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INTERACTION OF MONOCYTE Fc RECEPTORS WITH MONOVALENT AND POLYVALENT LIGANDS+

B. KOCH, H. LEIBL, M.M. EIBL AND J.W. MANNHALTER

Department of Immunological Research, Immuno AG, Orth, Austria, and Institute of Immunology, University of Vienna, Vienna, Austria

INTRODUCTION

The receptors for IgG (FcγR) on human mononuclear phagocytes participate in several biological processes of cellular and humoral immune defense. Thus they play a role in endocytosis of immune complexes /1/, in phagocytosis of opsonized pathogens /2/, in antibody-dependent cellular cytotoxicity /3/ and in the activation of T lymphocytes by anti-CD3 MoAb /4/. Our group is especially interested in the interaction of FcγR with their respective ligands (interaction of FcγR with monomeric IgG, polymeric IgG and immune complexes) and in the ways in which these interactions modulate FcγR function.

In the study presented here we used radioligands to determine the binding capacity of IgG in its various forms (monomeric, dimeric) to FcγR expressed in the membrane of human monocytes. Special attention was paid to possible differences in the interaction of these ligands with the two different types of FcγR (FcRI and FcRII) known to be present in the monocyte membrane /5/.

Both number and affinity of receptor sites for a given ligand can be determined by using the Scatchard plot analysis. With respect to FcγR determination by Scatchard plots, the data in the literature are contradictory /6, 7/. Some authors report a curvilinear Scatchard plot, while in other publications

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linear Scatchard plots are shown. The data presented here deal with these discrepancies and demonstrate binding of IgG dimers to both FcRI and FcRII.

MATERIALS AND METHODS

Cells

Mononuclear cells were isolated from the peripheral blood of healthy donors by Lymphoprep sedimentation /8/. Monocytes were then further purified by an elutriation technique using a counterflow centrifuge /9/ (purity of monocytes above 90%, granulocyte contamination below 1%).

Immunglobulin preparations

IgG monomers were isolated from pooled normal human plasma by cold ethanol precipitation /10/, DEAE-Affi gel blue chromatography (according to the manufacturer's instructions, BIO RAD Bull-1062) and molecular sieving.

Dimerization of IgG was performed using dimethyl suberimidate (Pierce) /11/.

Aggregated immunoglobulin was prepared by heating a 1% solution of IgG for 20 min at 63°C /12/.

Iodination

Iodination was performed by the glucose oxidase/lactoperoxidase method using Immunobeads (according to the manufacturer's instructions, BIO RAD Bull-1062).

Binding assays

For Scatchard plot analysis /13/, monocytes (at a concentration of 8×10^6 per ml) were incubated for 1 hr at 37°C or for 4 hrs at 4°C in the presence of varying concentrations of radioligand (0.23 nM to 118 nM) and either an equal volume of buffer or, as competing protein, a solution of heat-aggregated IgG.

Bound radioligand was separated from unbound IgG by spinning through a phthalate oil layer. Data evaluation was performed by data analysis and curve fitting with the LIGAND computer system /14/.

RESULTS

The studies presented here support previous investigations by others in showing that monomeric IgG binds mainly to one receptor with high affinity for the ligand: Kd (the data are values from one typical experiment, Kd = mole/liter) of 2.6×10^{-9} with 14 000 receptors/cell at 4°C. These data have been analysed by the LIGAND computer program, thereby confirming the

one-receptor binding model with high significance.

However, binding of IgG dimers to human monocytes results in a curvilinear Scatchard plot, thus suggesting taht IgG dimers bind to two receptors of different affinity: Kd_1 of 0.96×10^{-9} with 15 000 receptors/cell and Kd_2 of 3.7×10^{-8} with 48 000 receptors/cell. The high-affinity receptor most likely represents FcRI, a receptor with apparently comparable affinity for IgG monomers and dimers. The second receptor with lower affinity depicts FcRII, preferentially binding IgG oligomers and immune complexes. This assumption is further supported by studies employing Fc receptor-directed monoclonal antibodies. Preliminary data suggest that a blockade of FcRII with the MoAb IV. 3 completely abolishes IgG dimer binding to the low-affinity binding site.

CONCLUSION

While IgG monomers bind predominantly to the Fc receptor of high affinity, IgG dimers have the capacity to interact with both high- and low-affinity Fc receptors.

The two receptors binding IgG dimers most likely represent FcRI and FcRII.

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B. Koch MD
A-Vienna, Austria