VEGF-A recruits a proangiogenic MMP-9-delivering neutrophil subset that induces angiogenesis in transplanted hypoxic tissue

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Supplemental Data

Figures S1-S4
Transplanted PE-sphere in cremaster Site distant from transplanted PE-sphere

**Transplanted sterile material does not induce recruitment of leukocytes in muscle.** Highly fluorescent polyethylene spheres in the size of pancreatic islets (diameter 125-150 µm) were transplanted into the cremaster muscles of mice in the same fashion as islets. After 3-5 days, cremaster muscles were exposed, and leukocyte recruitment assessed by in vivo confocal microscopy. No specific recruitment of Gr-1-positive leukocytes could be observed by the spheres (n=6). Extravasated leukocytes were however found in the muscle, but this recruitment is most probably unspecific, and a consequence of the surgical trauma caused by the insertion method.

In the left panel above, two highly fluorescent PE-spheres (fluoresces in both blue and red) in cremaster muscle four days after transplantation are shown in a confocal z-projection, and some extravasated leukocytes are visible (green). In the right panel, a site distant from the transplanted spheres is shown, where approximately the same degree of leukocyte extravasation can be seen. Bars are 50 µm.
Pancreatic islets increase their expression of VEGF-A after isolation

Mouse pancreatic islets were isolated and kept free-floating at 37°C in culture dishes. The expression of VEGF-A mRNA and VEGF-A protein was measured on the day of isolation, and after 1 and 4 days in culture. Pancreatic islets have high basal expression of VEGF-A (seen on day 0), and levels increase after 1 day in culture, probably due to tissue hypoxia. After 4 days in culture, the levels are back to where they were after isolation, either due to negative feedback mechanisms from accumulated protein in the media, or due to attained homeostasis. *$P<0.05$ compared to day 0, n=3 mice/time point.
Expression of CXCR4 is not inducible *in vitro*

To investigate whether the stimuli used to recruit leukocytes could cause a change in surface expression of CXCR4, *in vitro* stimulation experiments were performed.

**(A)** Blood was collected from mice by heart puncture. After red blood cell lysis, leukocytes were incubated with either MIP-2 or VEGF-A for 30 min. Flow cytometry was then performed, by which the level of CXCR4 expression was measured in the CD11b⁺/Gr-1⁺ population. The diagram shows levels related to the expression of CXCR4 in the group incubated with only cell medium. There is no significant difference between the groups. n=6 mice.

**(B)** Leukocytes were recruited into the peritoneal cavity of mice by either MIP-2 or VEGF-A. The leukocytes were then incubated with MIP-2, VEGF-A, or only cell medium for 30 min in a criss-cross fashion. Flow cytometry was then performed where the level of CXCR4 expression was measured in the CD11b⁺/Gr-1⁺ population. The diagram shows levels related to the expression of CXCR4 in the group incubated with only cell medium. There is no significant difference between the groups. n=5-6 mice/group.
Leukocytes recruited to VEGF-A contain more MMP-9 than leukocytes recruited to MIP-2

Leukocytes were recruited into the peritoneal cavity of mice by either MIP-2 or VEGF-A. The cells were then counted and diluted in two different concentrations (2×10⁴ and 4×10⁴ leukocytes/ml) and stimulated with PMA. Supernatants were collected and analyzed for MMP-9 content by gelatin zymography. The figure displays four representative unedited gels from these experiments.