Focal Adhesion Kinase, Proline-Rich Tyrosine Kinase-2, Thrombospondin, and Fascin-1 Expression in Adherent Macrophages and Foreign Body Giant Cells

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Statement of Purpose: Macrophages play an important role in the inflammatory reaction and healing response to implanted biomaterials. The adhesion, aggregation and fusion of these cells at the biomaterial-tissue interface result in the formation of foreign body giant cells (FBGCs). These multinucleated cells remain adherent to the biomaterial surface for the lifetime of the medical device. Effects of the FBGCs can be detrimental to the integrity and function of implanted materials and devices, thereby endangering the health of patients. The mechanism of formation of FBGCs, from monocyte adhesion to macrophage aggregation and fusion, is not completely understood; therefore further characterization of the process of macrophage aggregation and fusion will lead to better understanding of the inflammatory response as well as further control over the foreign body reaction to carefully designed biomaterial properties.

Focal adhesion kinase (FAK) and proline-rich tyrosine kinase-2 (PYK-2) as well as actin bundling fascin-1 and the adhesive protein thrombospondin (TSP) are of particular interest. These proteins play important roles in facilitating cellular adhesion through intracellular organization, cell-to-cell and cell-to-extracellular matrix signaling. Investigation of these adhesive proteins will help elucidate mechanisms of cellular adhesion that are necessary for the fusion of adherent macrophages into FBGCs.

Methods: Human derived monocytes were isolated and cultured on RGD-coated polystyrene (MP Biomedicals, Aurora, OH) in serum-free medium for macrophages (Invitrogen, Grand Island, NY) containing 20% autologous serum. Addition of 15ng/ml of IL-4 on day 3 induced FBCG formation. On days 0 (2 hours), 3, 7, 10 or 13 cell lysates were collected. After aspiration of supernatant adherent cells were washed twice with PBS^{++} at 37° C. Ice cold RIPA (Pierce, Rockford, IL) lysing buffer was added to the wells and the plates were then vigorously shaken at 4° C for 30 minutes or until complete lysis was verified with light microscopy. Lysates were then transferred to microcentrifuge tubes, centrifuged for 15-30 minutes at 13,200 rpm then aliquoted for storage at -80° C.

SDS-PAGE was performed on 7.5% Tris-HCl mini-gels (Biorad, Hercules, CA). Following electrophoresis, proteins were transferred to a polyvinylidene diflouride membrane for immunoblotting. The membranes were blocked in 5% nonfat milk/TTBS and incubated overnight in primary antibody or negative control normal IgG in 1% nonfat milk/TTBS. After incubation in secondary antibody conjugated to horseradish peroxidase for one hour an Amplified Opti-4CN kit (Biorad) was used to detect proteins of interest.

Results/Discussion: With immunoblotting, strong signals have been detected for the adhesive proteins FAK, PYK-2 and TSP as well as fascin-1. Adhesive protein βig-h3 as well as the negative control of normal rabbit IgG (Rb IgG) were not detected.

Expression of Adhesive Proteins

Primary Antibody	FAK	PYK-2	TSP	βig-h3	Rb IgG
Strength of Signal	+++	+++	+++	-	-

(+ + +)-strong signal, (-)-no signal

The detection of FAK over time was investigated to determine levels of expression in relation to the processes of monocyte adhesion and macrophage aggregation and fusion into FBGCs. In day 0 (2 hours) lysates a weak signal was detected for FAK. A moderate signal was detected in day 3 samples and strong expression was detected at days 7, 10 and 13. The FAK signal over time suggests the increase in expression as the macrophages adhere to the culture surface, spread and aggregate. The expression of FAK appears to remain constant once FBGC formation has occurred. There was no signal detected in the negative control of Rb IgG at any of the time points.

Expression of FAK Over Time

	d0	d3	d7	d10	d13
FAK	+	++	+++	+++	+++
Rb IgG	-	-	-	-	-

(+)-weak, (+ +)-moderate, (+ + +)-strong, (-)-no signal

The expression of these adhesive proteins, as well as the expression over time, support their role in FBGC formation. As macrophage morphology has been shown to be material dependent, the role of adhesive proteins in "outside-in" signaling may form the basis for material dependent responses of cellular adhesion and fusion on varying surface characteristics.

Conclusions: The detection of these adhesive and structural proteins in adherent macrophages and FBGCs suggests their role in mechanisms of monocyte adhesion, macrophage aggregation and FBGC formation. These results prompt further investigation to determine material dependent expression of these proteins. Further investigation of expression on different materials will help to determine the role of "outside-in" adhesion signaling by monocytes, macrophages, and FBGCs adherent to biomaterials.

Acknowledgements: This work was supported by National Institutes of Health Grant EB00275.