Structural and Functional Analysis of C3 Using Monoclonal Antibodies

J. ALSENZ[1]*, J. D. BECHERER[1], B. NILSSON[2], and J. D. LAMBRIS[1]

1 Introduction 235
2 Generation of Anti-C3 Monoclonal Antibodies 235
3 Structural and Functional Analysis of C3 Using Anti-C3 Monoclonal Antibodies 236
3.1 Detection of Conformational Changes in C3 and its Fragments 236
3.2 Analysis of the C3 Functions 237
3.3 Mapping of the Epitopes Recognized by the Monoclonal Antibodies 239
4 Analysis of the C3 Complement Activation Products 243
5 Conclusions 244
References 245

1 Introduction

Monoclonal antibodies (MoAbs) have greatly facilitated the structural and functional analysis of proteins in general and of the third protein of complement (C3) in particular. Various aspects of the structure and functions of C3 have been addressed using MoAbs; these include: (a) the study of conformational changes occurring in the C3 molecule and its fragments during complement activation, (b) the analysis of the interactions of C3 with other complement components and receptors as well as with proteins of foreign origin, and (c) the detection of C3 activation products in biological fluids. The purpose of this review is to summarize the contribution that MoAbs have made in understanding the structure and functions of C3.

2 Generation of Anti-C3 Monoclonal Antibodies

Although the generation of MoAbs has become a routine procedure, a few interesting points have been observed concerning the nature of the immunizing antigen and the subsequent specificity of the anti-C3 MoAb. In the most commonly used immunization

[1] Basel Institute for Immunology, Grenzacherstr. 487, 4005 Basel, Switzerland
[2] Department of Clinical Immunology and Transfusion Medicine, University Hospital, Uppsala, Sweden
* J. A. has an EMBO long-term fellowship (ALTF 298-1987). The Basel Institute for Immunology was founded and is supported entirely by F. Hoffmann-LaRoche Ltd. Co., Basel, Switzerland

Current Topics in Microbiology and Immunology, Vol. 153
© Springer-Verlag Berlin · Heidelberg 1989

Table 1. Summary of the monoclonal anti-C3 antibodies reccgnizing neoantigens and/or influencing C3-ligand interactions

MoAb	Fragment recognized	Neoantigen expressed in	Inhibition of the C3 interaction with						References
			H	B	P	CR1	CR2	gC	
C3–5	C3a	C3*/C3a							HACK et al. 1988
4SD 17.1	C3a	C3a							NILSSON et al. 1988
H453, H454	C3a	C3a							BURGER et al. 1988
C3-1	C3c	C3*/C3b/C3c							HACK et al. 1988
C3-9	C3c	C3*/C3b/C3c	+	+		+	+	–	HACK et al. 1988; BECHERER et al. 1989c; HUEMER et al. 1989
C-5G	C3c	C3b/C3c				+	–		IIDA et al. 1987a
bH6	C3c	C3b/iC3b/C3c	+	–			–	+	GARRED et al. 1988b, HUEMER et al. 1989, BECHERER et al. 1989c
7D326.1, 7D331.1	C3c	sb^+ C3b/iC3b							NILSSON et al. 1989a
MoAb 130	C3d	iC3b/C3dg/C3d	–	–			+		TAMERIUS et al. 1982, LAMBRIS et al. 1985
G-3E	C3d	iC3b/C3dg/C3d				–	+		IIDA et al. 1987a
clone 9	C3dg	iC3b/C3dg/C3g	–	–		–	–		LACHMANN et al. 1980
C3-11	C3dg	C3*/C3b/C3dg							HACK et al. 1988
7D323.1, 7D84.1, 7D264.6	C3dK	sb iC3b							NILSSON et al. 1989b
105	C3c	–	–	+	–	–	–	+	BURGER et al. 1982, HUEMER et al. 1989
111	C3c	–	–	+	+	–	–	+	BURGER et al. 1982, HUEMER et al. 1989
Ab 12	C3c	–	+	–	–				TAMERIUS et al. 1982
498	C3c	–	–	+		–	–		BECHERER et al. 1989c
Ab 84	C3c	–	–	–	+				TAMERIUS et al. 1982
311	C3d	–	+	–		–	+	+	BECHERER et al. 1989c. HUEMER et al. 1989
31	C3d	–	+	–		–	–		BECHERER et al. 1989c
Ab 14, Ab 72	C3d	–	+	–	+				TAMERIUS et al. 1982
4C2	C3d	–	+	+		+			KOISTINEN et al. 1989
H11	β-chain	–	+	+	–	+	–	+	WÖRNER et al. 1989, HUEMER et al. 1989
H2	β-chain	–	–	+	–	+	–	+	WÖRNER et al. 1989, HUEMER et al. 1989

C3* = $C3(H_2O)$; sb^+ = surface bound.

Other anti-C3 MoAbs reacting with:

C3: anti-FG11 (MA et al. 1985); HAV4-1 (KOCH and BEHRENDT 1986)

C3a: H13 (BURGER et al. 1987); MoAb 868 (KLOS et al. 1988)

C3b: MoAb 755 (KLOS et al. 1988)

C3c: clone 4 (LACHMANN et al. 1980); N-7A (IIDA et al. 1987a); WM-1 (WHITEHEAD et al. 1981); MoAb-BRL (AGUADO et al. 1985); anti-C3c (22 different MoAb) (DOBBIE et al. 1987)

C3d: Fc 112, Fc 280, Fc 283, Ortho (CHAPLIN and MONROE 1986); anti-C3d (nine different MoAbs) (DOBBIE et al. 1987); 3D4H3 (RUDDY et al. 1983); BRIC 8 (HOLT et al. 1985)

have been identified in C3b, one in C3d and another in C3c (GANU and MÜLLER-EBERHARD 1985; LAMBRIS et al. 1988; BECHERER et al. 1989a; LAMBRIS et al. 1989; BECHERER et al. 1989c). The H binding site in C3d is associated with the CR2 site while the site in C3c is within the domain of C3 containing the B and CR1 binding sites. It is interesting that the latter site contains a sequence (between residues 744 and 755) that is similar to the sequence of the CR2 binding site in C3d (for more details see BECHERER et al. 1989a). Furthermore, a third sequence similar to the CR2 binding site has been identified in the β-chain of C3 (between residues 295 and 306), and a peptide spanning this sequence has been found to bind to CR2 (ESPARZA et al. 1989). Although a direct binding of CR1, H, or B to this peptide has not yet been determined, this may explain the finding that MoAbs to the β-chain of C3 have also been found to inhibit H, CR1, and B binding to C3b (WÖRNER et al. 1989).

The selective inhibition by MoAbs of either B (498, 105, 111) or H binding (311, Ab 12, 14, 72, and 84) to C3b (TAMERIUS et al. 1982; BURGER et al. 1982) suggests the presence of distinct binding sites for B and H on C3b. This is not necessarily in contrast to the above findings and might be attributed to the interaction of these molecules with C3b via multiple sites. In this context, it has been observed that MoAb 130 inhibits CR2 but not H binding to C3d; both sites are located within residues 1187–1249 of C3. The latter fact further supports the findings with synthetic peptides which have shown that these two sites are related but are not the same (LAMBRIS et al. 1985, 1988). Of interest also is the recent finding that MoAbs to C3 which binding H were also found to inhibit the binding of glycoprotein C (gC) of herpes simplex virus type 1 (HSV-1) to C3b (HUEMER et al. 1989). However, the inhibition of H but not gC binding to C3b by other antibodies (Table 1) may explain the functional differences between these two molecules; H has both cofactor and decay-accelarating activity while gC has only the latter.

The interaction of properdin with C3b, as investigated by MoAbs to either C3c or C3d (Table 1; TAMERIUS et al. 1982), suggests a two-side interaction between these two molecules. Since the site in C3c for P has recently been localized to residues 1402–1435 of C3 (DAOUDAKI et al. 1988), it will be of interest to see whether these anti-C3c antibodies react with this region of C3, or whether the observed inhibition is indirect (steric or allosteric effects, etc.). The presence of a second P interaction site in C3b remains to be determined.

The epitopes recognized by several other MoAbs (Table 1) have not yet been localized, and this makes it difficult to correlate the structural elements involved in antibody binding to those involved in ligand binding. In addition, MoAbs which bind close to a functional site in C3 (see also Fig. 1) were fount not to inhibit its reactivity with the ligand. However, this is not necessarily unexpected since it has been shown that a peptide, 24 amino acids in length, is able to bind two different antibodies simultaneously (JACKSON et al. 1988).

3.3 Mapping of the Epitopes Recognized by the Monoclonal Antibodies

A major breakthrough in understanding antibody-antigen interactions was provided by the crystallographic studies of Fab fragments complexed to the protein antigens lysozyme (AMIT et al. 1986) and influenza virus neuraminidase (COLMAN et al. 1987).

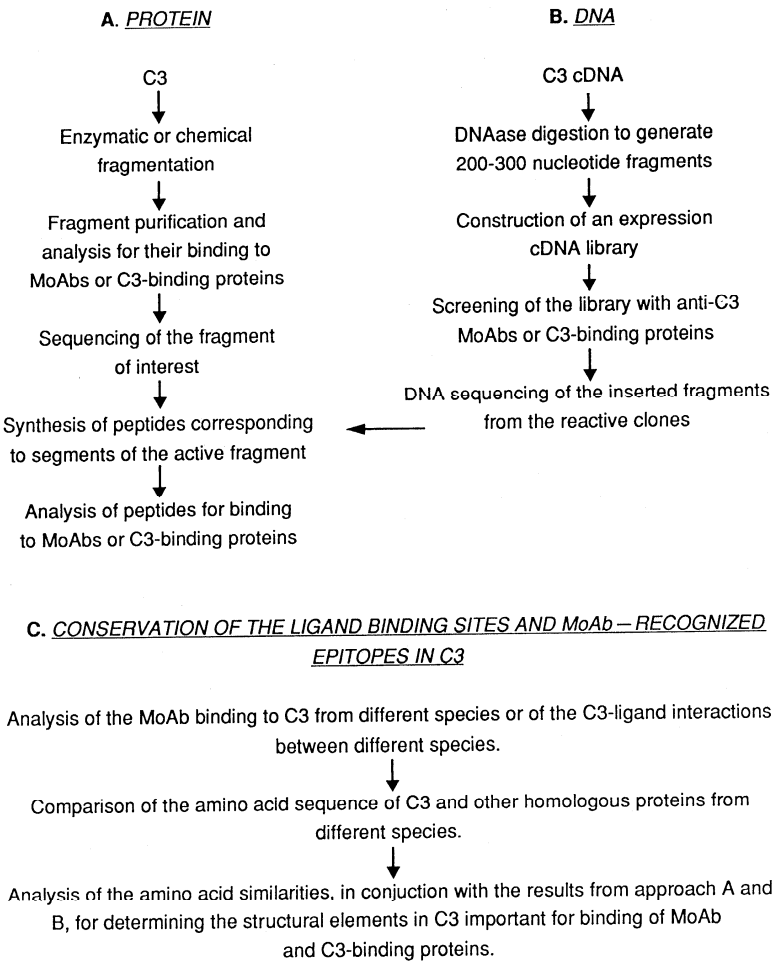

Fig. 2. Schematic representation of a general strategy to localize MoAb-recognized epitopes and ligand binding sites in C3

The third approach deals with the conservation of antigenic and functional sites within C3 from different species and other homologous proteins such as C4, C5, and α_2-macroglobulin. First, C3 from different species is purified and tested for its ability to bind to MoAbs or to the various human C3 ligands (ALSENZ et al. 1989; BECHERER et al. 1987). These proteins can then be sequenced at the DNA level, thus allowing analysis of the conservation of epitopes or binding sites between different species. This, together with the conserved sequences of other homologous proteins that do or do not bind the MoAb or ligand of interest, offers a wealth of information on the structural features of the C3 molecule. Concerning this third approach, the complete amino acid sequences of human and mouse C3 (DE BRUIJN and FEY 1985; LUNDWALL et al. 1984; WETSEL et al. 1984) and the partial sequences of rabbit (KUSANO et al. 1986) and *Xenopus* C3 (GROSSBERGER et al. 1989) have been resolved recently.

Using the above approaches, various epitopes recognized by anti-C3 MoAbs have been localized (Table 2). Several of the antibodies recognize epitopes located within or close to functional regions of C3 and therefore have been used as tools to analyze its functions. For example, the MoAb 130 was localized to a discontinuous epitope within residues 1192–1249 of C3 (LAMBRIS et al. 1989). This MoAb inhibits the binding of C3d to CR2 and assists in the localization of the CR2 binding site within this fragment to residues 1199–1210 (LAMBRIS et al. 1985).

Table 2. Summary of the monoclonal anti-C3 antibodies whose epitopes in C3 have been mapped

Antibodies	C3-fragment(s) recognized	Residues	References
H11[a,h]	C3b/C3c/β-chain	1-645	WÖRNER et al. 1989
GV1.8[b,h]	β-chain/CHO	63*	GRIER et al. 1987
GV1.10[b,h]	α/β-chain, CHO	63*, 917*	GRIER et al. 1987
H7[a,h]	C3b/C3c/β-chain	89–645	WÖRNER et al. 1989
H15[a,h]	C3b/C3c/β-chain	98–293	WÖRNER et al. 1989
H2[a,h]	C3b/C3c/β-chain	98–293	WÖRNER et al. 1989
H21[a,h]	C3b/C3c/β-chain	294–645	WÖRNER et al. 1989
H453, H454[c,i]	C3a	718–725	BURGER et al. 1988
398.1[d,i]	sb C3b	741–758	BECHERER, NILSSON, LAMBRIS unpublished
406.4[d,i]	sb C3b	741–758	BECHERER, NILSSON, LAMBRIS unpublished
595.2[d,i]	sb C3b	741–758	BECHERER, NILSSON, LAMBRIS unpublished
615.1[d,i]	sb C3b	741–758	BECHERER, NILSSON, LAMBRIS unpublished
7D 84.1[d,i]	sb C3b/iC3b	926–946	NILSSON et al. 1989a
7D 264.6[d,i]	sb C3b/iC3b	926–936	NILSSON et al. 1989a
7D 323.1[d,i]	sb C3b/iC3b	926–946	NILSSON et al. 1989a
Clone 9[e,i]	iC3b/C3dg	933–946	MYONES et al. 1989
7D 9.2[d,i]	sb C3b	1082–1118	NILSSON et al. 1989a
MoAb 130[f,i]	iC3b/C3dg/C3d	1192–1249	LAMBRIS et al. 1985, 1989
7D 326.1[d,j]	sb C3b/iC3b	1234–1294	NILSSON et al. 1989a
7D 331.1[d,j]	sb C3b/iC3b	1234–1294	NILSSON et al. 1989a
4SD 11.1[d,j]	sb C3b	1476–1510	NILSSON et al. 1989a
4SD 18.1[d,j]	sb C3b	1476–1510	NILSSON et al. 1989a
H18[a,j]	C3c	1476–1531	WÖRNER et al. 1989
H6b[a,j]	C3c	1476–1531	WÖRNER et al. 1989
H3[a,j]	C3c	1476–1531	WÖRNER et al. 1989
H206[d,j]	C3c	1476–1531	WÖRNER et al. 1989
H215[g,j]	C3c	1476–1531	WÖRNER et al. 1989

sb = surface bound;
* The MoAb binds to the carbohydrate moiety linked to this residue.

Immunization with:
[a] native C3;
[b] cobra venom factor;
[c] synthetic peptide $C3^{69-76}$ coupled to KLH;
[d] SDS-denatured C3;
[e] inulin-fixed C3b/iC3b;
[f] trypsin-generated C3b;
[g] expressed C3 fragment.

Mapped by:
[h] enzymatic fragmentation;
[i] synthetic peptides;
[j] expressed C3 fragments

The segment of C3 spanning residues 929–946 contains at least four different overlapping epitopes (Table 2). These epitopes are expressed by surface-bound and/or fluid-phase iC3b and are within the segment of C3 which also contains: (a) the carbohydrate moiety mediating conglutinin binding (HIRANI et al. 1985), (b) the factor I cleavage site(s) (DAVIS and HARRISON 1982 and BECHERER et al. 1989a), and (c) the leukocytosis-inducing activity of the C3 molecule (MEUTH et al. 1983). Using these antibodies to localize the fragments generated upon cleavage of iC3b by factor I, it was found that factor I cleaves C3 within this region at three different positions (NILSSON-EKDAHL et al. 1989; BECHERER et al. 1989a). The existence of several epitopes within such a limited amino acid sequence is not surprising since a segment as small as 19 amino acids has been shown to accommodate three different epitopes (FIESER et al. 1987).

Other epitopes in C3 have been mapped using either synthetic peptides or expressed C3 fragments (Table 2). These epitopes – several being expressed solely by surface-bound C3 fragments – have been located to residues 741–758 (four MoAbs), 1234–1294 (two MoAbs), and 1476–1531 (seven MoAbs) of the C3 sequence (for references see Table 2). The localization of these epitopes has been greatly facilitated by their expression in denatured C3 also. The possibility that some of these antibodies recognize the same epitope has not been excluded.

Related to the third approach (conservation of epitopes and binding sites), anti-C3 antibodies have been shown to cross-react with C3 from other species. Cross-reactivity studies using MoAbs have facilitated the identification of which residues are involved in the binding, and which MoAbs recognize the same or different epitopes. For example, from three MoAbs which recognize epitopes within residues 929–946 of human C3, only MoAb 7D323.1 and not 7D84.1 or 7D264.6 react with rabbit C3. Based on their reactivity with rabbit C3 and overlapping synthetic peptides, it was predicted that these MoAbs recognize different epitopes, and that residues Arg^{929}, Arg^{932}, and Glu^{933} are essential for the binding of MoAbs 7D84.1 and 7D264.6 but not that of 7D323.1 (NILSSON et al. 1989b).

4 Analysis of the Complement Activation C3 Products

The selective binding of MoAbs to either native C3 or its degradation products, whether fluid-phase or surface-bound, has rendered possible the development of sensitive assays detecting these products in biological fluids. Most of these are simple (e.g., enzyme-linked immunosorbent assay, or ELISA) and available to laboratories that are not specialized in dealing with some of the tedious and complicated assays used in analyzing the complement system. To date three MoAbs have been found to react specifically with the C3a fragment of C3 (Table 1). The detection of this fragment is relevant for the diagnosis and/or prognosis of adult respiratory distress syndrome and various other diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus; SLE) (for review see BITTER-SUERMANN 1988 and HUGLI 1989). Two versions of ELISA have been described for detecting C3a. In a competition ELISA using MoAb 4SD 17.1, which recognizes C3a but not native C3 (NILSSON et al. 1988), the amount of C3a in the test sample is quantitated based on its ability to inhibit the binding of

this MoAb to microtiter plate-fixed C3a. In the ELISA reported by BURGER et al. (1988), the C3a-containing sample is first incubated with an immobilized polyclonal anti-C3a antibody, and the bound C3a is detected by the MoAb H453. The sensitivity of both assays briefly described above is approximately 1 ng/ml.

In addition to the above antibodies which detect complement activation based on the generation of the C3a fragment, the MoAbs 130 (KANAYAMA et al. 1986), clone 9 (MOLLNES and LACHMANN 1987), and bH6 (GARRED et al. 1988a), all of which recognize neoantigens (Table 1), have also been used to develop assays that detect complement activation. These assays use either MoAbs fixed to microtiter plates (MOLLNES and LACHMANN 1987; GARRED et al. 1988a) or competition assays similar to that described for C3a.

The reactivity of several MoAbs with surface-bound, but not fluid-phase, C3 fragments led to the development of assays detecting and quantitating these fragments fixed to immune complexes (AGUADO et al. 1985; IIDA et al. 1987b) or particles and micro-organisms (NEWMAN and MIKUS 1985). A highly sensitive ELISA measuring C3 fragments in immune complexes has been developed using MoAbs 130 and 105. This assay detects as little as 6.26 μg aggregated human Ig/ml serum (AGUADO et al. 1985) and is comparable or superior to the most commonly used Raji (THEOFILOPOULOS et al. 1976) and C1q assays (ZUBLER et al. 1976). Using this assay, increased levels of complement-fixing immune complexes were detected in plasma of patients with autoimmune diseases (rheumatoid arthritis, Sjögrens syndrome, systemic lupus erythematosus) and paracoccidioidomycosis (AGUADO et al. 1985). Interestingly, patients with paracoccidioidomycosis were found to have a markedly decreased degradation of immune complex bound C3b/iC3b to C3dg compared to patients with autoimmune diseases. This observation resulted from the differential specificities of MoAb 105, an antibody which binds preferentially to surface-bound C3b, and MoAb 130, an antibody specific for iC3b. In addition to the different fragments of C3 found on immune complexes, differences were also observed, using MoAb clone 9, in the fragments bound to the surface of complement activators (NEWMAN and MIKUS 1985). These findings emphasize the importance of monoclonal antibodies in distinguishing the C3 activation products in biological fluids.

5 Conclusions

MoAbs have been useful tools for studying the C3-ligand interactions as well as the conformational changes associated with its degradation. A great deal of information on the functional aspects of C3 has been obtained based on the ability of MoAbs differentially to inhibit certain C3 interactions. Since the results obtained with MoAbs are often due to steric or allosteric effects, and since most antibodies recognize conformation-dependent epitopes which are difficult to localize by present techniques, other approaches are necessary to confirm functional sites identified only by MoAbs. For diagnostic purposes, MoAbs directed against particular fragments of C3 have facilitated the development of assays detecting these fragments in biological fluids, and their use will augment our understanding of complement involvement, particularly the role of C3, in various diseases.

Acknowledgements. We thank Drs. D. Goundis and H. Huemer for helpful discussions and critical reading of the manuscript. Also, we thank Drs. J. Tamerius and B. Kolb (Cytotech) for generously providing serveral of the MoAbs used in our studies.

References

Aguado MT, Lambris JD, Tsokos GC, Burger R, Bitter-Suermann D, Tamerius JD, Dixon FJ, Theofilopoulos AN (1985) Monoclonal antibodies against complement 3 neoantigens for detection of immune complexes and complement activation. Relationship between immune complex levels, state of C3, and numbers of receptors for C3b. J Clin Invest 76: 1418–1426

Alsenz J, Lambris JD (1988) Modulation of factor H (H) functional activities by monoclonal antibodies (MoAbs) to H. Complement 5: 202–203 (Abstract)

Alsenz J, Becherer JD, Esparza I, Daoudaki ME, Avila D, Oppermann S, Lambris JD (1989) Structure and function analysis of C3 from different species. Complement Inflamm 6: 307 (Abstract)

Amit AG, Mariuzza RA, Phillips SEV, Poljak RJ (1986) Three-dimensional structure of an antigen-antibody complex at 2.8A resolution. Science 233: 747–753

Becherer JD, Daoudaki ME, Lambris JD (1987) Conservation of the C3 ligand binding sites within different species. Fed Proc 46: 771 (Abstract)

Becherer JD, Alsenz J, Lambris JD (1989a) Molecular aspects of C3 interactions and structural/functional analysis of C3 from different species. Curr Top Microbiol Immunol 153: 45–72

Becherer JD, Alsenz J, Servis C, Myones BL, Lambris JD (1989b) Cell surface proteins reacting with activated complement components. Complement Inflamm 6: 142–165

Becherer JD, Alsenz J, Hack CE, Drakopulou E, Lambris JD (1989c) Identification of common binding domains in C3b for members of the complement family of C3b-binding proteins. Complement Inflamm 6: 313 (Abstract)

Bitter-Suermann D (1988) The anaphylatoxins. In: Rother K, Till GO (ed) The complement system. Springer Berlin Heidelberg New York, pp 367–395

Burger R, Deubel U, Hadding U, Bitter-Suermann D (1982) Identification of functionally relevant determinants on the complement component C3 with monoclonal antibodies. J Immunol 129: 2042–2050

Burger R, Bader A, Kirschfink M, Rother U, Schrod L, Wörner I, Zilow G (1987) Functional analysis and quantification of the complement C3 derived anaphylatoxin C3a with a monoclonal antibody. Clin Exp Immunol 68: 703–711

Burger R, Zilow G, Bader A, Friedlein A, Naser W (1988) The C terminus of the anaphylatoxin C3a generated upon complement activation represents a neoantigenic determinant with diagnostic potential. J Immunol 141: 553–558

Chaplin H Jr, Monroe MC (1986) Comparisons of pooled polyclonal rabbit anti-human C3d with four monoclonal mouse anti-human C3ds. I. Preparation, purification and binding properties. Vox Sang 50: 42–51

Collawn JF, Wallace CJA, Proudfoot AEI, Paterson Y (1988) Monoclonal antibodies as probes of conformational changes in protein-engineered cytochrome c. J Biol Chem 263: 8625–8634

Colman M, Laver WG, Varghes JN, Baker AT, Tulloch PA, Air GM, Webster RG (1987) Three-dimensional structure of a complex of antibody with influenza virus neuraminidase. Nature 326: 358–363

Daoudaki ME, Becherer JD, Lambris JD (1988) A 34-amino acid peptide of the third component of complement mediates properdin binding. J Immunol 140: 1577–1580

Davis AEIII, Harrison RA (1982) Structural characterization of factor I mediated cleavage of the third component of complement. Biochemistry 21: 5745–5749

De Bruijn MHL, Fey GH (1985) Human complement component C3: cDNA coding sequence and derived primary structure. Proc Natl Acad Sci USA 82: 708–712

Acknowledgements. We thank Drs. D. Goundis and H. Huemer for helpful discussions and critical reading of the manuscript. Also, we thank Drs. J. Tamerius and B. Kolb (Cytotech) for generously providing serveral of the MoAbs used in our studies.

References

Aguado MT, Lambris JD, Tsokos GC, Burger R, Bitter-Suermann D, Tamerius JD, Dixon FJ, Theofilopoulos AN (1985) Monoclonal antibodies against complement 3 neoantigens for detection of immune complexes and complement activation. Relationship between immune complex levels, state of C3, and numbers of receptors for C3b. J Clin Invest 76: 1418–1426

Alsenz J, Lambris JD (1988) Modulation of factor H (H) functional activities by monoclonal antibodies (MoAbs) to H. Complement 5: 202–203 (Abstract)

Alsenz J, Becherer JD, Esparza I, Daoudaki ME, Avila D, Oppermann S, Lambris JD (1989) Structure and function analysis of C3 from different species. Complement Inflamm 6: 307 (Abstract)

Amit AG, Mariuzza RA, Phillips SEV, Poljak RJ (1986) Three-dimensional structure of an antigen-antibody complex at 2.8A resolution. Science 233: 747–753

Becherer JD, Daoudaki ME, Lambris JD (1987) Conservation of the C3 ligand binding sites within different species. Fed Proc 46: 771 (Abstract)

Becherer JD, Alsenz J, Lambris JD (1989a) Molecular aspects of C3 interactions and structural/functional analysis of C3 from different species. Curr Top Microbiol Immunol 153: 45–72

Becherer JD, Alsenz J, Servis C, Myones BL, Lambris JD (1989b) Cell surface proteins reacting with activated complement components. Complement Inflamm 6: 142–165

Becherer JD, Alsenz J, Hack CE, Drakopulou E, Lambris JD (1989c) Identification of common binding domains in C3b for members of the complement family of C3b-binding proteins. Complement Inflamm 6: 313 (Abstract)

Bitter-Suermann D (1988) The anaphylatoxins. In: Rother K, Till GO (ed) The complement system. Springer Berlin Heidelberg New York, pp 367–395

Burger R, Deubel U, Hadding U, Bitter-Suermann D (1982) Identification of functionally relevant determinants on the complement component C3 with monoclonal antibodies. J Immunol 129: 2042–2050

Burger R, Bader A, Kirschfink M, Rother U, Schrod L, Wörner I, Zilow G (1987) Functional analysis and quantification of the complement C3 derived anaphylatoxin C3a with a monoclonal antibody. Clin Exp Immunol 68: 703–711

Burger R, Zilow G, Bader A, Friedlein A, Naser W (1988) The C terminus of the anaphylatoxin C3a generated upon complement activation represents a neoantigenic determinant with diagnostic potential. J Immunol 141: 553–558

Chaplin H Jr, Monroe MC (1986) Comparisons of pooled polyclonal rabbit anti-human C3d with four monoclonal mouse anti-human C3ds. I. Preparation, purification and binding properties. Vox Sang 50: 42–51

Collawn JF, Wallace CJA, Proudfoot AEI, Paterson Y (1988) Monoclonal antibodies as probes of conformational changes in protein-engineered cytochrome c. J Biol Chem 263: 8625–8634

Colman M, Laver WG, Varghes JN, Baker AT, Tulloch PA, Air GM, Webster RG (1987) Three-dimensional structure of a complex of antibody with influenza virus neuraminidase. Nature 326: 358–363

Daoudaki ME, Becherer JD, Lambris JD (1988) A 34-amino acid peptide of the third component of complement mediates properdin binding. J Immunol 140: 1577–1580

Davis AEIII, Harrison RA (1982) Structural characterization of factor I mediated cleavage of the third component of complement. Biochemistry 21: 5745–5749

De Bruijn MHL, Fey GH (1985) Human complement component C3: cDNA coding sequence and derived primary structure. Proc Natl Acad Sci USA 82: 708–712

Klos A, Ihrig V, Messner M, Grabbe J, Bitter-Suermann D (1988) Detection of native human complement components C3 and C5 and their primary activation peptides C3a and C5a (anaphylatoxic peptides) by ELISAs with monoclonal antibodies. J Immunol Methods 111: 241–252

Koch C, Behrendt N (1986) A novel polymorphism of human complement component C3 detected by means of a monoclonal antibody. Immunogenetics 23: 322–325

Koistinen V, Wessberg S, Leikola J (1989) Common binding region of complement factors B, H and CR1 on C3b revealed by monoclonal anti-C3d. complement Inflamm 6: 270

Kusano M, Choi NH, Tomita M, Yamamoto K, Migita S, Sekiya T, Nishimura S (1986) Nucleotide sequence of cDNA and derived amino acid sequence of rabbit complement component C3 alpha-chain. Immunol Invest 15: 365–378

Lachmann PJ, Oldroyd RG, Milstein C, Wright BW (1980) Three rat monoclonal antibodies to human C3. Immunology 41: 503–515

Lachmann PJ, Pangburn MK, Oldroyd RG (1982) Breakdown of C3 after complement activation. Identification of a new fragment, C3g, using monoclonal antibodies. J Exp Med 156: 205–216

Lambris JD (1988) The multifunctional role of C3, the third component of complement. Immunol Today 9: 387–393

Lambris JD, Ganu VS, Hirani S, Müller-Eberhard HJ (1985) Mapping of the C3d receptor (CR2)-binding site and a neoantigenic site in the C3d domain of the third component of complement. Proc Natl Acad Sci USA 82: 4235–4239

Lambris JD, Avilla D, Becherer JD, Müller-Eberhard HJ (1988) A discontinuous factor H binding site in the third component of complement as delineated by synthetic peptides. J Biol Chem 263: 12147–12150

Lambris JD, Becherer JD, Daoudaki ME, Servis C, Alsenz J (1989) Use of synthetic peptides in exploring and modifying complement reactivities. In: Sim RB (ed) Activators and inhibitors of complement activation. Kluer Academic, Lancaster (in press)

Lundwall A, Wetsel RA, Domdey H, Tack BF, Fey GH (1984) Structure of murine complement component C3. I. Nucleotide sequence of cloned complementary and genomic DNA coding for the β chain. J Biol Chem 259: 13851–13862

Ma D, Sessler MJ, Meyer TF, Schrod L, Hänsch GM, Burger R (1985) Expression of polypeptide segments of the human complement component C3 in *E. coli*: genetic and immunological characterization of cDNA clones specific for the β-chain of C3. J Immunol 135: 3398–3402

Meuth JL, Morgan EL, DiScipio RG, Hugli TE (1983) Suppression of T lymphocyte functions by human C3 fragments. I. Inhibition of human T cell proliferative responses by a kallikrein cleavage fragment of human iC3b. J Immunol 130: 2605–2611

Molenaar JL, Helder AW, Müller MAC, Goris-Mulder M, Jonker LS, Brouwer M, Pondman KW (1975) Physico-chemical and antigenic properties of human C3. Immunochemistry 12: 359–364

Mollnes TE, Lachmann PJ (1987) Activation of the third component of complement (C3) detected by a monoclonal anti-C3′g′ neoantigen antibody in a one-step enzyme immunoassay. J Immunol Methods 101: 201–207

Myones BL, Avila D, Lachmann PJ, Lambris JD (1989) Localization of the epitopes recognized by the anti-C3G (clone 9) monoclonal antibody using synthetic peptides. Complement Inflamm 6: 373 (Abstract)

Newman SL, Mikus LK (1985) Deposition of C3b and iC3b onto particulate activators of the human complement system. Quantitation with monoclonal antibodies to human C3. J Exp Med 161: 1414–1431

Nilsson UR, Nilsson B (1982) Analogous antigenic alterations elicited in C3 by physiologic binding and by denaturation in the presence of sodium dodecylsulfate. J Immunol 129: 2594–2597

Nilsson B, Nilsson UR (1986) SDS denaturation of complement factor C3 as a model for allosteric modifications occurring during C3b binding: demonstration of a profound conformational change by means of circular dichroism and quantitative immunoprecipitation. Immunol Lett 13: 11–14

Nilsson B, Svensson KE, Borwell P, Nilsson UR (1987) Production of mouse monoclonal antibodies that detect distinct neoantigenic epitopes on bound C3b and iC3b but not on the corresponding soluble fragments. Mol Immunol 24: 487–494

Nilsson B, Svensson KE, Inganäs M, Nilsson UR (1988) A simplified assay for the detection of C3a in human plasma employing a monoclonal antibody raised against denatured C3. J Immunol Methods 107: 281–287

Nilsson B, Grossberger D, Nilsson-Ekdahl K, Riegert P, Becherer JD, Nilsson U, Lambris D (1989a) Localization of neoantigenic epitopes in C3b and iC3b. Complement Inflamm 6: 376 (Abstract)

Nilsson B, Nilsson-Ekdahl K, Avila D, Nilsson UR, Lambris JD (1989b) Conformational changes in C3 as detected by monoclonal antibodies: mapping of the recognized epitopes by synthetic peptides. J Biol Chem (submitted for publication)

Nilsson-Ekdahl K, Nilsson B, Becherer JD, Nilsson UR, Lambris JD (1989) Further studies on the degradation of complement factor C3 by factor I. Inhibition of factor I with DFP. Seventh International Congress of Immunology Gustav Fischer Verlag Stuttgart p. 131 (Abstract 22–24)

Pangburn MK (1987) A fluorimetric assay for native C3. The hemolytically active form of the third component of human complement. J Immunol Methods 102: 7–14

Pangburn MK, Schreiber RD, Müller-Eberhard HJ (1981) Formation of the initial C3 convertase of the alternative pathway. Acquisition of C3b-like activities by spontaneous hydrolysis of the putative thioester in native C3. J Exp Med 154: 856–867

Perkins SJ, Sim RB (1986) Molecular modelling of human complement C3 and its fragments by solution scattering. Eur J Biochem 157: 155–168

Ross GD, Medof (1985) Membrane complement receptors specific for bound fragments of C3. Adv Immunol 37: 217–267

Ruddy S, Moxley GF, Purkall DB (1983) Inhibition of classic and alternative pathway convertases by rat monoclonal antibody to human C3. Immunobiology 164: 291 (Abstract)

Tamerius JD, Pangburn MK, Müller-Eberhardt HJ (1982) Selective inhibition of functional sites of cell-bound C3b by hybridoma-derived antibodies. J Immunol 128: 512–514

Tamerius JD, Pangburn MK, Müller-Eberhard HJ (1985) Detection of a neoantigen on human C3bi and C3d by monoclonal antibody. J Immunol 135: 2015–2019

Theofilopoulos AN, Wilson CB, Dixon FJ (1976) The Raji cell radioimmune assay for detecting immune complexes in human sera. J Clin Invest 57: 169–182

Wetsel RA, Lundwall A, Davidson F, Gibson T, Tack BF, Fey GH (1984) Structure of murine complement component C3. II. Nucleotide sequence of clones complementary DNA coding for the alpha chain. J Biol Chem 259: 13857–13862

Whitehead AS, Sim RB, Bodmer WF (1981) A monoclonal antibody against human complement component C3: the production of C3 by human cells in vitro. Eur J Immunol 11: 140–146

Wörner I, Burger R, Lambris JD (1989) Localization and functional characterization of epitopes on α and β-chains of C3. Complement Inflamm 6: 416 (Abstract)

Zubler RH, Lange G, Lambert PH, Miescher PA (1976) Detection of immune complexes in untreated sera by a modified ^{125}I-C1q binding test. J Immunol 116: 232–235