Immunology Letters, 44 (1995) 111–117
Elsevier Science B.V.

IMLET 2283

Recognition sites on human IgG for Fcγ receptors: the role of glycosylation

Royston Jefferis *, John Lund and Margaret Goodall

Department of Immunology, The Medical School, Edgbaston, Birmingham B15 2TT, UK

(Received 14 October 1994; accepted 18 October 1994)

Key words: Fcγ Receptor; IgG glycosylation; Protein engineering

1. Summary

Human IgG subclass proteins exhibit more than 95% primary amino acid sequence homology in their Fc regions, but each has a unique profile for recognition by the 3 human Fcγ receptors. The FcγRs are themselves highly homologous members of the immunoglobulin supergene family. Consistent with these data we have proposed that FcγRI, FcγRII and FcγRIII recognise overlapping non-identical interaction sites in the lower hinge region of the C_H2 domain of the IgG molecule. Evidence in support was provided by protein engineering effecting single amino acid replacements in the proposed site. Alternatively, we have demonstrated that the primary amino acid sequence alone is not sufficient for IgG molecules to fold with the generation of FcγR interaction sites and that glycosylation of Asn 297 of the C_H2 domain is essential. We have further defined a 'core' oligosaccharide structure that provides for the generation of FcγR interaction sites which suggests that the addition of outer-arm sugar residues does not affect this primary activity; although in vivo it could influence other essential biological activities.

These findings have opened up a new approach to engineering antibody function – by protein engineering of amino acid residues that form contacts with the oligosaccharide moiety. In the present report we demonstrate that replacement of contact residues for galactose on the $\alpha_{(1-6)}$ arm does not affect FcγRI and FcγRII recognition while replacement of Asp 265, a contact for a 'core' N-acetylglucosamine residue, results in a loss of FcγRI and FcγRII recognition.

* *Corresponding author:* Prof. R. Jefferis, Department of Immunology, The Medical School, Edgbaston, Birmingham B15 2TT, UK. Tel.: (44) 21-414-4063; Fax: (44) 21-414-3599.

2. Introduction

Currently there is a burgeoning interest in the discovery and definition of new protein molecules. Most often they are discovered through the genes encoding them, and the primary amino acid sequence is derived from the genetic code and preliminary structures generated by computer modelling. The genes may be cloned and expressed to produce recombinant molecules in a wide range of expression systems, e.g., bacteria, yeasts, insect cells or eukaryotic cells. Site-directed mutagenesis may be applied to effect single amino acid replacements in an attempt to 'define' functional sites. However, these studies rarely take account of *post-translational modifications* (PTM), that may differ with the expression system, that can have a profound influence on biological activity. More than 30 such modifications can be listed; some are relatively common (e.g., glycosylation, chelation of metal ion prosthetic groups, deamidation, phosphorylation, sulphation) while others are more specific to individual proteins. The differential effects of PTMs may not be apparent in in vitro assay protocols, but can dramatically affect their activity in vivo. When considering the application of recombinant protein and glycoprotein molecules for human therapy it is essential to demonstrate that the recombinant molecule has the full range of biological activities exhibited by the natural product and that PTMs are controlled in the production process.

One of the most common PTMs is glycosylation which can influence biological activity, stability, pharmokinetics, antigenicity, etc. [1]. However, bacterial expression systems do not glycosylate proteins while yeasts can only add a high mannose oligosaccharide moiety. This contrasts with eukaryotic cells that can effect the addition of a wide range of oligosaccharides, dictated by the protein structure and cell type. These

SSDI 0165-2478(94)00201-0

assertions are apparent from our studies of recombinant antibody molecules [2,3].

3. The IgG antibody molecule: structure and function

The IgG antibody molecule is the most intensively studied of the antibody isotypes and we have a good understanding of the relationships between gross structure and function. It stands as a paradigm for molecules of the immunoglobulin supergene family and defines the basic immunoglobulin fold or domain. The intact IgG molecule comprises 3 globular protein moieties, 2 Fabs and an Fc, that are linked through a flexible 'hinge' that is essential for the functional expression of the intrinsic activities of the Fab and Fc regions; each Fab and the Fc fragment are composed of 4 domains. The hinge is comprised of a flexible upper portion which provides mobility for the Fab regions and allows each to engage an epitope on the target antigen; a central 'core' rich in proline and cystine residues that has a rigid secondary structure and forms inter-heavy chain disulphide bridges; a flexible lower region allows mobility for the Fc region such that ligand binding sites, essential for effector function activation, are accessible in antigen/antibody complexes. Several studies have correlated segmental flexibility to the ability to activate C1 and this appeared to be corroborated by the finding that IgG1 molecules in which the hinge is deleted do not activate C1 [4–6].

The Fc region of the IgG molecule expresses multiple interaction sites for various ligands, including Fcγ receptors on leucocytes, C1q, rheumatoid factors, Fc receptors expressed by bacteria, e.g., staphylococcal protein A (SpA) or induced by virus, etc. In man 4 subclasses of IgG are defined whose Fc primary structures are greater than 95% homologous but express distinct profiles of Fc-determined biological activities. In addition heterologous IgGs have been shown to activate human IgG Fc-mediated functions so that correlation of primary sequence characteristics with the expression of particular biological activities has been attempted.

Following such a study we proposed that the lower hinge region of the IgG molecule may contribute directly to FcγRI recognition. Subsequent studies employing site-directed mutagenesis to effect amino acid replacements appeared to corroborate this proposal and identified the sequence Leu–Leu–Gly–Gly– as the optimal motif for recognition by FcγRI, FcγRII and FcγRIII [18]. At this point we were interpreting all of our findings in the light of changes to primary amino acid sequence and inferred influences on the tertiary or quaternary structure. However, we also demonstrated that Fc-mediated biological activities were dependent

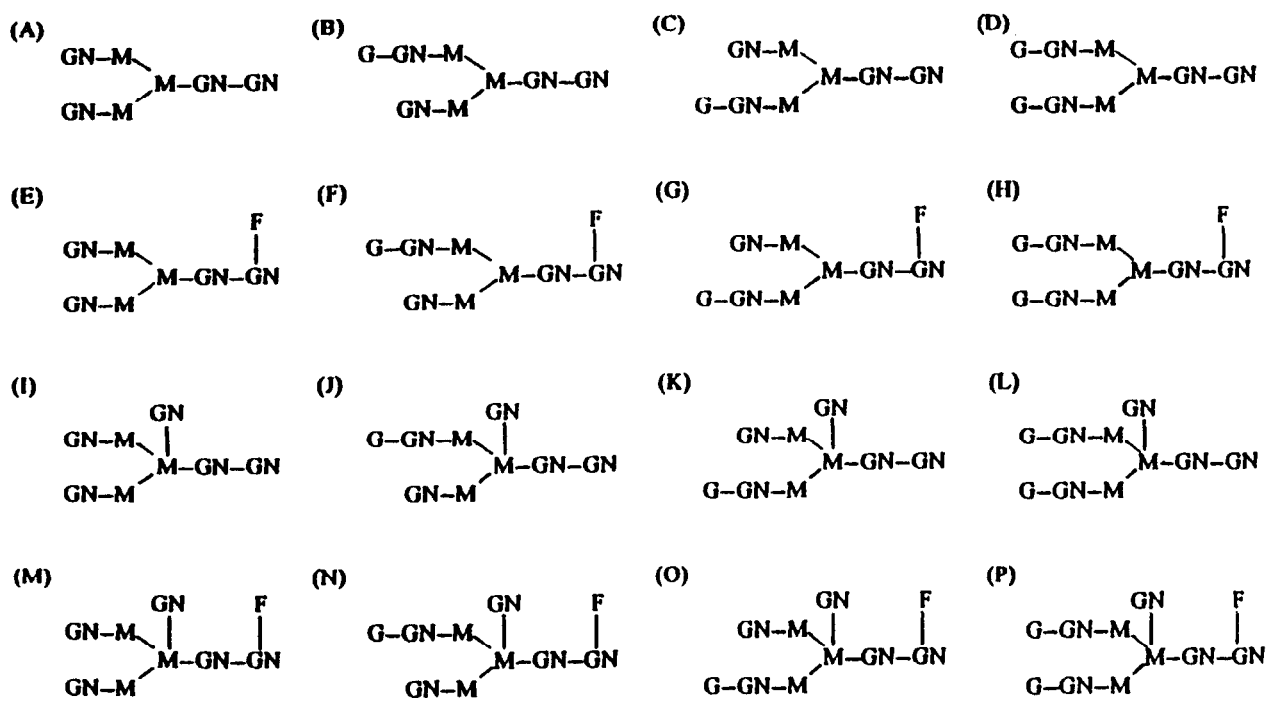

Fig. 1. Profile of neutral oligosaccharide structures present in human IgG molecules (G, galactose; M, mannose; F, fructose; GN, N-acetylglucosamine).

on the molecule being glycosylated at Asp 297 in the C_H2 domain [7,8]. Our further studies have shown that glycosylation is an essential property that must be controlled in the production of recombinant proteins [3]. However, it also provides a potential route for the production of new bio-molecules expressing modulated profiles of activities. It should be noted that glycosylation is only one of an important series of PTMs that protein molecules are subject to.

4. Antibody glycosylation

A conserved glycosylation site, at Asn 297 on the b4 bend of the Fy face, has been observed for all IgG molecules investigated, and a homologous glycosylation site is present in IgM, IgD and IgE molecules but not IgA. This conservation suggests functional significance, if not necessity. The oligosaccharide is of the complex type which has a minimal hexasaccharide 'core' structure with variable attachment of outer-arm sugar residues (Fig. 1).

X-ray crystallographic analyses of Fc fragments of human IgG1 and rabbit IgG allow resolution of the 'core' oligosaccharide structure bearing fucose on the primary N-acetylglucosamine residue and galactose on the $\alpha_{(1-6)}$ arm (structure F, Fig. 1) [9–11]. It was suggested that some unresolved electron density could be due to further sugar residues that were mobile within the crystal structure. The resolved oligosaccharide was estimated to make a total of 85 contacts through 14 amino acid residues of the C_H2 domain [11]. The contact residues are highlighted in the C_H2 cartoon of Fig. 2 and listed in Table 1. The remarkable conserva-

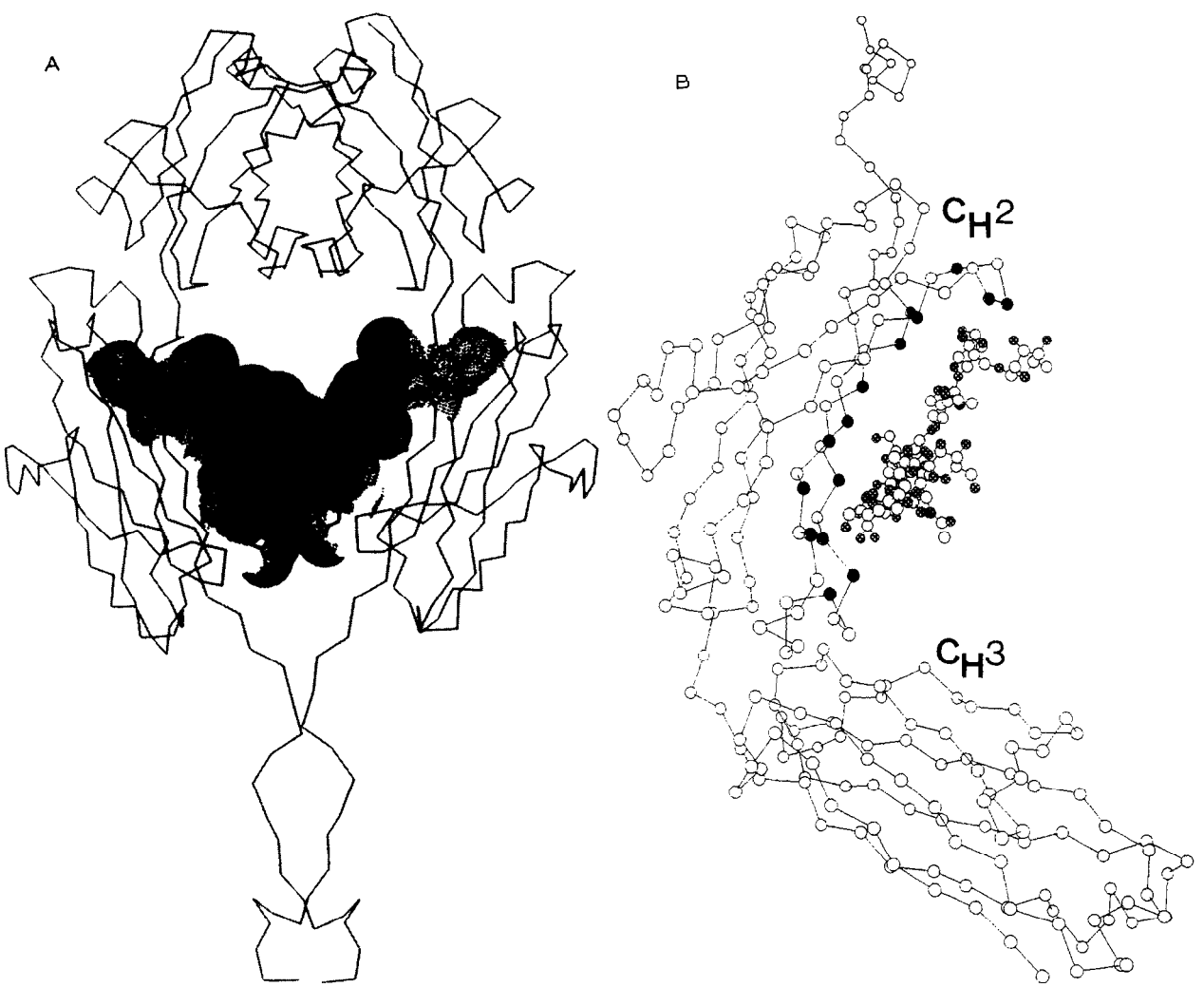

Fig. 2. A: human IgG1-Fc: the carbohydrate is highlighted as the surface accessible to a water molecule. B: one-half of an Fc with the carbohydrate contact residues of the C_H2 domain as filled circles.

tion of oligosaccharide contact residues for the IgG molecules is apparent and contrasts with the lack of conservation observed for the other human isotypes. It is interesting to note that the site of attachment of oligosaccharide in the C_H2 domain of IgA is Asp 258 which is located at the C-proximal end of the domain. In IgG glutamic acid 258 makes multiple contacts with a galactose residue on the $\alpha_{(1-6)}$ arm.

While antibody molecules bearing carbohydrate within variable domains have been reported the position of attachment and structure of the oligosaccharide moieties has not been the subject of systematic study. Germline V_H gene segments have been defined that encode glycosylation sequons but glycosylated products of these gene segments have not been identified. Analysis of the germline V_H gene sequences [12] reveals that 5 of 83 germline V_H gene segments encode for an N-glycosylation site; the frequencies being 0/22, 0/5, 2/29, 1/20, 2/6 and 0/1 for V_H1, V_H2, V_H3, V_H4, V_H5 and V_H6 family gene segments respectively. Inspection of 37 V_H region protein sequences revealed 15 glycosylation sequons. However, none were encoded by identified germline genes. It appears, therefore, that glycosylation sequons in expressed proteins arise as a result of somatic mutation and positive selection by antigen, while protein sequences expressing germline-encoded glycosylation sequons may be selected against [13].

5. Human IgG, Fc effector functions

Aglycosylated IgG has been produced by several groups either by culture in the presence of the glycosylation inhibitor tunicamycin or following site-directed mutagenesis to remove the Asp 297 glycosylation site. There is a consensus that Fc receptor recognition, C1q binding, C1 activation are compromised and there is an enhanced susceptibility to proteolytic degradation, although, binding to SpA is essentially unaffected [7,8,14–16,19]. It is important to evaluate the effects of aglycosylation on a particular biological activity in several different assay systems in order to reach a conclusive finding. Thus it has been variously reported that aglycosylation results in the retention of C1 activating potential, but requiring high sensitisation levels or that whilst binding activity for C1q is diminished, C1 activation is abrogated [15,17].

In a detailed study of an aglycosylated chimeric human IgG3 anti-NP antibody we demonstrated a reduction in affinity of 2 orders of magnitude for human FcγRI [8]. This resulted in a loss of rosette formation between sensitised red blood cells and FcγRI-expressing U937 cells. However, rosette formation was observed when the U937 cells were activated with γ-interferon (IFNγ) to up-regulate FcγRI expression [8]. The residual recognition capacity is sufficient to trigger superoxide production from the IFNγ stimulated

TABLE 1
CARBOHYDRATE CONTACT RESIDUES IN Ig MOLECULES

	241	243	244	245	246	249	256	258	260	262	264	265	296	299	301
Human γ1	F	F	P	P	K	D	T	E	T	V	V	D	Y	T	R
Human γ2	–	–	–	–	–	–	–	–	–	–	–	–	F	–	–
Human γ3	–	–	–	–	–	–	–	–	–	–	–	–	F	–	–
Human γ4	–	–	–	–	–	–	–	–	–	–	–	–	F/Y	–	–
Mouse γ1	–	–	–	–	–	–	–	K	–	–	–	–	F	–	–
Mouse γ2a	–	–	–	–	–	–	S	I/M	–	–	–	–	–	–	–
Mouse γ2b	–	–	–	–	–	–	T	K	–	–	–	–	–	–	–
Mouse γ3	–	–	–	–	–	–	–	K	–	–	–	–	–	–	–
Rat γ1	–	–	–	–	–	–	–	K	–	–	–	–	F	–	–
Rat γ2a	–	–	–	–	–	–	–	K	–	–	–	–	S	–	–
Rat γ2b	–	–	–	–	–	–	N	K	–	–	–	–	–	–	–
Rat γ2c	–	–	–	–	–	–	–	K	–	–	–	–	L	–	–
Rabbitt γG	–	–	–	–	–	–	–	–	–	–	–	–	F	–	–
Guinea pig γ1	–	–	–	–	–	–	–	K	–	–	–	–	F	–	–
Guinea pig γ2	–	–	–	–	–	–	–	K	–	–	–	–	–	–	–
Chimp. γG	–	–	–	–	–	–	–	–	–	–	–	–	F	–	–
Other human Ig isotopes															
Human μ	–	I	–	–	S	S	S	K	–	L	T	–	P	–	S
Human α	S	H	R	–	A	–	E	N	–	T	T	G	C	–	S
Human δ	Y	L	T	–	A	–	K	T	–	F	–	G	S	S	S
Human ε	S	Y	R	S	R	P	S	T	Y	L	–	–	R	–	T

cells but not from the same cells activated with dibutyryl-cyclic AMP, that down-regulates FcγRI expression. The same aglycosylated IgG3 protein was shown not to be recognised by FcγRII expressed on the Daudi and K562 cell lines [19]. Rosette formation through FcγRIII expressed on K cells was reduced to 40% of that observed with glycosylated IgG3; however, antibody-dependent cellular cytotoxicity (ADCC) was completely abrogated [18].

The biological consequences of aglycosylation are profound and require a mechanistic explanation. Since the carbohydrate moiety is integral to the tertiary and quaternary structure of the protein moiety it is possible that in the absence of glycosylation the IgG molecule folds in a radically different way with the loss of the required ligand binding sites. Alternatively, the ligand binding sites may be composites of both the protein and oligosaccharide moieties. In attempts to determine the structural consequences of aglycosylation we have failed to detect any differences in epitope expression between glycosylated and aglycosylated IgG Fc with a panel of 20 mouse monoclonal antibodies (mAb) to human Fc [7]. Interestingly, parallel epitope expression was observed for both the glycosylated and aglycosylated IgG in ELISA assays carried out at temperatures to 65°C. This suggests that there are no gross conformational differences between the 2 forms. This was confirmed by high-field NMR studies, using histidine residues as reporter groups for conformational differences between the 2 forms, that revealed the environment of His 268 as the only evident structural difference [23]. This residue is on a beta bend at the N-proximal end of the C_H2 domain in the vicinity of the lower hinge region which has been proposed to be directly involved in FcγR recognition.

A direct role for the carbohydrate moiety depends on the accessibility of constituent sugar residues. The X-ray structure suggests that the 'core' residues are sequestered within the quaternary structure and are not likely to be available for ligand binding. This is supported by the finding that the carbohydrate cannot be removed with endo-glycosidases and glycosaminidase [20,21]. Probing with lectins suggests that terminal sialic acid and galactose are accessible while terminal N-acetylglucosamine is not [22]. However, terminal N-acetylglucosamine of human IgG1 [23] and IgG4 Fc [24] is accessible to bovine milk galactosyltransferase for the addition of galactose. Clearly, accessibility to ligand is a qualitative parameter determined by precise steric requirements; however, these data suggest that terminal sugars may be accessible to participate in ligand recognition and binding. It is tempting to propose that the 'core' structure determines precise protein conformation while outer-arm sugars may modulate ligand recognition specificity and affinity and hence thresholds for biological activation.

6. Outer-arm sugars and biological activity

As stated above the X-ray crystal structure of IgG Fc reveals interactions between the oligosaccharide and protein moieties. This includes the outer-arm residues fucose and galactose on the $\alpha_{(1-6)}$ arm; it is not known whether the Fc fragments analysed had other sugar residues attached that were not resolved due to their mobility. Since glycosylation is essential for full expression of biological activity considerable interest is attached to the possibility that glycoforms differing in outer-arm sugars may have modulated activities. A small but significant reduction in FcγRI and C1q binding recognition has been reported for IgG following the removal of galactose with β-galactosidase [20]. In an opposite approach we have compared agalactosylated IgG with the fully galactosylated molecule generated by enzymatic addition. We did not detect a significant difference in the ability of these preparations to inhibit FcγRI-mediated superoxide release by U937 cells triggered through sensitised red blood cells [24]. Similarly, we could not distinguish between molecules bearing or devoid of fucose. Many studies have employed mouse/human chimeric antibodies produced in different rodent cell lines that do not attach bisecting N-acetylglucosamine sugar residues suggesting that it does not contribute significantly to the activities investigated [2,3,19].

In further studies we have generated ^{13}C galactose-labelled IgG and applied ^{13}C NMR to probe its environment both in the intact molecule and when released as the free oligosaccharide. This study provides direct evidence for the galactose residues being in heterogeneous environments consistent with them being both free and interacting with the protein moiety [25]. A significant body of data shows that the proportion of human IgG molecules lacking galactose (Go,IgG) is increased in patients with rheumatoid arthritis and certain other chronic inflammatory diseases [26,27] and it has been suggested that Go,IgG may have a role in the pathogenesis of diseases in which its level is increased [27]. This is an interesting possibility since IgG antibody responses can be subclass and clonally restricted [28] and the glycosylation profiles of these antibodies may also be restricted; as observed for IgG myeloma proteins [29]. If receptor/ligand interactions are modulated by glycosylation differences the protective effector mechanisms activated by an oligoclonal specific antibody population could be dependent on the predominant glycoforms present.

The structural subtlety that can determine receptor/ligand recognition for antibodies can be illustrated by the polymorphic high and low responder forms of human FcγRII. While both forms are equally responsive to IgG1 and IgG3 antibodies only the low-responder form is activated by IgG2 [30]. The recognition and functional difference between the 2 FcγRII forms appears to be a single amino acid residue difference (arginine/histidine, 131) in a domain of 110 amino acids [31]. Interaction of mouse IgG2b with mouse FcγRII has also been shown to be abrogated by a Glu/Ala interchange at residue 318 [33]. Although the lower hinge has been shown to contribute to FcγRII recognition by human IgG1 and IgG3 the radically different lower hinge of IgG2 would not be expected to contribute. Therefore, recognition may be determined by structure in the vicinity of 318. This residue is part of the complement motif so that a single amino acid replacement can result in the loss of both C1 activation and FcγRII recognition. Aglycosylation also results in loss of both FcγRII recognition and C1 activation [16,33]. It is interesting to speculate whether the presence of outer-arm sugar residues could affect the architecture of this site and modulate the threshold for biological activation.

7. Protein engineering of oligosaccharide contact residues

Our understanding of IgG Fc structure suggests that the conformation of both the protein and oligosaccharide moieties are interdependent. Changes in the composition of either component may affect biological activity. We have explored this possibility through amino acid replacements of contact residues for sugar residues. An initial target was residues forming contacts with the $\alpha_{(1-6)}$ galactose residue. Thus, the replacements lysine/alanine (246) + aspartic acid/alanine (249) and glutamic acid/asparagine (258) were introduced. These replacements did not affect FcγRI or FcγRII recognition monitored by rosette formation or FcγRI-mediated superoxide generation by U937 cells. The double-mutant aspartic acid/alanine (265) + asparagine/serine (280) introduced into mouse IgG2b was shown to abrogate binding and phagocytosis mediated through mouse FcγRII. Residue 265 is a contact for the primary N-acetylglucosamine residue of the 'core' oligosaccharide moiety. The single residue replacement aspartic acid/alanine (265) in the human IgG3 molecule also results in a loss of human FcγRI and FcγRII recognition; revealed by a rosette assay.

We have produced the double-mutant protein asparagine/alanine (297) + glutamic acid/asparagine (258) in the IgG3 molecule to remove the natural glycosylation site and to introduce a glycosylation sequon at the position of IgA glycosylation. The resulting protein is glycosylated at 258; however, FcγRI and FcγRII recognition are not 'rescued' and its activity is the same as aglycosylated IgG. Interestingly the oligosaccharide is of the oligomannose rather than complex type. It has previously been observed that IgG produced in yeast cells and bearing high mannose oligosaccharide at asparagine 297 is fully active in cell lysis mediated through FcγRIII but does not activate C1; suggesting that $(GlcNac)_2(Man)_3$ is sufficient for recognition of IgG by FcγRIII [34].

8. Environmental influences on glycosylation

Analyses of human and mouse mAbs have demonstrated isotype and clone-specific glycosylation profiles [3,29]. This suggests that the profile observed for polyclonal IgG is the sum of all the unique clones contributing to IgG production. One of the parameters influencing glycosylation is the polypeptide chain itself which acts as template directing or being permissive of particular glycosylation pathways. Thus, while IgG has a single glycosylation site mouse IgM bears 5 oligosaccharide moieties of which only 1 is of the biantennary type (attached at residue 171): 1 being high mannose (residue 363) and 3 triantennary (residues 332, 364, 402) [35]. The finding that mouse/human chimeric anti-NP antibodies of each of the IgG subclasses exhibit individual glycosylation profiles is again suggestive of template direction. However, it could also be argued that each transfection event results in a unique J558L clone and that glycosylation may be one of the unique properties. We analysed a series of chimeric human IgG3 proteins that were each products of a separate transfection experiment in an attempt to address this point. The results indicated that the different transfectant gave essentially the same oligosaccharide profile if produced in the same in vitro culture protocol. However, significant differences were observed for proteins produced in ascitic fluids, hollow fibre bio-reactors or still culture [3]. Most striking was the increased galactosylation of proteins produced in still culture, including the addition of gal $\alpha_{(1-3)}$ gal structures, with only 2–3% of oligosaccharide moieties lacking galactose (Go). In contrast the same protein produced in a hollow fibre bio-reactor had 30% Go oligosaccharide structures. This demonstrates the extreme effects that environment can have on glycosylation and the need for rigorous control of production systems. It is possible, therefore, that the changes in IgG galactosylation observed in specific diseases could reflect underlying

disease processes and be exploited as an indicator of disease activity.

References

[1] Jenkins, N. and Curling, M.A. (1994) Enz. Microbiol. Technol. 16, 354.

[2] Lund, J., Winter, G., Jones, P.T., Pound, J.D., Tanaka, T., Walker, M.R., Artymiuk, P.J., Arata, Y., Burton, D.R., Jefferis, R. and Woof, J.M. (1991) J. Immunol. 147, 2657.

[3] Lund, J., Takahashi, N., Nakagawa, H., Bentley, T., Hindley, S., Tyler, R., Goodall, M. and Jefferis, R. (1993) Mol. Immunol. 30, 741.

[4] Burton, D.R. and Woof, J.M. (1992) Adv. Immunol. 51, 1.

[5] Jefferis, R. and Pound, J. (1992) in: Inflammation: Basic Principles and Clinical Correlates. (J.I. Gallin, I.M. Goldstein, R. Snyderman, Eds.), Raven Press, New York.

[6] Jefferis, R., Pound, J., Lund, J. and Goodall, M. (1994) Ann. Biol. Clin. 52, 57.

[7] Walker, M.R., Lund, J., Thompson, K.M. and Jefferis, R. (1989) Biochem. J. 259, 1356.

[8] Pound, J.D., Lund, J. and Jefferis, R. (1993) Mol. Immunol. 30, 233.

[9] Deisenhofer, J. (1981) Biochem. 20, 2361.

[10] Sutton, B.J. and Phillips, D.C. (1983) Biochem. Soc. Trans. 11, 130.

[11] Padlan, E.A. (1990) in: Fc Receptors and the Action of Antibodies (H. Metger, Ed.), pp. 12–30, American Society for Microbiology, Washington, DC.

[12] Tomlinson, I.M., Walter, G., Marks, J.D., Llewelyn, M.B. and Winter, G. (1992) J. Mol. Biol. 227, 776.

[13] Jefferis, R. (1993) Glycoconjugate J. 10, 357.

[14] Leatherbarrow, R.J., Rademacher, T.W., Dwek, R.A,. Woof, J.M., Clark, A., Burton, D.R., Richardson, N. and Feinstein, A. (1985) Mol. Immunol. 22, 407.

[15] Nose, M. and Wigzell, H. (1983) Proc. Natl. Acad. Sci. USA 80, 6632.

[16] Duncan, A.R and Winter, G. (1988) Nature 332, 738.

[17] Dorai, H., Mueller, B.M., Reisfeld, R.A., Gillies, S.D. (1991) Hybridoma 10, 211.

[18] Sarmay, G., Lund, J., Gergely, J. and Jefferis, R. (1992) Mol. Immunol. 29, 633.

[19] Lund, J., Tanaka, T., Takahashi, N., Sarmay, G., Arata Y. and Jefferis, R. (1990) Mol. Immunol. 27, 1145.

[20] Tsuchiya, N., Endo, T., Matsuta, K., Yoshinoya, S., Aikawa, T., Kosuge, E., Takeuchi, F., Miyamoto, T. and Kobata, A. (1989) J. Rheum. 16, 285.

[21] Newkirk, M.M., Lemmo, A. and Rauch, J. (1990) Arthr. Rheum. 33, 800.

[22] Sumar, N., Bodman, K.B., Rademacher, T.W., Dwek, R.A., Williams, P., Parek, R.B., Edge, J., Rook, G.A.W., Isenberg, D.A., Hay, F.C. and Roitt, I.M. (1990) J. Immunol. Methods 131, 127.

[23] Fujii, S., Nishiura, T., Nishikawa, A., Miura, R. and Taniguchi, T. (1990) J. Biol. Chem. 265, 6000.

[24] Lund, J., Takahashi, N., Nkagawa, H., Pound, J., Goodall, M. and Jefferis, R. (1995) FASEB J., in press.

[25] Gilhespy-Muskett, A.M., Partridge, J., Jefferis, R. and Homans, S.W. (1994) J. Glycobiol. 4, 485.

[26] Rademacher, T.W. and Dwek, R.A. (1988) Ann. Rev. Biochem. 57, 785.

[27] Rademacher, T.W., Parek, R.B., Dwek, R.A., Isenberg, D., Rook, G., Axford, J.S. and Roitt, I. (1988) Springer Sem. Immunopathol. 10, 231.

[28] Jefferis, R. and Kumararatne, D.S. (1990) Clin. Exp. Immunol. 81, 357.

[29] Jefferis, R., Lund, J., Mizutani, H., Nakagawa, H., Kawazoe, Y., Arata, Y. and Takahashi, N. (1990) Biochem. J. 268, 529.

[30] Parren, P.W.H.I., Warmerdam, P.A.M., Boeiji, L.C.M., Capel, P.J.A., van de Winkle, J.G.J. and Aarden, L.A. (1992) J. Immunol. 148, 695.

[31] Warmerdam, P.A.M., van de Winkle, J.G.J., Vlug, A., Westerdaal, N.A.C. and Capel, P.J.A. (1991) J. Immunol. 147, 1338.

[32] Duncan, A. and Winter, G. (1988) Nature 332, 738.

[33] Lund, J., Pound, J.D., Jones, P.T., Duncan, A.R., Bentley, T., Goodall, M., Levine, B., Jefferis, R. and Winter, G. (1992) Mol. Immunol. 29, 53.

[34] Horowitz, A.H., Chang, C.P., Better, M., Hellstrom, K.E. and Robbinson, R.R. (1988) PNAS 85, 8678.

[35] Anderson, D.R., Atkinson, P.H. and Grimes, W.J. (1985) Arch. Biochem. Biophys. 13, 605.