on Matrigel in MEM supplemented with growth factors (5% FBS, VEGF, FGF, EGF). Negative controls consisted of LEC cultures on Matrigel in basal MEM only with reduced serum (2% FBS). In some wells of Th17-LEC coculture, soluble-IL-17 receptor-Fc or soluble-VEGFR3-Fc was added in media. After 8- and 24-hour incubation at 37°C, wells were visualized under bright-field inverted microscope to study the LEC tube formations on Matrigel, and digital micrographs were then captured for quantitative analysis of tube length. Data are mean ± SEM values of 3 independent experiments. *P < .05, as determined by Student t test. n.s. indicates not significant.
findings of inhibiting lymphangiogenesis by targeting Th17/IL-17 may have broader clinical implications beyond the treatment of ocular immune diseases alone.

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References


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