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C_H3 domain of IgG as binding site to Fc receptor on mouse lymphocytes

MEMBRANE receptors recognising the Fc portion of immunoglobulin molecules (Fc receptors) are found in many cells of the immune system[1–4]. Fc receptors on lymphocytes are readily detected by a rosette test[3,5,6] and this reaction is inhibited by pretreatment of the lymphocytes with IgG (ref. 3). IgG proteins lacking almost the entire C_H1 and C_H3 homology regions have been obtained from mutant cell lines of MOPC 21, a plasmacytoma secreting IgG1 (refs 7 and 8) and the extent of the deletions determined (Fig. 1). To identify that part of the IgG molecule which interacts with the Fc receptor, we have tested the ability of these IgG proteins to inhibit Fc rosette formation on murine lymph node cells. We show (Table 1) that an intact C_H3 region is essential for the binding of IgG to Fc receptors on lymph node cells.

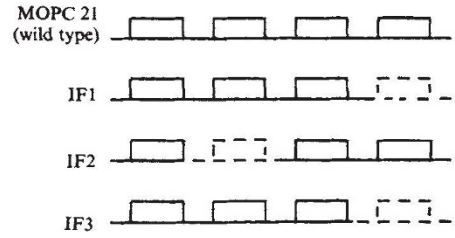

Fig. 1 Structure of the mutant proteins. Dotted lines indicate deletions in the heavy chains, shown diagrammatically, with the four intra-chain disulphide bridges. IF1, IF2 and IF3 were cloned tissue culture lines of the mouse plasmacytoma MOPC 21. IF2 has a deletion in the heavy chain of residues 121 to 214 corresponding to the wild type protein[8,16], that is, of almost the entire C_H1 region. IF1 has a deletion of residue 358 onwards[16] and IF3 of residue 342 onwards (K. A. and C. Milstein, unpublished). The secreted protein used in the experiments was purified from the serum of mice bearing the respective tumours, using ammonium sulphate precipitation and DEAE cellulose chromatography. The purity was checked using electrophoresis on cellulose acetate strips, and isoelectric focusing on polyacrylamide gels.

The ability of IF2 protein to inhibit Fc rosette formation shows that the C_H1 region is not the site through which IgG binds to Fc receptors. The failure of IF1 and IF3 proteins to inhibit Fc rosettes shows that an intact C_H3 region is required for the binding of IgG to Fc receptors. Since both IF1 and IF3 possess intact C_H2 regions, this region may not be required for binding to Fc receptors. It is possible, however, that the absence of the C_H3 region in IF1 and IF3 can result in a rearrangement of the C_H2 region, destroying an active site wholly or partly within this region. The inability to detect the C_H3 region of membrane-bound IgG on lymphocytes, with a fluorescent antiglobulin possessing an anti-C_H3 region specificity, also suggests that it is the C_H3 region which interacts with the lymphocyte membrane[9].

Table 1 Inhibition of rosette formation with IgG proteins

Inhibitor		Percentage reacting cells ± s.e
Experiment 1		
No inhibitor		33.8 ± 2.3
MOPC 21 (parent IgG)	0.58 mg ml^{-1}	18.0 ± 2.9
IF1 (C_H3 deletion)	0.58 mg ml^{-1}	30.9 ± 1.9
IF2 (C_H1 deletion)	0.58 mg ml^{-1}	3.3 ± 3.7
Experiment 2		
No inhibitor		34.1 ± 2.5
MOPC 21 (parent IgG)	10 mg ml^{-1}	0.1 ± 0.1
IF1 (C_H3 deletion)	10 mg ml^{-1}	35.9 ± 1.1
Experiment 3		
No inhibitor		43.6
MOPC 21 (parent IgG)	5 mg ml^{-1}	9.4
IF3 (C_H3 deletion)	5 mg ml^{-1}	41.4 ± 1.8
IF1 (C_H3 deletion)	5 mg ml^{-1} (heat aggregated)	43.8 ± 2.9

Pooled lymph node cells from BALB/c mice were used in the experiments. Fc rosette formation was carried out as previously described[5,6]. For inhibition of Fc rosettes, lymphocytes were pretreated with inhibitor protein at the concentrations stated for 15 min at 0° C before rosette formation. For one experiment IF1 protein was heat-aggregated at 63° C. The results are the means of more than three replicate preparations when the standard error of the mean is given. Two observations in experiment 3 were the results of counting over 200 cells in a single preparation.

In an extension of the domain hypothesis, Edelman *et al.*[10] proposed that each of the homology regions of the polypeptide chains of immunoglobulin molecules folds to form a compact domain which has evolved to perform independent functions. Thus the V_H and C_H2 regions of IgG possess the antigen binding and complement fixing sites[11], respectively. The binding of IgG to human monocytes[12] and guinea pig macrophages[13], possibly through a site in the C_H3 region, has been suggested as evidence for the domain hypothesis. The binding of IgG to Fc receptors on K cells[14], neutrophils[14] and a non-secreting plasmacytoma[15] also requires an intact C_H3 region. The C_H3 region of IgG seems therefore to possess the property of binding IgG to Fc receptors, which may be functionally relevant, on many types of cells.

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RANJAN RAMASAMY

ARC Institute of Animal Physiology, Babraham

DAVID S. SECHER
KAYODE ADETUGBO

MRC Laboratory of Molecular Biology, Hills Road, Cambridge, UK

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