The role of osteopontin in macrophage mediated inflammation

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Statement of Purpose: Osteopontin (OPN) is a multifunctional matricellular protein that is expressed at sites of inflammation and plays an important role in the pathology of several chronic inflammatory diseases. OPN is a potent macrophage chemoattractant and is highly expressed in both macrophages and foreign body giant cells (FBGCs). Previous studies in our lab have demonstrated that OPN regulates the foreign body response *in vivo.* When PVA sponges were implanted subcutaneously in OPN-null mice, fewer macrophages accumulated at the site of implantation compared to wild type mice.[1] Despite the defect in macrophage recruitment, OPN-null mice formed more FBGCs on the surface of the implant. *In vitro* studies further demonstrated that OPN inhibits macrophage fusion in a dose dependent manner. Whether OPN regulates other important macrophage functions, and the receptor and OPN domains important for these functions are unknown.

The goal of the current study was to investigate the role of OPN in macrophage survival, migration, and activation state, and determine the domains mediating these effects.

Methods: *Mice:* OPN-null mice were generated on a C57Bl/6 background as previously described.[2] OPN homozygous wild-type (WT) and homozygous null (OPN-/-) mice were used in these studies.

Murine bone marrow-derived macrophage (BMDM) isolation: Bone marrow was isolated from the femurs of WT and OPN-/- mice and was expanded in BMDM expansion media (50% RPMI, 30% L929 conditioned media, and 20% FBS). Cells were fed on day 4 and mature macrophages were harvested on day 7.

Survival studies: BMDMs were plated on poly-D-lysine (PDL) coated permanox chamber slides. Two hours after plating, cells were challenged with Fas-crosslinking antibody (Jo2, BD Bioscience) in serum free medium and incubated overnight. Percent apoptosis was determined by nuclear fragmentation. For OPN rescue experiments, OPN-/- BMDM were plated on PDL, PDL with 100 nm soluble OPN, or directly on OPN prior to Fas ligation. For antibody blocking studies P388D1 cells were used. P388D1 cells were pre-incubated with neutralizing antibody to α4 or β3 integrin, and 100 nm OPN was added prior to challenge with Fas crosslinking antibody.

FBGC studies: Bone marrow was obtained from the femurs of WT and OPN-/- C57Bl6 mice and was expanded in Iscove's modified Dulbecco's medium (IMDM) supplemented with FBS, M-CSF and flt-3 ligand. To induce the formation of FBGCs, macrophages were plated at 1 x 10^6 cells/well in 24-well non-tissue cultured treated plates in IMDM supplemented with IL-4 and GM-CSF. After 7 days of treatment, cells were fixed and stained and fusion was analyzed.

Migration assays: The migration of WT and OPN-/- BMDM was assayed using a Transwell migration assay as previously described.[3]

Macrophage activation state: WT and OPN-/- BMDMs were stimulated toward the M1 phenotype by stimulation with IFN-γ (20 ng/ml) and LPS (100 ng/ml) or towards the M2 phenotype by treatment with IL-4 (60 ng/ml). After 24 hours, BMDMs were harvested and expression of M1 and M2 markers was assayed by FACS. IL-12, CD86, and iNOS were used as markers of M1 activation, while mannose receptor and IL-1 type II decoy receptor were used as M2 markers.

Results: Previous studies have shown that OPN is a pro-survival factor for a variety of cell types. Similarly, we show here that OPN-/- macrophages were more sensitive to Fas-mediated apoptosis than WT macrophages. OPN-/- macrophages could be rescued from cell death by the addition of either soluble OPN or plating them on an OPN substratum. To explore the receptors though which OPN mediates macrophage survival, we performed studies with the macrophage-like cell line P388D1. Using neutralizing antibodies we demonstrated that the pro-survival effect of OPN is mediated through a α_4 integrin-initiated signaling pathway.

Preliminary data indicated that OPN inhibits macrophage fusion in vivo in response to biomaterial implantation and *in vitro.* Here we show that macrophages from OPN-null mice formed more FBGCs and these FBGCs contained more nuclei per cells than WT ones.

Using a Transwell migration assay we reconfirmed that OPN is a potent macrophage chemoattractant for OPN-/- and WT macrophages. However, we did not observe a reduced migration of OPN-/- macrophages as has been previously reported.[3]

OPN has been previously reported to stimulate IL-12 secretion from macrophages and inhibit IL-10 production.[4] Consequently, we were interested in the hypothesis that OPN stimulates macrophages toward an M1 macrophage phenotype. However, we found that OPN deficiency does not affect macrophage phenotype, as both WT and OPN-/- BMDM could be efficiently polarized to both M1 and M2 phenotype and expressed similar levels of M1 or M2 markers when treated with the appropriate stimuli. Further, exogenously added OPN was unable to induce IL-12 production.

Conclusions: OPN plays a key role in macrophage biology. OPN serves as a potent macrophage chemoattractant and inhibits FBGC formation. OPN also functions as a pro-survival factor in macrophages in a α_4 integrin dependent manner. Future studies to determine the OPN functional domains mediating these effects will provide unique insights into the molecular mechanisms of OPN in macrophage biology.

References:

1. Tsai A. Biomaterials. 2005;26(29):5835-43.
2. Rajachar R. J Mater Sci Mater Med. 2008;19:3279-85.
3. Bruemmer D. J Clin Invest. 2003; 112(9):1318-31.
4. Ashkar S. Science. 2000; 287(5454):860-4.