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MOLECULAR MEDIATORS FOR INFLAMMATION-INDUCED LYMPHANGIOGENESIS IN THE MURINE BLADDER

Hypothesis / aims of study

Although Bacillus Calmette-Guérin (BCG) has been used extensively for treating superficial bladder cancer and recently tested for interstitial cystitis, its efficacy varies and its mechanisms of action remain poorly understood. Neuropilins (NRPs), co-receptors for vascular endothelium growth factor (VEGF), as well as mediators of immune responses, are strongly expressed in human and mouse bladder urothelium. As VEGF receptors and NRPs seem to play an important role in bladder inflammation and immunity, this study sought to confirm that the VEGF pathway is part of the basic mechanism underlying the effects of intravesical BCG treatment.

Study design, materials and methods

24 female C57BL/6 mice were randomly assigned to one of the two groups illustrated in Figure 1. The mice were challenged, once a week for four weeks, by intravesical instillation of PBS or BCG. To identify cells with functionally active VEGF receptors, such cells were tagged *in vivo* with an internalizable fluorescent tracer, scVEGF/Cy5.5 that is capable of detecting, *in vivo* and in real time, alterations in VEGF receptor activity induced by inflammation. [1,2] On day 37, mice were injected intravenously with this tracer, and near infrared fluorescence (NIRF) images were acquired over a 24 hour period to follow the accumulation of the tracer throughout the tissues. On day 38, mice were euthanized and tissues were removed for histology and immunofluorescence (IF), for evaluation of inflammatory cell markers and lymphatic and vascular features of the urinary bladder.

Results

NIRF imaging of both groups of anesthetized mice (PBS/PBS, PBS/BCG) revealed that intravenous injection of scVEGF/Cy5.5 resulted in a time-dependent accumulation of fluorescence in the urinary bladder. The time course of scVEGF/Cy5.5 uptake in mice instilled with PBS versus those that received chronic BCG was compared, and initially, similar levels of tracer uptake in the urinary bladder was found in PBS- and BCG-treated mice up to 120 minutes post-injection. However, at two hours post-injection, the observed scVEGF/Cy5.5 fluorescence rapidly declined in control animals whereas in BCG-treated mice, there was higher steady-state level of fluorescence that lasted for 24 hours (Figures 2 and 3). *Ex vivo* evaluation of cross-sections revealed an intense accumulation of scVEG-Cy5.5 in NRP-expressing CD11c- and F4/80-positive cells. BCG treatment significantly increased the numbers of both CD11c and F4/80 inflammatory cells invading the urinary bladder (Figure 4). Significant dilation of suburothelial blood vessels, as quantified by endothelial marker CD31-positive cells, was identified in the urinary bladders of the BCG-treated mice (Figure 5). Also, LYVE-1- and podoplanin-positive lymphatic vessel density was found to be increased in the lamina propria, concomitantly with an increase in cell proliferation (Ki67; Figures 5 and 6).

Interpretation of results

Chronic treatment with BCG resulted in an increased accumulation of fluorescence in the urinary bladder when compared to mice that received intravesical instillation of PBS alone. The prolonged accumulation of the tracer in the bladder of the chronic-BCG treated mice suggests that inflammation induces up-regulation of VEGF and NRP receptors. The strong accumulation of scVEGF-Cy5.5 in CD11c- and F4/80-positive cells, suggests that the VEGF pathway may also be involved in the infiltration and migration of these inflammatory cells into the urinary bladder, to the site of BCG exposure. The increased vascularity and lymphatic vessel density as a consequence of BCG instillation is suggestive of the lymphangiogenic function VEGF-NRPs have in response to the inflammation induced by BCG.

Concluding message

The physiologic changes seen in the murine bladder such as the increase in lymphatic and blood vessel density and inflammatory cell migration is proposed as a basic mechanism underlining the beneficial effects of intravesical BCG. These results are highly suggestive of the important role VEGF-NRPs play in inducing these changes.

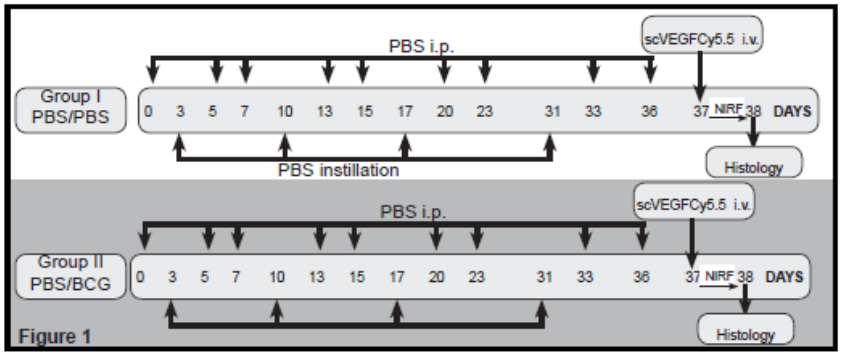

Figure 1

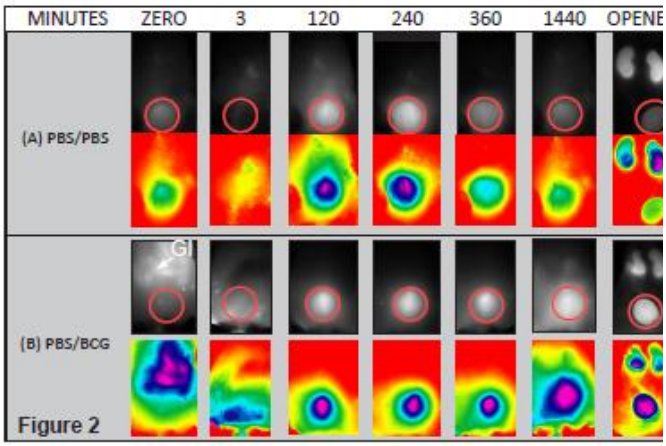

Figure 2

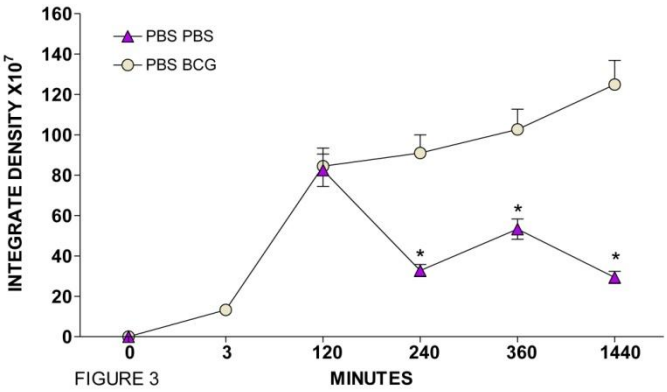

FIGURE 3

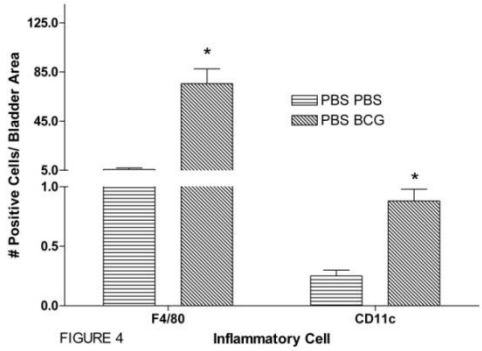

FIGURE 4

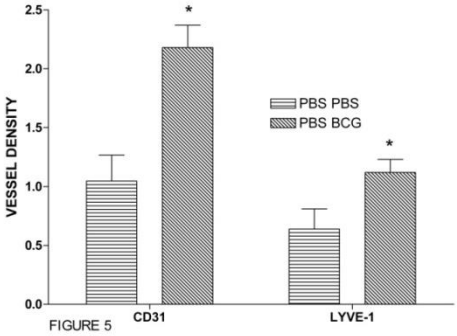

FIGURE 5

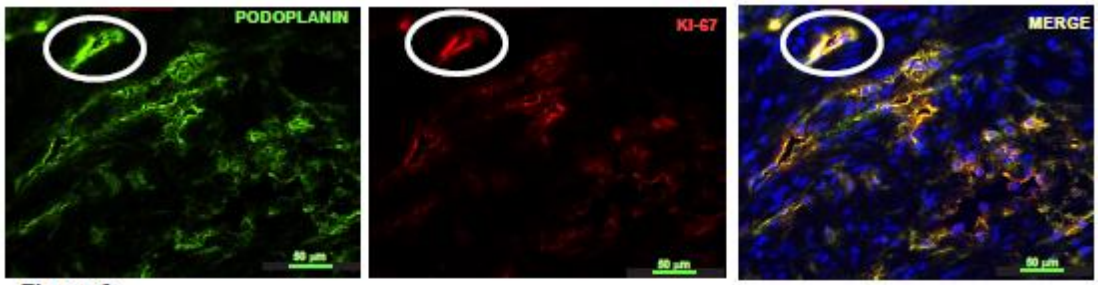

Figure 6

References

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2. Saban MR, Backer JM, Backer MV, Maier J, Fowler B, Davis CA, Simpson C, Wu XR, Birder L, Freeman MR, Soker S, Hurst RE, Saban R: VEGF receptors and neuropilins are expressed in the urothelial and neuronal cells in normal mouse urinary bladder and are upregulated in inflammation, Am J Physiol Renal Physiol 2008, 295:F60-72

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Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**All animal experimentation was performed in conformity with the APS's Guiding Principles in the Care and Use of Animals 19 and OUHSC Animal Care & Use Committee protocol #08-105.**