Engineering of Cell Adhesive Immunoglobulin G by Grafting the Arg-Gly-Asp Cell Adhesive Signal

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A new artificial cell adhesive protein was engineered by grafting the Arg-Gly-Asp (RGD) sequence, the minimal recognition signal of fibronectin for interaction with integrin, to immunoglobulin G (IgG) by in vitro mutagenesis. The mutagenized protein showed cell adhesive activity on baby hamster kidney (BHK) cells.

A large number of cell adhesive proteins in the extracellular matrix and body fluids are known to be important in cell adhesion and migration during development, wound healing, platelet aggregation, and tumor metastasis. Especially, the tripeptide RGD is contained as the cell-adhesive signal in such proteins as fibronectin, vitronectin, type I collagen, fibrinogen, von Willebrand factor, and osteopontin. A number of receptors recognizing the RGD signal have been isolated and characterized as members of the integrin adhesion receptor family.

We have shown that the cells adhesive activity can be transferred to truncated form of the Staphylococcal protein A and the human calpstatin domain I by grafting the RGD sequence using a genetic engineering method. For this report, we constructed a cell-adhesive IgG by grafting RGD sequence using sit-directed mutagenesis. Such IgG may be useful for potentiation phagocytosis of the immune complexes by macrophages through interaction with RGD-directed integrins expressed on macrophages.

On grafting the RGD sequence, the following considerations were taken into account for as the criteria; 1) Antigen-binding capacity of IgG needs to be retained. 2) Folding of the polypeptides and binding of the sugar chain should be affected. 3) The RGD sequence should form a loop structure. We chose the CH3 domain of the heavy chain of IgG as a site of the insertion.

The site of the RGD grafting is shown in Fig. 1. Plasmid pSV2·HG1·Vpc·EF1 was constructed from pSV2·HG1·gpt and pSV2·HG1·Vpc·, the expression vector for heavy chain of anti-phosphorylcholine antibody, by site-directed mutagenesis. The vector and pSV2·C·Vpc, the expression vector for the light chain of anti-phosphorylcholine antibody, were co-transfected into the mouse myeloma cell SP2/O by electroporation. The transfected cells were selected in RPMI1640 medium containing 10% fetal bovine serum, 250 μg/ml of xanthine, and 10 μg/ml of mycophenolic acid and, then IgG-producing transformants were screened for with enzyme-linked immunosorbent assay specific for mouse/human chimeric IgG. RGD-grafted IgG was purified with a protein A affinity column.

**Fig. 1.** Position Grafted for the RGD Sequence in the Human IgG CH3 Domain.

a) Amino acid and nucleotide sequences of the human IgG CH3 domain. The numbering of amino acid residues begins with Gly-1 of CH3 domain as described by Elliston et al.. RGD sequence is inserted after Ser-75. The vector was mutagenized using an oligonucleotide primer, S′-ACCGTTGAGAAGAGACCCGGGCGAGACCGCTTAGGGCGCCCGGCGGGCCAGACGGCTTAGGTGGCACAGCGGCGGGG-3′, a sense strand of sequence encoding the TVDKSTGPDQPRGQ sequence.

b) Schematic diagram of CH3 domain of the RGD-grafted IgG. The position grafted for the RGD sequence is indicated by an arrowhead. β-Strands described by Deisenhofer are shown by arrows.

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Abbreviations: BHK, baby hamster kidney; POD, peroxidase.

**Fig. 2.** Cell Adhesive Activity of the RGD-grafted IgG.

BHK fibroblasts were incubated on plastic substrates precoated with the following proteins: RGD-grafted IgG (panel A) and non-grafted IgG (panel B). Concentration of the proteins for the precoating was 1000 ng/ml. Bar represents 50 μm.
A column. The cell-adhesive activity of the RGD-grafted IgG was measured as previously described. Preliminary experiments showed that the cell attachment activity of the RGD-grafted IgG was weaker than that of RGD-grafted protein A or RGD-grafted calpastatin (data not shown). Since Gailit and Ruoslahti reported that divalent cations such as Mg$^{2+}$, Ca$^{2+}$, or Mn$^{2+}$ enhance RGD-dependent cell-adhesion, the cell-adhesive activity of the RGD-grafted IgG was measured in the presence of 1 mM Mn$^{2+}$. The cell adhesive activity of the RGD-grafted IgG and non-grafted IgG assayed with BHK cells under these conditions is shown in Fig. 2. The IgG containing the RGD sequence was capable of mediating cell attachment and spreading when coated on an inert plastic surface, but the non-grafted IgG was totally inactive. When inert plastic surfaces were coated with various concentrations of the RGD-grafted IgG, a significant cell adhesion was observatiul at >250 nm. The RGD-grafted IgG retained the binding activity to the antigen, i.e., phosphorylcholine (Fig. 3), but the amount of IgG bound was lower than that of the control IgG when measured with peroxidase (POD)-conjugated anti-human IgG antibody. This suggests that the affinity of the RGD-grafted IgG to the antigen or the affinity of the 2nd-antibody to the RGD-grafted IgG was affected by the insertion of the RGD sequence.

Our results indicated that the RGD-signal grafted to IgG retained the cell adhesive activity. However, the activity of the RGD-grafted IgG to promote cell attachment and spreading was comparable to the RGD-grafted protein A or the RGD-grafted calpastatin only when the activity was assayed in the presence of Mn$^{2+}$. The RGD sequence grafted to protein A and calpastatin was assumed to be in a flexible region of the peptide backbone and, therefore, easily accessible by RGD-directed receptors. Nuclear magnetic resonance study of the conformation of the tenth fibronectin type III module demonstrated that the folding topology of the module is similar to that of immunoglobulin constant domains and the RGD sequence is in a tight turn on an exposed loop. Although the RGD signal grafted to IgG was also in a loop structure, the cell-adhesive activity of the signal was significantly lower than that grafted to protein A or calpastatin. This could be due to poor accessibility of the RGD signal to the integrin receptors. Close association of two heavy chains of the IgG may inhibit the interaction of the grafted RGD signal with the receptors, resulting in accessibility of the RGD signal to the receptors lower than that of other RGD-grafted proteins. Although the role of Mn$^{2+}$ in potentiation of the integrin receptor function is not well understood, it is likely that the binding of Mn$^{2+}$ ions to the receptors induces a conformational change of the receptors leading to an increased accessibility of the RGD signal to the receptors.

We have produced a series of artificial cell adhesion proteins by grafting the RGD sequence to protein A, calpastatin, and IgG, respectively. The artificial proteins should be useful materials for studies on the function of RGD-dependent receptors.

References