CREATION OF PATHOGEN MIMETICS AS NOVEL DRUG DELIVERY PLATFORMS

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Introduction

Antibody- or Fc- coated beads have been used in a variety of studies to investigate the function of signal transduction cascade responses in particle uptake and phagocytosis, stimulation of immune responses, and general biotechnological assays. Currently, the process of Fc-mediated phagocytosis is not well understood, and quantitative spatio-temporal measurements of this process remain elusive. The process of phagocytosis must be studied more extensively before progress may be made in the field of targeted drug delivery.

The objective of the present study was to determine the effects of antibody density and microsphere size on macrophage phagocytosis of antibody-coated polystyrene microspheres, which served as pathogen mimetics. Although part of an overall effort to design multi-functional microparticles that not only target and bind infectious microorganisms, but also activate the local immune system, this study concentrated on activation of the local immune system.

Procedure

Yellow fluorescent polystyrene microspheres (Bangs Labs, Polysciences) with diameters of 1 μm, 3 μm, or 4.5 μm were coated with bovine serum albumin (BSA) followed by primary BSA antibody labeled with fluorescent fluorescein isothiocyanate (FITC, Invitrogen) dye. Antibodies were passively adsorbed to protein-coated microspheres in order to expose the Fc region of each antibody, which activates the local immune system and mediates phagocytosis[1]. To vary antibody density, antibodies were added at 10x, 5x, 1x, 0.5x, 0.1x, or 0x the saturating mass of antibody for each bead size. To determine antibody density, FITC fluorescent intensity was measured with flow cytometry. As an additional measurement of antibody density, FITC-labeled secondary antibodies were conjugated to a second set of beads with non-fluorescent primary antibody.

For each microsphere size and saturation, 10^6 microspheres with FITC-labeled primary antibody were added to a constant number of mouse leukaemic monocyte macrophages (RAW 264.7). After incubation for 60 min, cells were dyed with CellMask Deep Red plasma membrane stain (Invitrogen) and trypan blue was used to quench FITC fluorescence outside living cells. Thus, populations of beads, cells, and beads within cells could be isolated and counted using flow cytometry. The phagocytosis of a bead by a cell was represented as the reaction equation, $B + C \rightarrow BC$. Subsequently, phagocytotic efficiency was defined similarly to the chemical equilibrium constant as $\varepsilon = \frac{BC}{B \cdot C}$, where BC is the number of beads phagocytosed by cells, B is the total number of beads, and C is the total number of cells.

Results and Discussion

Antibody density was determined using FITC intensity data by assuming 100% density at 10x saturation, 0% density at 0x saturation, and direct proportionality between FITC intensity and antibody density. Intensity data for both primary and secondary-labeled antibodies and for each of three bead sizes were used to calculate antibody density at each saturation value. Since the saturating mass of protein is a function of bead size, antibody density is theoretically a function of only saturation. Thus, antibody density was averaged at each saturation value. Antibody density generally increased logarithmically with saturation (data not shown).

Phagocytotic efficiency was observed to be dependent on both microsphere size and antibody density (Fig. 1).

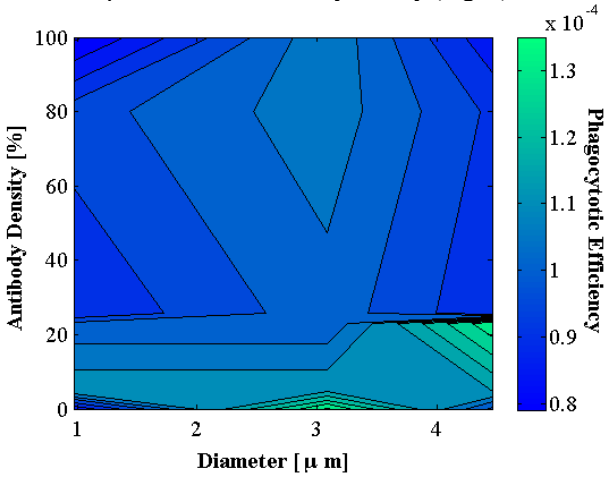

Figure 1. Phagocytotic efficiency contour plot. High phagocytotic efficiency (green) was observed for large, sparsely covered beads, whereas low phagocytotic efficiency (blue) was observed for small or densely covered beads.

Phagocytotic efficiency was generally higher for 3 μm beads than for 1 μm or 4.5 μm beads. However, the highest phagocytotic efficiencies were observed for 3 μm and 4.5 μm beads with low antibody densities. High phagocytotic efficiency was expected for high antibody density because the exposed Fc region of the antibodies should trigger phagocytosis[1]. Since beads with low antibody densities displayed low fluorescent intensity even before quenching with trypan blue, populations of beads adhering to cells were not entirely distinct from population of beads phagocytosed by cells. Such overlapping of bead populations may have skewed phagocytotic efficiency data.

Conclusion

It was demonstrated that antibody density may be varied in a controllable manner by adjusting the mass of antibody relative to the saturating mass of antibody. Phagocytosis was most efficient for 3 μm beads and for low antibody densities. Although phagocytosis is generally associated with high antibody densities, labeling BSA with FITC would allow adequate florescent quenching for any antibody density, which could result in more accurate phagocytotic efficiency data.

References

[1] Aderem A, Underhill D. Mechanisms of phagocytosis in macrophages. Annu.Rev.Immunol. 1999;17:593-623.