Pathol. Oncol. Res. (2009) 15:639–644
DOI 10.1007/s12253-009-9164-y

ORIGINAL PAPER

Histochemical Contributions to the Binding Mechanism of Complement (CR1, CR2) Receptors

F. Baranyay

Received: 25 February 2008 / Accepted: 13 March 2009 / Published online: 8 April 2009

Abstract Complement receptors (CR1, CR2, CR3), and their ligands (C3b, C3d, iC3b) are essentially involved in germinal center development and in binding, trapping, and retaining immunocomplexes. Methods studying complement receptor (CR1/CR2)-ligand (C3b/C3d) interactions mostly involve coating of sheep erythrocytes (E), sheep erythrocyte-antisheep erythrocyte antibody (EA complexes) and whole human (h) or mouse (m) sera as a source of complement, EACh/m complexes, as reagents. The observation of Dukor et al. (1970), that EACm complexes in native cryostat sections bind selectively and very strongly to the B lymphocyte regions of lymphoid organs allowed the topo-histochemical analysis of receptor (CR1/CR2)—ligand (C3b/C3d) interactions in such an immunologically important area as the germinal centers. The main finding of this study is, that periodic acid pretreatment of unfixed cryostat tonsil sections-oxidizing vicinal glycol groups of polysaccharide chains into dialdehydes-completely abolished the binding of all EAC/EC complexes to germinal center area. It may suggest the involvement of receptor carbohydrate in C3 receptor/ligand binding. In addition to, the subsequent sodium borohydride reduction-converting aldehydes (produced by periodic acid oxidation) into primary alcohols-restored selectively the binding of all applied EAC/EC complexes to follicular centers. These in vitro topo-histochemical studies give a strong hint for the participation of—OH groups of sugar residues in CR1/CR2 ligand (C3b/C3d) binding.

F. Baranyay (✉)
Department of Pathology, Medical University of Pécs,
Dorottya Kanizsai Hospital,
Szekeres J. u. 2-8,
8801 Nagykanizsa, Hungary
e-mail: fbaranyay@hotmail.com

Keywords CR1 · CR2-reversible tissue adherence of E(A) C3b · E(A)C3d

Abbreviations

C3	the third component of the complement system
CR1	complement receptor type one, the immune adherence C3b receptor
CR2	complement receptor type two, the C3d receptor
CR3	complement receptor type 3, the iC3b receptor
E	erythrocyte
EA	erythrocyte-antibody complex
EAC	erythrocyte-antibody-complement complex
EC3	sheep E-C3 complexes prepared by C3 activation and fixation into sheep E
TA-E	thiol-activated erythrocytes
TA-EC3	TA-E coated with C3
SPDP	N-succinimidyl 3-(2-pyridildithio) propionate

Introduction

The interactions of complement components, mainly C3 fragments (C3b, C3d) and their receptors (CR1/CD35, CR2/CD21) on follicular dendritic cells (FDCs) and germinal center B cells are essentially involved in the formation of germinal centers, antigen trapping and retention, isotope switching and generation of strong B cell memory response [8, 9, 26, 32].

The activation of the central complement molecule (C3) either by classical or alternative complement pathway leads to the specific proteolytic cleavage of C3 by convertases into C3a and C3b. The biochemical mechanism of the C3b activation and fixation to acceptor molecules were clarified

decades ago. The short lived "nascent" C3b binds through its carbonyl group in the internal thioester (labile or metastable binding site) to hydroxyl or amino-groups of macromolecules, pathogen agents, immunoglobulins etc.) forming covalent (ester or amid) bond [22]. Target bound C3b (C3 molecules that have been fixed to the substrate) may then interact with C3 receptors through their stable binding site. The binding mechanism and transport of antigens, immunocomplexes in germinal centers has not been clarified.

Most of the methods for complement receptor studies involve coating of erythrocytes with C3 by means of activation of the alternative or classical pathway. These methods use sheep erythrocyte-antisheep erythrocyte antibody (EA) complexes and complement factors either from fresh human (EACh) or mouse (EACm) whole sera [6, 28], or require relatively large amounts of different complement components C1, C2, C4, and C3 or factors B, D, nephritic factor and C3 [27].

A specific and simple method was described for preparing EC3b, EC3d, which is based on the fact that C3b expresses an SH-group and that C3b binds to thiol activated erythrocytes [15].

So far the binding mechanism between complement receptor types and their ligands has been analysed by the inhibition of the EAC rosette formation in suspensions after different pretreatments of the C3 receptor bearing cells [5, 11, 16]. Biochemical analysis has suggested carbohydrates [7, 18], while molecular cloning studies of CR1/CR2 revealed proteins in their ligand binding [3, 14, 21]. The C3b binding sites in CR1 were mapped to two adjacent long homologous repeats /LHRs/ at the terminus of the external membrane domain [13], the C3d protein bound to the amino terminus of CR2 requiring both SCR1 and SCR2 [10, 29]. These studies were all performed in solutions.

Dukor et al. (1970) demonstrated that C3 receptors retain their ability to bind EAC in frozen sections and discovered the tissue localization of lymphocytes bearing a membrane receptor for antigen-antibody-complement complexes. The EAC red blood cells adhered exclusively to B cell areas, mainly to germinal center and follicular mantle cells but not to interfollicular regions. These observations made possible the topo-histochemical analysis of receptor (CR1/CR2)–ligand (C3b/C3d) binding.

Pretreatment of cryostat tonsil sections with periodic acid (1% for 10 min), completely abolishes adherence of EACh, EACm, TA-EC-3b, TA-EC3d to follicules. This raises a possibility that ligand binding sites of CR1/CR2 involve carbohydrate. On the other hand the effect of periodate could be reversed, when the periodate treated cryostat tonsil sections were exposed to sodium borohydride (1% for 10 min), before adding the EACh, EACm, TA-EC3b, TA-EC3d complexes.

Materials and Methods

Materials Pieces of fresh human tonsils were frozen within an hour and 8 μm cryostat sections cut and stored at −20°C until used.

Preparation of EACh, EACm reagents Freshly-prepared sheep red blood cell (E) suspensions (1% vol/vol), were treated with the IgM fraction of the anti-E serum [28] in subagglutinating concentration at 37°C for 30 min. The erythrocyte-antibody (EA) complexes were incubated either with heat inactivated (56°C, 30 min) human serum or with complement containing fresh human and mouse serum (EACh, EACm). Because human serum diluted 1:4 effected the lysis of sheep erythrocytes of the EA complexes, the erythrocytes were made haemolysis resistant by glutaraldehyde fixation, which did not influence their antigenicity [30].

EAC1-3d analogous reagent was generated by incubating 1×10^8 EACm with 2 mg trypsin 200 E/g (Merck, Darmstadt, Germany) in 1 ml of 0.01 M TRIS-buffered saline, pH 7.4 for 30 min at 37°C. The reaction was stopped by the addition of 40 μg/ml of soybean trypsin inhibitor (SERVA, Heidelberg, Germany, [25]).

EAC1-3b analogous reagent was generated from purified C3 (1 mg/ml) by incubation with trypsin (10 mikrog/ml; Serva, Heidelberg, Germany) for 1 min followed by the addition of soybean trypsin inhibitor.).

Preparation of C3b C3b was generated from purified C3 (1 mg/ml) by incubation with trypsin (10 ug/ml; Serva, Heidelberg, Germany) for 1 min followed by the addition of soybean trypsin inhibitor.

Coupling of C3b to sheep erythrocytes The thiol-activation of the sheep erythrocytes was carried out as described previously by Lambris et al., 1983 [15]. Briefly, 3.9 mg N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP from Sigma) dissolved in 25 μl DMSO. The SPDP solution was then added to 2.5 ml of a suspension of sheep erythrocytes (2×109/ml) in phosphate buffered saline (PBS) and the mixture was shaken vigorously. The thiol-activated erythrocytes (TA-E) were washed four times with PBS, resuspended to a concentration of 5×10^9 cells / ml PBS, and then C3b prepared with trypsin was mixed with thiol-activated erythrocytes on a rotating mixer at room temperature for 60 min. The resulting C3-coated cells (TA-EC3b) were washed three times with PBS, resuspended to a concentration of 2×10^8 cells/ml and stored at 4°C until use. TA-EC3b cells were converted into C3d with KSCN-inactivated serum and plasmin [15].

Adherence of EACh, EACm,TA-EC3b, TA-EC-3d to tonsil sections Cryostat sections of tonsils were air-dried at room temperature for 2 h and incubated with EACh, EACm, TA-EC3b, TA-EC3d complexes in a humidified chamber at 37°C for 30 min. The slides were then washed in saline solution until no more erythrocytes could be washed off. Sections were then fixed in 2 % glutaraldehyde for 4 min. Attached sheep erythrocytes were visualized by the benzidin-peroxidase reaction; Mayer's haematoxylin was used as a counter stain.

For the purpuse of stating whether sialic acid do have a role in CR1/CR2 ligand binding *neuraminidase pretreatment* was carried out by the method of Hsu et al., (1986) [12]: 2 U/ml neuraminidase (from Vibrio cholerae, SERVA, Heidelberg, Germany) in 0.1 M acetate buffer, pH 5.3 was used for 20 min at 37°C.

Periodic acid pretreatment was used to characterize the carbohydrate or peptidic nature of CR'/CR2 active binding sites. *Periodic acid*: 0.01–1 % HIO_4 (SIGMA) in distilled water and incubated for 1–30 min.

Whether C3 receptor sugar-OH groups are involved in the ligand binding periodic acid formed aldehydes were restored with sodium borohydride into hydroxil groups. *Sodium borohydride*: 0.01–1 % $NaBH_4$ (SIGMA) in distilled water and incubated for 1–30 min.

Preincubation of the sections with antiserum to human alpha-1-antitrypsin, *mouse* (with C3d, iC3b) and *human sera* (with C3b, C3d) or with *MAbs* to CD35, CD21 and CD11b and *mannose specific lectins* (Concanavalin A, Lens culinaris /SIGMA/, 0.5–100 ug/ml) were performed.

In order to define, whether do *divalent cations* play a role in C3 receptor-ligand binding one part of 0.1–0.5 M Na_2H_2 EDTA (pH 7.6) was added to nine part of EACh, EACm, TA-EC3b, TA-EC3d erythrocyte suspensions.

Immunodetection of complement receptors I have pretreated the sections with periodic acid and with different anti-C3 receptor antibodies in order to define whether the epitope of the complement receptors are peptidic or carbohydrate in nature. I intended to test, that the binding of some monoclonal antibodies is periodic acid sensitive or resistant. Furthermore whether these antibodies are able to block the adherence of EACh complexes and they can react with supposed real carbohydrate or the peptidic binding site.

Cryostat sections of tonsils were incubated followed by periodic acid oxidation with monoclonal antibodies (MAbs) clone: J3D3 (Dakopatts) and clone RFD5 to CD35/(CR1), clones OKB7 (Ortho), IOB1a (Dakopatts), RFB6 to CD21(CR2), MAbs OKM1 (Ortho) and 2LPM19c (Dakopatts) to CD11b (CR3) diluted 1 :100, without prior fixation or blocking of the endogenous peroxidase. Peroxidase conjugated anti-mouse immunoglobulin (Dakopatts) diluted 1:100 was used with 3-amino-9-ethylcarbazole as a chromogen in the peroxidase reaction. The slides were counterstained with Mayer's hematoxylin. Controls were carried out omitting the incubation with the MAbs.

Results

The binding of EACh and EACm complexes to tonsil sections was more intensive than that of TA-EC3b and TA-EC3d. The adherence of the trypsinized EACm complexes (EAC1-3d analogous reagent) was comparable to the less dense adherence of TA-EC3d to the germinal center area. The tissue localization (adherence) of EACh and TA-C3b complexes seemed to be similar. The binding of the EACh, TA-EC3b complexes was more extensive (follicular center, mentle zone, some parafollicular adherence), while EACm, TA-EC3d adherence confined mainly to the germinal center cells (Fig. 1). There was no adherence of EA complexes incubated with heat inactivated human or mouse sera. Pretreatment of the sections with 1% periodic acid for 10 min the EACh, EACm, TA-EC3b, TA-EC3d did not adhere to the to the cryostat sections of tonsils (Fig. 2). Neuraminidase predigestion, $NaBH_4$ pretreatment of the sections did not influence the adherence of EC/EAC complexes.

Pretreatment of the sections with 1% periodic acid for 10 min the EACh, EACm, TA-EC3b, TA-EC3d reagents did not adhere to the cryostat sections of tonsils (Fig. 2).

However, periodic acid oxidation followed by subsequent sodium borohydride reduction the adherence of all reagents (EACh, EACm, TA-EC3b, TA-EC3d) selectively restored to the area of follicles, mainly to the germinal centers (Fig. 3).

Schematic representation of CR1, CR2 and CR3 structure is demonstrated on Fig. 4.

The adherence of all the applied EC/EAC reagents could not be abolished by EDTA, so the adherence of different E(A)C complexes do not require divalent cations.

Neither neuraminidase predigestion nor $NaBH_4$ pretreatment (1%, for 10 min) influence the adherence of EACh, EACm and TA-EC3b and TA-EC3d, which indicates the role of the alkali stable N-glycosidic oligosaccharide chain in CR1/CR2 binding.

EDTA could not abolish the specific adherence of neither EACh, EACm nor TA-EC3b, TA-EC3d to the germinal center areas after periodic acid-borohydride treatment, so no divalent cations are required for the reversed ligand (C3b, C3d) binding, either.

Preincubation of the sections with mouse and human sera, Lens culinaris and Concanavalin A lectins did not change the adherence of EAC/EC complexes.

After periodic acid oxidation of cryostat sections of tonsils the binding of (Monoclonal antibodies /MAbs/) J3D3 (Dakopatts) and RFD5 to CD35, MAbs HB5 (Becton-Dickinson) and OKB7 (Ortho), IOB1a (Dakopatts), RFB6 to CD21 was well preserved, whereas MAbs OKM1 (Ortho) and 2LPM19c (Dakopatts) to CD11b (similarly to E(A)C complexes) were sensitive to periodic acid.

Discussion

The discovery of Dukor et al. (1970) was the very specific and strong tissue adherence of EACm complexes to the germinal center cells allowing the topo-histochemical analysis of ligand (C3b/C3d)–receptor (CR1/CR2) binding. The main finding of this study is that the periodic acid treatment of human tonsil sections abolishes binding of EAC/EC complexes to follicles (germinal centers) and that borohydride restores their binding. This seems to prove that ligand binding site of CR1/CR2 involve carbohydrate sugars. Aminoacids are oxidized by periodate at alkaline pH (pH 7.0 and not at pH 3.0 –pH 4.0) [4]. Bianco et al. [2] reported in 1970, that some lymphocytes bound sheep erythrocytes (E) coated with IgM (A) and complement (C). The same authors showed that binding of EAC is mediated by the third component of the complement system (C3). In the same year Dukor et al. demonstrated that C3 receptors retain their ability to bind EAC in frozen sections. This observation made possible the tissue localization of complement receptor bearing lymphocytes. They observed that EAC adhered exclusively to germinal centers and follicular mantles but not to interfollicular regions. EA coated with mouse complement (EACm) exclusively adhere to C3d receptor positive cells, while EAC human complexes bind to C3b and C3d receptor-bearing cells [28] Thiol-activated erythrocytes which were coupled with C3b, C3d, (TA-EC3b, TA-EC3d) prepared from purified C3 without (anti-erythrocyte) antibody [15], also adhered only in the area of follicles in cryostat sections of tonsils.

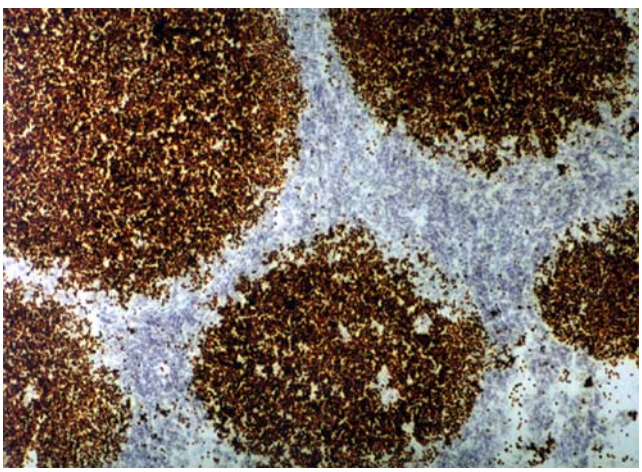

Fig. 1 Thiol-activated sheep erythrocytes covered with C3b (TA-C3b) adhere to the germinal center cells of normal human tonsil. The overlayered red blood cells are appeared as dark brown dots with the benzidin-peroxidase reaction. In the interfollicular region only a few red blood cells are seen. × 160

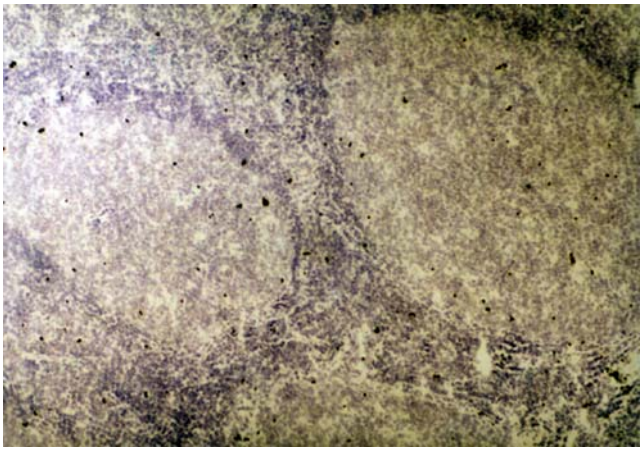

Fig. 2 The adherence of TA-EC3b stopped after periodic acid oxidation. × 160

Histochemical use of periodic acid was investigated by McManus (1946) [20] Periodic acid has been used earlier as a carbohydrate oxidant [19] to form aldehyde, when it split a chain between two carbon atoms each bearing-OH groups. Periodic acid in tissue sections breaks the C-C bonds, where vicinal glycol groups (CHOH-CHOH) are present and converts them into dialdehydes. Sodium borohydride is a suitable, specific reducing agent for converting aldehydes (produced by periodic acid oxidation) into Schiff negative primary alcohols [17]. Periodic acid pretreatment of gastrointestinal sections was applied to characterize the carbohydrate or peptidic nature of mucin epitopes [1].

Periodate oxidative treatment—as a chemically very specific attack on lymphocyte surfaces-leads to stimulate blastic transformation, which is mediated by the induction of free aldehyde groups on their surface [23]. Transformation of periodate-treated lymphocytes is blocked by subsequent incubation with sodium borohydride [24]. Using mild periodate oxidation (1–2 mM, pH 7.0)–which, selectively oxidizes only the side chain of terminal sialic acid-followed by $NaBH_4$ reduction of rat lymphocytes [31] found that the lymphocyte recirculation in vivo was

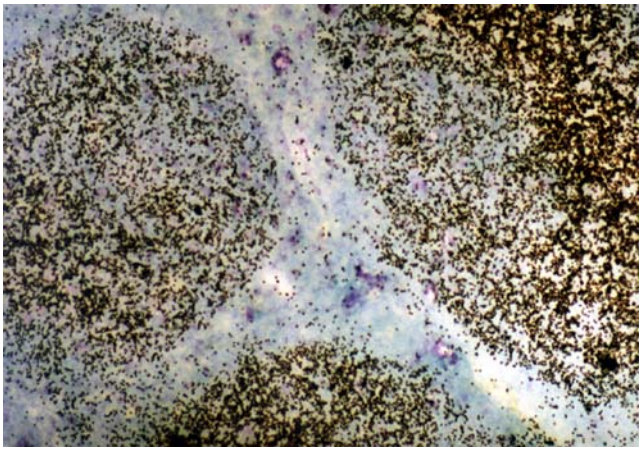

Fig. 3 Restored adherence of TA-EC3b in the areas of germinal centers—on an adjacent cryostat section of the same tonsil—after periodic acid oxidation followed by borohydride reduction. ×160

dependent on the oxidation reduction state of protein bound sialic (neuraminic) acid. Taking together these findings and our observations suggest similar biochemical mediation of the bindings between complements and CR1/CR2 receptors and of those used by lymphocytes at recirculation.

The selective restored binding of EAC/EC complexes in the area of germinal centers in the periodic acid-borohydride system gives a strong hint for the involvement of sugar residues in the ligand binding of CR1/CR2 receptors. Preincubation of cryostat tonsillar sections with fresh native and diluted human and mouse sera (native complement) and with mannose specific lectins none of them abolished the adherence of different EA/EAC complexes. Periodic acid pretreatment of cryostat tonsillar sections preserved the binding of the applied monoclonal antibodies to CR1/CR2. Presumable mainly the protein part (short and long consensus repeats /SCRs, LHRs/) participates in the binding of Mabs to CD35, CD21 clusters (to the strongest antigenic /peptidic) antigenic structure of CR1/CR2.

All these findings indicate, that—in such an immmunologically important areas as the normal germinal centers the carbohydrate part of CR1/CR2 is also (or mainly) involved in ligand (C3b/C3d) binding. The reversible adherence of various E(A)C complexes in the periodic acid oxidation-sodium borohydride reduction (topo-chemical) system seems to prove the redox regulatory role of CR1/CR2 receptor oligosaccharide sugar molecules. It may serve as an in vitro model for the traffic of the immunocomplexes in the area of germinal centers. It seems possible that both the C3b/C3d acceptor and CR1/CR2 receptor molecules take part with their-OH groups in binding and C3b behaves as an endogenous lectin.

The protein part of CR1/CR2 consists of short consensus repeats (SCRs) in a linear alignment as a semi-rigid structure. Finally I postulate, that the protein part of the complement receptors presumably is unable to bind to protein ligands in a reversible, rolling way with regard to the fact, that the binding of the immunocomplexes must be quick and reversible in the germinal centers.

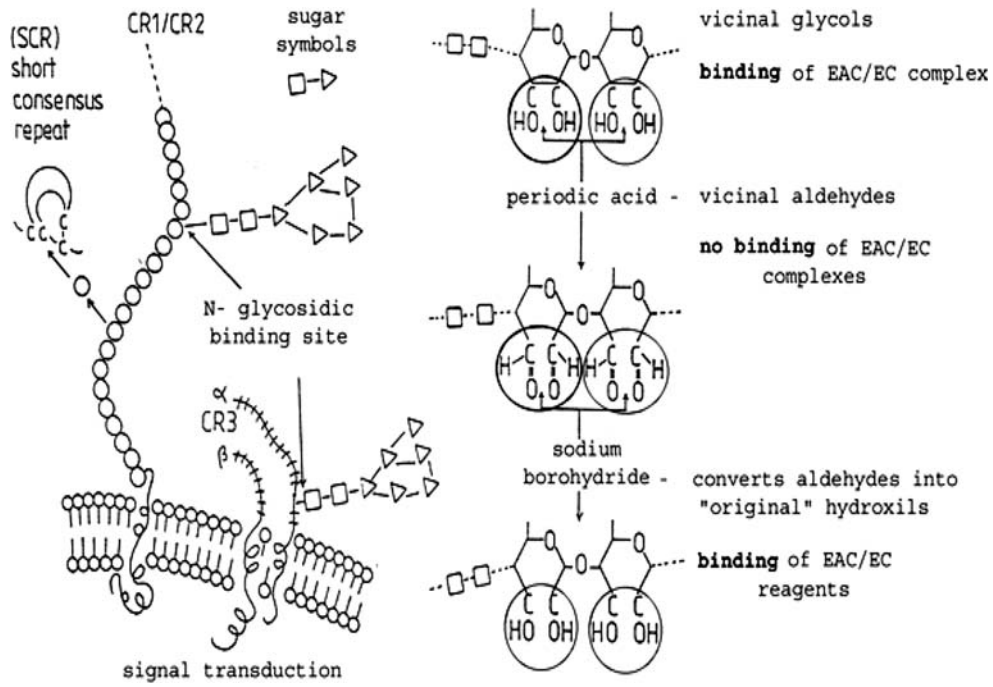

Fig. 4 Schematic representation of CR1, CR2 and CR3 structure. The protein part of CR1/CR2 consists of short consensus repeats (SCRs) in a linear alignment as a semi-rigid structure. Every SCR has a triple loop formation in which four conserved half-cystines form disulfide linkage [13] CR3 (iC3b) receptor belongs to the integrin family with α and β chain proteins. All C3 receptors are glycosilated

Acknowledgements RFD5 and RFB6 MAbs were kindly provided by Prof. G. Janossy, Royal Free Hospital (London).

I would like to express my gratitude to prof. Gábor Kelényi for his useful suggestions, prof. György Füst for his kindly prividing me purified C3 and prof. Gerda Szakonyi for her critical reading this manuscript

I also would like also to thank Mrs. Maria Garai and Tibor Honti for their excellent technical assistance.

References

1. Bara J, Decaens C, Loridon-Rosa B, Oriol R (1992) Immunohistological characterization of mucin epitopes by pre-treatment of gastro-intestinal sections with periodic acid. J Immunol Methods 149:105–113
2. Bianco C, Patrick R, Nussenzweig V (1970) A population of lymphocytes bearing a membrane receptor for antigen-antibody-complement complexes. J Exp Med 132:702–720
3. Birmingham DJ, Irshaid F, Gavit KF, Nagaraja HN, Yu CY, Rovin BH, Hebert LA (2007) A polymorphism in the type one complement receptor (CR1) involves an additional cysteine within the C3b/C4b binding domain that inhibits ligand binding. Mol Immunol 44:3510–3516
4. Clamp JR, Hough L (1965) The periodate oxidation of amino acids with reference studies to glycoproteins. Biochem J 94:17–24
5. Dierich MP, Scheiner O, Mussel HH, Hamman KP, Schopf RE, Schutz Z (1982) Characterisation of complement receptors. Molecular Immunology 19:1201–1204
6. Dukor P, Bianco C, Nussenzweig V (1970) Tissue localization of lymphocytes bearing a membrane receptor for antigen -antibody-complement complexes. Proc Natl Acad SAci USA 67:991–997
7. Fearon DT (1979) Regulation of the amplification C3 convertase of human complement by an inhibitory protein isolated from human erythrocyte membrane. Proc Natl Acad Sci USA 76:5867–5871
8. Fischer MB, Goerg S, Shen L, Prodeus AP, Goodnow CC, Kelsoe G, Carroll MC (1998) Dependence of germinal center B cells on expression of CD21/CD35 for survival. Science 280:582–585
9. Fischer MB, Ma M, Hsu NC, Carroll MC (1998) Local synthesis of C3 within the splenic lymphoid compartment can reconstitute the impaired immune response in C3-deficient mice. J Immunol 160:2619–2625
10. Guthridge JM, Rakstang JK, Young KA, Hinshelwood J, Aslam M, Robertson AG, GipsonMG SMR, Moore WT, Meagher M, Karp D, Lambris JD, Perkins SJ, Holers VM (2001) Structural studies in solution of the recombinant N-terminal pair of short consensus / complement repeat domains of complement receptor type 2 (CR2/CD21) and interactions with its ligand C3dg. Biochemistry 20:5931–5941
11. Huber H, Polley MJ, Linscott WD, Fudenberg HH, Müller-Eberhardt HJ (1968) Human monocytes: distinct receptor sites for the third component of complement and for immunoglobulin G. Science 162:1281–1285
12. Hsu SM, YS H, Li PJ, Monheit J, Ree HJ, Sheibani K, Winberg CD (1986) L-H variants of Reed-Sternberg cellsexpress sialylated Leu M1 antigens. Am J Path 122:199–203
13. Klickstein LB, Wong WW, Smith JA, Weis JH, Wilson JG, Fearon DT (1987) Human C3b/C4b receptor (CR1). Demonstration of long homologous repeating domains that are composed of the short consensus repeats characteristics of C3/C4 binding proteins. J Exp Med 165:1095–1112
14. Krych-Goldberg M, Atkinson JP (2001) Structure-function relationships of complement receptor type 1. Immunol Rev 180:112–122
15. Lambris JD, Scheiner O, Schulz TF, Alsenz J, Dierich MP (1983) Coupling of C3b to erythrocytes by disulfide bond formation of EC3b for hemolytic and complement receptor assays. J Immunol Methods 65:277–283
16. Lay WH, Nussenzweig W (1968) Receptors for complement of leukocytes. J Exp Med 128:991–1007
17. Lillie RD, Pizzolato P (1972) Histochemical use of borohydrides as aldehyde blocking reagents. Stain Techn 47:13–16
18. Lublin DM, Griffith RC, Atkinson JP (1986) Influence of glycosylation on allelic and cell specific Mr variation receptor processing and ligand binding of the human C3b/C4b receptor. J Biol Chem 261:5736–5744
19. Malaprade L (1934) Étude de l'action de polyacools sur l'acide periodique et les periodates alcalins. Bull Soc Chim Franc 5:833–852
20. McManus JFA (1946) Histological demonstration of mucin after periodic acid. Nature 158:202
21. Molina H, Perkins SJ, Guthridge J, Gorka J, Kinoshita T, Holers M (1995) Characterisation of a complement receptor 2 (CR2, CD21) ligand binding site for C3. An initial model of ligand interaction with two linked short consensus repeat moduls. J Immunol. 154:5426–5435
22. Müller-Eberhard HJ (1988) Molecular organisation and function of the complement system. Ann Rev Biochem 57:321–347
23. Novogrodsky A, Katchalski E (1972) Membrane site modified on induction of the transformation of lymphocytes by periodate. Proc Natl Acad Sci USA 69:3207–3210
24. O'Brien RL, Parker JW (1976) Oxidation-induced lymphocyte transformation. Cell 7:13–20
25. Okuda T, Sato S, Tachibanam T (1984) The effect of divalent cations on the rosette formation via C3 receptors. Tohoku Exp Med 143:221–224
26. Reynes M, Aubert JP, Cohen JH, Audpuin J, Tricottet V, Diebold J, Kazatchkine MD (1985) Human follicular dendritic cells express CR1, CR2 and CR3 complement receptor antigens. J Immunol 135:2687–2694
27. Ross GD, Medof E (1985) Membrane complement receptors specific for bound fragments of C3. Adv Immunol 37:217–267
28. Stein HE, Grosskopf E, Lennert K (1980) Demonstration of C3 receptors of frozen sections of normal lymphoid tissue and malignant lymphomas using vartious EAC complexes Blut. Ann Hematol 41:101–118
29. Szakonyi G, Guthridge JM, Li D, Young K, Holer M, Chen XS (2001) Structure of complement receptor 2 in complex with its C3d ligand. Science 292:1725–1728
30. Turner RH, Liener IE (1975) The use of glutaraldehyde treated erythrocytes for assaying the agglutination activity of lectins. Anal Biochem 68:651–653
31. Zatz MM, Goldstein AL, Blumenfeld OO, White A (1972) Regulation of normal and leulaemic lymphocyte transformation and recirculation by sodium periodate oxidation and sodium borohydride reduction. Nature New Biology 240:252–255
32. Zwirner J, Felber E, Schmidt P, Riethmüller G, Feucht HE (1989) Complement activation in human germinal centres. Immunology 66:270–77