APPLICATION OF MONOCYTES ON ANTIGEN MICROARRAYS - DETECTING INFLAMMATORY ACTIVATION

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Background:

In our earlier studies we demonstrated the applicability of monocytoid cell line U937 to detect antigen bound IgG. We found that this adhesion of the cells is mainly determined by the Fcγ receptor - IgG interactions. This interaction depends on the affinity of Fcγ receptors towards the IgG subclasses that are sensitve to glycolysation as well. Therefore detection of antibodies with monocytes is possibly a useful tool to simply determine effector functions of an antibody from the cells' point of view. Activating Fcγ receptors after binding their ligands are known to activate the Nf-κB pathway, an inflammatory pathway. In our present work we investigated how this inflammatory activation of the cells could be detected.

Methods:

To investigate the inflammatory activation of the cells we transfected U937 monocytoid cells with plasmids coding EGFP under the regulation of Nf-κB responsive elements and an other plasmid coding iRFP, a fluorescent protein. Following the cloning of the cells we characterized the cells Nf-κB response to various stimuli.

Results:

First we investigated the activating properties of LPS towards these cells and determined the kinetics and the dose dependence of the activation and found the mean fluorescence intensity of these cells to be close to the peak after 8 hours of incubation in the 0,1 - 10 µg/ml LPS range. We compared the activating properties of IgG subclasses in solution and in coat as well. In summary we found that while IgG 1,3 and 4 in coat activates the Nf-κB pathway, in solution none of them did just like IgG2 which we found to be less significant in activation of the cells both in solution and in coat. We also found that this activation is blockable by masking Fc parts of IgG molecules.

Conclusion:

In agreement with our previous results we demonstrated how U937 cells through their Fcγ receptors differentiate between IgG subclasses bound to a solid surface and that this activation does not only result in the adhesion of the cells but in the activation of their Nf-κB pathway as well.

This work was supported by KMOP 1.1.1-08/1-2008-028 grant to Diagnosticum, and OTKA-K104838, OTKAPD-104779 grants, and by the Hungarian Academy of Sciences. Krisztián Papp is supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.