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ScienceDirect
Molecular Immunology 45 (2008) 307–319
Molecular Immunology
www.elsevier.com/locate/molimm

An FcγRIIa-binding peptide that mimics the interaction between FcγRIIa and IgG

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Received 6 February 2007; received in revised form 13 June 2007; accepted 17 June 2007
Available online 30 July 2007

Abstract

A disulphide-constrained peptide that binds to the low affinity Fc receptor, FcγRIIa (CD32) has been identified and its structure solved by NMR. Linear (7-mer and 12-mer) and disulphide-constrained (7-mer) phage display peptide libraries were panned on recombinant soluble FcγRIIa genetically fused to HSA (HSA–FcγRIIa). Peptides were isolated only from the constrained peptide library and these contained the consensus sequence, CWPGWxxC. Phage clones displaying variants of the peptide consensus sequence bound to FcγRIIa and the strongest binding clone C7C1 (CWPGWDLNC) competed with IgG for binding to FcγRIIa and was inhibited from binding to FcγRIIa by the FcγRIIa-blocking antibody, IV.3, suggesting that C7C1 and IgG share related binding sites on FcγRIIa. A synthetic disulphide-constrained peptide, pep-C7C1 bound to FcγRIIa by biosensor analysis, albeit with low affinity ($K_D \sim 100\ \mu M$). It was significant that the FcγRIIa consensus peptide sequence contained a Proline (Pro^3), which when substituted with alanine abrogated FcγRIIa binding, consistent with Pro^3 contributing to receptor binding. Upon binding of IgG and IgE to their respective Fc receptors (FcγRs and FcεRI) Pro^{329} in the Fc makes a critical interaction with two highly conserved Trp residues (Trp^{90} and Trp^{113}) of the FcRs. The NMR structure of pep-C7C1 revealed a stabilizing type II β-turn between Trp^2 and Trp^5, with Pro^3 solvent exposed. Modelling of the pep-C7C1 structure in complex with FcγRIIa suggests that Pro^3 of C7C1 binds to FcγRIIa by inserting between Trp^{90} and Trp^{113} of FcγRIIa thereby mimicking the molecular interaction made between FcγRIIa and IgG.

Keywords: Fcγ receptor; Phage display; Disulphide-constrained peptides; NMR

1. Introduction

FcγRIIa (CD32) is a key activatory receptor in immunity and host resistance linking humoral and cellular based effector systems including antibody-dependent cell cytotoxicity (ADCC), phagocytosis, endocytosis and clearance of immune complexes, and enhanced antigen presentation (Hulett and Hogarth, 1994a). Furthermore, transgenic mouse models (Hogarth, 2002; McKenzie et al., 1999; Tan Sardjono et al., 2003; Taylor et al., 2000) and genetic susceptibility studies (Brun et al., 2002; Carlsson et al., 1998; Dijstelbloem et al., 2000; Manger et al., 1998; Moser et al., 1998; Salmon et al., 1996; van Sorge et al., 2003; Williams et al., 1998) have established the importance of FcγRIIa in the pathogenesis of systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and idiopathic thrombocytopenia (ITP). Hence FcγRIIa serves as a promising target for the development of therapeutics to treat these diseases.

Several X-ray crystal structures of the ectodomains of Fc receptors (FcRs) alone and in complex with Ig-Fc are known (Garman et al., 1998; Garman et al., 2000; Maxwell et al., 1999; Radaev et al., 2001a; Sondermann et al., 1999; Sondermann et al., 2000; Zhang et al., 2000). In the crystal structure of the FcγRIII–Fcγ complex (Radaev et al., 2001a; Sondermann et al.,

Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; BSA, bovine serum albumin; DQF-COSY, double-quantum-filtered correlated spectroscopy; ELISA, enzyme-linked immunosorbent assay; Fc, fragment crystallizable or fragment constant; HBS, HEPES-buffered saline; $FcγRIIa^{LR}$, low-responder allele of FcγRIIa; HSA, human serum albumin; HSQC, heteronuclear multiple quantum coherence spectroscopy; IFNγ, interferon; NMR, nuclear magnetic resonance; NOE, nuclear overhausser effect; NOESY, nuclear overhausser effect spectroscopy; PBS, phosphate-buffered saline; pfu, plaque forming units; PCR, polymerase chain reaction; ROESY, rotating frame overhauser effect spectroscopy; RU, resonance units; SPR, surface plasmon resonance; TBS, Tris-buffered saline; TOCSY, total coherence spectroscopy

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doi:10.1016/j.molimm.2007.06.152

2000), the residues involved in the interaction between FcγRIII and the Fc portion of IgG1, were consistent with interactions made between FcγRs and Ig-Fc identified from mutagenesis studies (Hibbs et al., 1994; Hulett et al., 1993, 1994b, 1995; Maxwell et al., 1999; Shields et al., 2001; Tamm et al., 1996; Wines et al., 2000). In particular, residues of D2 of FcγRIII bound asymmetrically to the lower hinge and C_H2 domains on the IgG1-Fc. One striking feature of the FcγRIII–Fcγ complex was that Pro^{329} from the FG loop of one C_H2 domain of Fc was positioned tightly between Trp^{90} and Trp^{113} of FcγRIII, this interaction being coined the "Proline Sandwich" (Radaev et al., 2001a; Sondermann et al., 2000); and was also apparent in the FcεRI–Fcε complex (Garman et al., 2000). Interestingly, these Trp residues are conserved in all other FcγRs (and FcεRI) and similarly Pro^{329} is conserved in all IgG subclasses (and IgE), suggesting that this is a common interaction for all FcγRs (and FcεRI) binding to Fc (Garman et al., 2000; Radaev et al., 2001a; Sondermann et al., 2000). Indeed mutagenesis of Pro^{329} drastically reduced binding of human IgG1 to all FcγRs (Shields et al., 2001) and mutagenesis of Trp^{90} and Trp^{113} in both FcγRI (Tan, unpublished data) and FcγRIIa (Cendron, unpublished data) has been found to reduce IgG binding, consistent with the structural data.

Several strategies have been utilised to identify potential peptide inhibitors of FcRs including the rational structure-based drug design of inhibitors to FcεRI (McDonnell et al., 1996; Takahashi et al., 1999), the screening of synthetic peptide libraries for IgG binding peptides and inhibitors to the FcγR and IgG-Fc interaction (Marino et al., 2000), the rational design of peptide mimics of the lower hinge region of IgG-Fc (Medgyesi et al., 2004; Radaev and Sun, 2001b; Sheridan et al., 1999; Uray et al., 2004) and the screening of peptides that span the extracellular domain of mouse FcγRII for inhibitors of IgG binding (Goldsmith et al., 1997). Phage display libraries have also been panned on a number of FcRs including the high affinity FcRs FcγRI (Berntzen et al., 2006) and FcεRI (Nakamura et al., 2001, 2002; Stamos et al., 2004) to identify potential inhibitors to these receptors. The consensus sequence CLRSGxGC was identified from panning phage display libraries on IFNγ-stimulated U937 cells, which express FcγRI, and showed specific binding to FcγRI, but at a site that is distinct from the IgG binding site (Berntzen et al., 2006). Panning of phage display peptide libraries on FcεRIα-Ig led to the discovery of peptides IgE06 (Ac-NLPRCTEGPWGWCM) and e131 (VQCPHFCYELDYELCPDVCYV), with micro- and nanomolar affinity for FcεRIα, respectively (Nakamura et al., 2001, 2002) and for which the structures of these peptides have been solved alone and in complex with FcεRIα (Stamos et al., 2004). The X-ray crystal structure of the FcεRI–e131 complex demonstrated the insertion of Pro^{16} (e131) between Trp^{90} and Trp^{113} of FcεRI (Stamos et al., 2004), thereby mimicking the interaction made between FcεRI and Pro^{329} from IgE-Fc (Garman et al., 2000).

In this study we have panned phage display peptide libraries to identify novel peptide ligands and identified a consensus peptide sequence that binds FcγRIIa at a related site to IgG. The solution structure of a synthetic disulphide-constrained peptide, pep-C7C1, revealed a surface exposed Pro^3 that is proposed on the basis of mutagenesis and modelling to bind FcγRIIa in the pocket formed by Trp^{90} and Trp^{113}, which is a feature common to all FcγRs and FcεRI. Thus, this approach has generated a peptide that targets a fundamental aspect of ligand recognition by this receptor class.

2. Materials and methods

2.1. Affinity selection

The basic strategy used for the isolation of FcγRIIa binding phage was (i) removal of HSA-binding phage, (ii) panning of HSA depleted phage on HSA–FcγRIIa and elution of bound phage with IgG or dithiothreitol (DTT), (iii) subsequent rounds of enrichment by panning on HSA–FcγRIIa coated at different densities and elution with IgG. To produce the HSA–FcγRIIa fusion protein cDNA of the HSA–FcγRIIa was produced using splice-overlap extension PCR (SOE-PCR) using HSA cDNA obtained from the American Type Culture Collection (Rockville, MA) (Dugaiczyk et al., 1982) and human $FcγRIIa^{LR}$ cDNA (Hibbs et al., 1988), as the templates. Primers ATCGATGAATTCATGAAGTGGGTAAC (HT4) and GGGGGAGCGCCTAAGGC AGCTTGAC (HT7) were used to prime from the HSA cDNA template and CCTTAGGCGCTCC-CCCAAAGGCTG (HT8) and CCCCATGAATTCCTATTGGA CAGTGATG (HT5) were used to prime from the $FcγRIIa^{LR}$ cDNA template, thereby linking the carboxyl terminus Gly^{584} (GGC) of HSA to the N-terminal Ala^1 (GCT) of FcγRIIa. The HSA–FcγRIIa was produced in *Pichia pastoris* as described previously (Wines et al., 1999).

"Ph.D.-7", "Ph.D.-12" and "Ph.D.-C7C" phage display peptide libraries (NEB, Beverly, MA, USA), with 7-mer and 12-mer random linear, and 7-mer random disulphide-constrained peptides, respectively, displayed on the minor coat protein (g3p or gIII) of filamentous bacteriophage M13, were used for panning experiments. For selection, 8-wells from a 96-well Maxisorp immunoplate (Nunc, Roskilde, Denmark) were coated with 100 μg/ml HSA (CSL, Parkville, Vic., Australia) or HSA–FcγRIIa (Biotech, Australia) in 0.1 M $NaHCO_3$, pH 8.6 (coating buffer) overnight at 4 °C, with gentle agitation. Wells were 'blocked' with 0.1 M $NaHCO_3$, pH 8.6 containing 0.5% (w/v) BSA (JRH Biosciences, Lenexa, KS, USA) (blocking buffer) for 1 h at 4 °C, washed six times with Tris-buffered saline (TBS) containing 0.1% (w/v) Tween20 (washing buffer), and phage (10^{10} pfu/well) was added to HSA-coated wells and incubated for 1 h at room temperature, with gentle agitation. Following this HSA "adsorption step" phage were transferred to HSA–FcγRIIa coated wells, and the plate incubated for a further 1 h at room temperature, with gentle agitation. Unbound phage were removed by washing wells 10 times. Bound phage was eluted with either IgG (Sandoglobulin, Sandoz, Sydney, Australia) or DTT (Bio-Rad, Hercules, CA, USA) or both, in particular approaches for eluting FcγRIIa specific phage included 0.1 mg/ml IgG (1 h at room temperature), 10 mg/ml IgG (4 °C overnight) and 1 mM DTT (1 h at room temperature).