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Novel monoclonal antibodies against mouse C3 interfering with complement activation: description of fine specificity and applications to various immunoassays

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Abstract

The role of complement proteins in various pathophysiological settings has been studied primarily using mouse models of disease. However, the specific contribution of C3-derived fragments to these biologic processes has not been addressed in a rigorous manner because of a lack of antibodies that can selectively recognize mouse C3 or any of its degradation fragments. Here we report the generation and characterization of a panel of rat monoclonal antibodies reacting with mouse C3 and its degradation products. We describe their performance in various immunological assays such as ELISA, Western blotting, flow cytometry and immunohistochemistry. Of all the antibodies generated, one selectively recognized the C3a anaphylatoxin, and all other reacted with C3c. Furthermore, two monoclonal antibodies preferentially reacted with the cleaved C3 fragments C3b/iC3b/C3c but not native C3. Except for the one recognizing C3a, all antibodies were suitable for detecting C3 deposited on cells and tissues, two effectively inhibited the hemolytic activity of mouse complement and one enhanced C3-deposition to the cell membrane. These novel monoclonal antibodies may serve as useful reagents for elucidating functions mediated by C3-derived fragments in various pathophysiological conditions.

Keywords: Mouse; C3; Monoclonal antibody; ELISA

1. Introduction

C3, the most abundant complement protein in serum, plays a central role in the complement activation cascade. Its cleavage product, C3b, forms an integral part of the C3 and C5 convertases (Rawal and Pangburn, 2001), promoting complement activation and the subsequent formation of the membrane attack complex. Covalent attachment of C3b to the activating surface results in opsonization of foreign antigens and provides vital co-stimulatory signals to elements of the acquired immune response via specific interactions with complement receptor-bearing cells (Nielsen and Leslie, 2002; Barrington et al., 2001; Sahu and Lambris, 2001; Volanakis, 2002). C3a, a peptide released from the N-terminus of the α-chain of C3, possesses anaphylatoxic as well as various immunoregulatory properties (Hugli, 1990).

Apart from serving as an essential link between innate and adaptive immunity and acting as an inflammatory mediator, C3 has recently been implicated in developmental and non-inflammatory processes such as hematopoiesis, skeletal and vascular development, and reproduction (Mastellos and Lambris, 2002). Complement activation also occurs in numerous pathological and clinical conditions (e.g., infection, autoimmunity, and transplantation) and is thereby associated with detrimental effects such as sustained tissue injury and excessive inflammation.

C3 deficiency results in impaired immune responses against a variety of antigens and pathogens (Singer et al., 1994). A critical role for C3 and its receptors in regulating acquired immunity is also underscored by the inability of mice deficient in CR1/2 (Haas et al., 2002) or CR3

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(Rosenkranz et al., 1998) to mount a proper immune response to pathogens.

The use of appropriate transgenic mouse models has facilitated the study of C3-mediated functions in vivo. However, a lack of antibodies that can selectively recognize murine C3 or any of its degradation fragments has considerably impeded the in-depth characterization of C3-mediated functions. Thus, developing tools for the detection of C3 and its split products in mice has become a necessity for complement researchers, to better understanding the functions of the complement system both in physiological and pathological conditions. Moreover, antibodies inhibiting complement activation in mice may provide useful therapeutic tools once successfully tested in appropriate mouse models. Here we describe the fine specificity and possible applications of a set of novel monoclonal antibodies (mAbs) generated against mouse C3.

2. Materials and methods

2.1. Animals

Eight to 12-week-old female LOU/M/WSL rats and Balb/c mice obtained from the National Institute of Oncology, Budapest were used in all experiments performed according to EC regulations.

2.2. Generation of hybridomas

Rats were immunized by intraperitoneal injection of mouse C3-coated Sepharose beads (Tosic et al., 1989) and 100 μg purified mouse C3 emulsified in complete Freund's adjuvant for priming or in incomplete Freund's adjuvant for boosting. Mouse C3—purified according to Van Berg et al. (1989)—was dissolved in phosphate-buffered saline and administered intravenously three days before the fusion of rat spleen cells and Sp2/0-Ag14 mouse myeloma cells in the presence of PEG 6000 (Sigma–Aldrich, Hungary) using standard techniques Tosic et al. (1989). Clones producing specific anti-mouse C3 antibodies were selected based on antibody reactivity with the target antigen, assessed by solid-phase enzyme assay and Western blotting.

2.3. ELISA to measure the binding of rat mAbs to mouse C3 fragments

Ninety-six-well microtiter plates (Propilén Kft., Pécs, Hungary) coated with purified mouse C3 (5 μg/ml) were used for screening hybridoma supernatants for the presence of monoclonal antibodies. Serial dilutions of rat hybridoma supernatanats were added, and biotinylated mouse or goat anti-rat IgG and streptavidin-peroxidase (Sigma–Aldrich, Hungary) were used to detect bound mAb. For the selection of hybridomas reacting with mouse C3c, microtiter plates were coated with purified mouse C3c (5 μg/ml), and bound mAbs were detected by the addition of HRP-conjugated goat anti-rat IgG. Similarly, for selecting mAbs reacting with synthetic mouse C3a (Spruce et al., unpublished observations), plates were coated with mouse C3a (1 μg/ml), and bound mAbs were detected with HRP-conjugated goat anti-rat IgG. Plates coated with rat isotype-specific, purified mouse mAbs (Serotec) were used to determine the IgG subclass of the selected mAbs.

2.4. ELISA to measure native and activated C3 fragments in mouse plasma

Microtiter wells coated with 50 μl of 2.3 μg/ml anti-rat IgG Fc (ICN Cappel) in PBS pH 7.4 for 2 h at 25 °C or overnight at 4 °C were then saturated with 200 μl of 10 mg/ml BSA (Sigma) in PBS (blocking buffer) The rat anti-mouse C3 mAb supernatants of clone 2/11 and 2/16, were diluted in blocking buffer and added at their optimal concentration based on previous titration experiments.

Mouse plasma was collected by intracardiac puncture of isoflurane anesthetized mice using 50 μg/ml Lepirudin (Refludan®, Aventis) (Mollnes et al., 2002). A portion of the plasma was activated with 2 mg/ml zymosan for 30 min at 37 °C. Samples of activated, non-activated, and 20 mM EDTA-treated plasma were serially diluted in blocking buffer (starting dilution 1:1000) and added to wells. Bound mouse C3 was detected with 3.2 μg/ml of HRP-conjugated goat anti-mouse C3 in blocking buffer (ICN Pharmaceuticals, Inc., OH). The reaction was developed by adding ABTS (Roche), and 0.033% H_2O_2 in 0.1 M CH_2COONa pH 4.2. The optical density was measured in an ELISA reader at 405 nm. All incubations following the blocking step were performed at 25 °C for 1 h, and unbound proteins were removed by washing with PBS, pH 7.4, containing 0.05% Tween 20.

To assess which antibodies recognize specifically the C3b fragment of mouse C3, degradation of C3b to iC3b in zymosan treated serum was prevented using K76COOH as previously described (Hong et al., 1981). Briefly, one volume of mouse serum was mixed with three volumes of 4 mg/ml K76COOH (kindly provided by Dr. T. Kinoshita, Osaka, Japan) dissolved in GVB^{++} or PBS as control and incubated with rotation for 30 min at 25 °C. The generated C3 fragments were detected as described above.

2.5. Western blotting

Electrophoresis of purified mouse C3 (1 μg; a kind gift of Barbara Uzonyi, ELTE, Budapest, Hungary) was performed under reducing or non-reducing conditions with 7.5% polyacrylamide gels. Proteins were transferred onto a nitrocellulose membrane (Bio-Rad), which was then blocked with 5% milk and incubated with supernatants of anti C3 mAbs diluted 1:10 for 1 h at room temperature. Biotin-conjugated rabbit anti-rat IgG (Vector Laboratories, Burlingame, CA)

diluted 1:1000 was used as a secondary antibody and the detection was carried out using chemiluminescence.

2.6. Flow cytometric detection of C3 fragments fixed to the cell membrane

Mouse splenocytes (3×10^5) were incubated in 100 μl of 10×-diluted autologous, freshly drawn mouse serum at 37 °C for 1 h, and washed three times with PBS. Cells were then incubated with 80 μl of supernatants of anti-C3 mAbs for 20 min at 4 °C, then washed again three times. FITC-conjugated goat anti-rat IgG (Organon Teknika Cappel, Durham, NC) was used to detect bound primary antibody. After washing, cells were incubated with Cy3-conjugated goat anti-mouse kappa light chain (Southern Biotechnology, Birmingham, USA) for 20 min at 4 °C. FACS analysis was carried out on a FACS Calibur flow cytometer (Becton Dickinson, San Jose, CA) and data were analyzed by WinMDI software.

2.7. Immunohistochemistry

Frozen kidney sections (5 μm) from mice treated with anti-glomerular basement membrane antibodies (generous gift of Dr. Wen-Chao Song) (Sogabe et al., 2001) were fixed in acetone for 5 min at room temperature. Endogenous peroxidases were quenched by incubation at room temperature in freshly prepared 3% hydrogen peroxide in methanol. Avidin and biotin blocking steps were performed using a Vectastain avidin–biotin blocking kit (Vector Laboratories, Burlingame, CA), followed by incubation with 4% rabbit serum in Tris Base Saline (TBS). Culture supernatants containing anti-C3 antibodies, diluted 1:5–1:25 in 1% bovine serum albumin in TBS, were applied to sections and incubated overnight at 4 °C. Biotinylated rabbit anti-rat IgG (Vector Laboratories), diluted 1:200 in 1% bovine serum albumin in TBS, was added to tissue sections. Standard peroxidase ABC reagent and 3,3′-diaminobenzidine substrate incubations were performed according to manufacturer's instructions (Vector Laboratories). Slides were counterstained with hematoxylin and assessed by light microscopy (Olympus BX 60).

2.8. Hemolytic assay to assess the ability of mAbs to inhibit mouse complement activation

The procedure described by Tanaka et al. (1986) was used with minor modifications. Antibody against rabbit erythrocytes (RE) was produced in guinea pigs. 300 μl of a 1% suspension of RE mixed with the same amount of complete Freund's adjuvant was injected subcutaneously, followed by seven sequential injections with incomplete adjuvant at weekly intervals. Seven days after the last injection, animals were exsanguinated under ketamin anesthesia by cardiac puncture. Serum was inactivated for 1 h at 56 °C and stored at −20 °C until use. RE were sensitized by adding antiserum diluted four-fold to 3×10^8 ml^{-1} RE in 10 mM EDTA*GVB solution at a 1:1 ratio. After incubation for 30 min at 37 °C, the RE were washed three times with PBS, and their concentration was adjusted to 1.5×10^8 cells/ml.

Hemolytic assays were carried out in 96-well U-bottom plates using 8.3 μl mouse serum diluted four-fold in GVB and 33 μl of 1.5×10^8 cells/ml sensitized RE. Hybridoma supernatants were added at the indicated dilutions, and samples were incubated for 1 h at 35 °C. Reactions were stopped by placing the plates on ice for 10 min. After centrifugation, 10 μl of the supernatants were mixed with 100 μl tetra-methyl-benzidine solution. The reaction was stopped with 100 μl 2 N H_2SO_4, and the OD of the samples was measured at 450 nm.

2.9. Modulation of C3 deposition on B cells by mAb 3/26

Mouse splenocytes (3×10^5) were incubated in a mixture of 10 μl freshly drawn mouse serum and 80 or 20 μl mAb supernatant, made up to 100 μl with complete RPMI medium. After 1 h of incubation at 37 °C, cells were washed three times with PBS. FITC conjugated goat anti-mouse C3 $F(ab')_2$ fragment (Cappel) was used to detect cell-bound C3. After washing, cells were incubated with Cy3-conjugated goat anti-mouse kappa light chain (Southern Biotechnology, Birmingham, USA) for 20 min at 4 °C. FACS analysis was carried out on FACS Calibur flow cytometer (Becton Dickinson, San Jose, CA) and data were analyzed by WinMDI software.

3. Results

3.1. Reactivity of mAbs with mouse C3 and its degradation fragments

The clones producing anti-mouse C3 antibodies were selected based on reactivity with ELISA plate-bound purified C3. However, when we used purified C3c fragments or zymosan-activated mouse serum instead of purified C3 in the detection assays, we saw noteworthy differences among the hybridomas. All mAbs except clone 3/11 recognized C3c (Fig. 1A and B). This finding prompted us to test the reactivity of clone 3/11 with the C3a anaphylatoxin, and, as demonstrated in Fig. 1C, clone 3/11 was indeed positive for binding to C3a (Table 1). Adsorption of C3 to a plastic surface can result in conformational modifications that expose the mAb site(s) of the molecule (Andersson et al., 2002). As a result, we were unable to distinguish between reactivity with the native and fragments of C3. Two separate approaches were employed in order to overcome this problem. First, a sandwich ELISA was designed with the mAbs captured by anti-rat IgG-Fc antibodies. The mAbs bound various C3 forms present in non-activated or zymosan-activated serum. Subsequently, a polyclonal

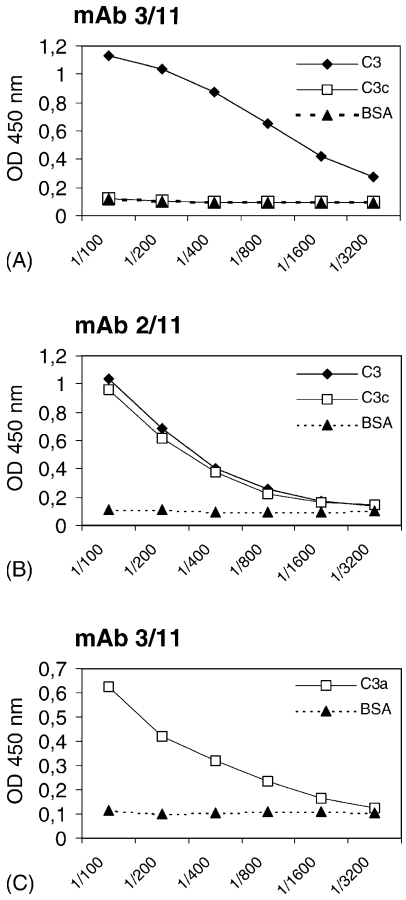

Fig. 1. mAb 3/11 specifically recognizes mouse C3a. Plates were coated with C3, C3c, and BSA (A and B) or C3a and BSA (C), and plate-bound proteins were detected with mAbs 3/11 (A and C) or 3/26 (B). Note that 2/11 is representative of all other mAbs except 3/11.

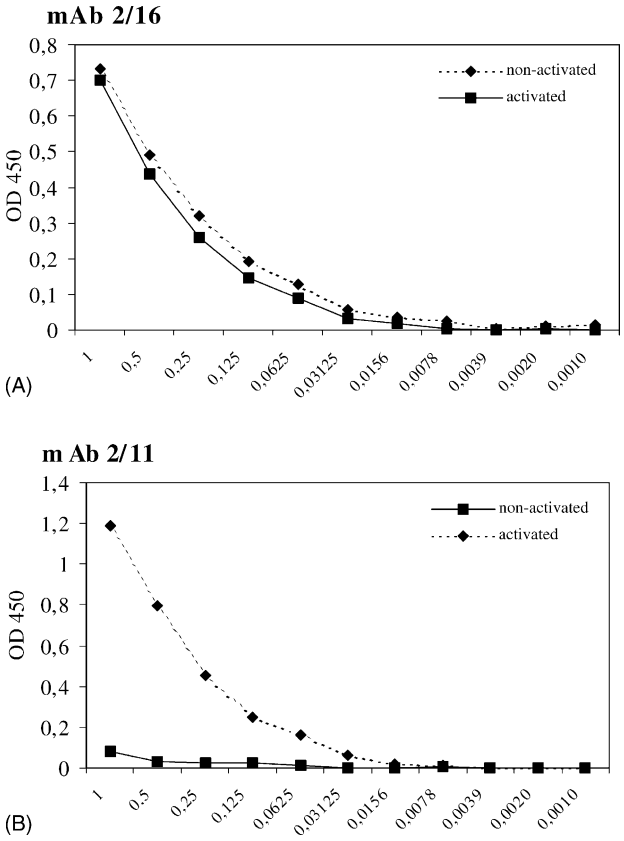

Fig. 2. mAb 2/11 preferentially recognizes activated C3 fragments. mAb 2/16 (A) or 2/11 (B) was bound to the plate by anti-rat IgG Fc antibody, then zymosan-activated or non-activated plasma was added. Captured C3 and C3 fragments were detected with HRP-conjugated goat anti-mouse C3 IgG.

antibody raised against mouse C3 was applied to detect all forms of C3 generated in the serum bound by the mAbs. Antibody 2/11 preferentially reacted with activated C3 fragments as shown in Fig. 2A. In the second approach, serum was pretreated with the monocarboxylic acid K76COOH to prevent cleavage of C3b to iC3b during zymosan-mediated activation of serum. Two of the antibodies, 2/11 and 3/26, showed significant reactivity with K76COOH-treated sera (Fig. 3).

Table 1
Summary of mAb reactivities

Clone (isotype)	Fragment specificity[a]	K76 COOH test[b]	N/A C3[c]	SB-C3b/iC3b[d]	Inhibition of hemolysis[e]	WB-NR	WB-R
2/1 (IgG1)	C3/iC3b/C3c	−	N + A	+	n.d.	+	+
2/11 (IgG1)	C3b/iC3b/C3c	+	A	+	Yes	+	−
2/16 (IgG1)	C3/iC3b/C3c	−	N + A	+	No	+	+
2/19 (IgG1)	C3/iC3b/C3c	−	N + A	+	No	+	+
2/20 (IgG1)	C3/iC3b/C3c	−	N + A	+	n.d.	+	+
2/26 (IgG1)	C3/iC3b/C3c	−	N + A	+	No	+	+
3/11 (IgG2a)	C3a	−	−	−	n.d.	+	−
3/26 (IgG2a)	C3b/iC3b/C3c	+	−	+	Yes	+	−

[a] Summary of all tests by ELISA using purified C3c and synthetic C3a.
[b] By ELISA after pretreatment of zymosan-activated serum with K76COOH inhibitor.
[c] Antibody against rat Fc was used to capture anti-C3 mAbs. After adding non-treated or zymosan-activated mouse serum, the C3-fragments were detected with an HRP-conjugated goat anti-mouse C3. N, native; A, activated.
[d] Tested by indirect cytofluorimetry using B cells incubated with fresh mouse serum (1:10 dilution). Binding of various mAbs to cell-bound C3 was visualized using FITC-labeled anti-rat Ig.
[e] Hemolytic assay using rabbit erythrocytes sensitized with antibodies generated in guinea pig.

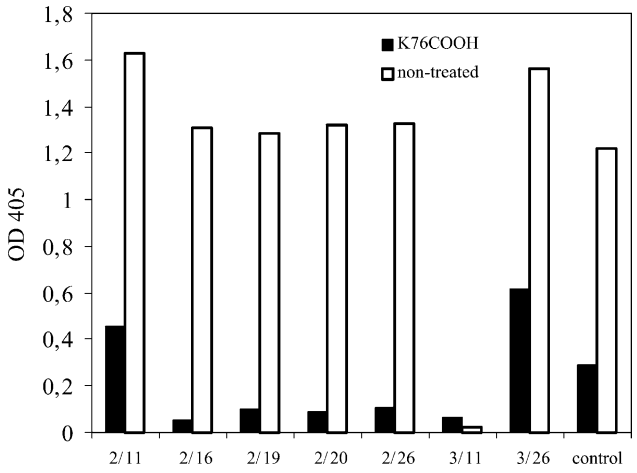

Fig. 3. Reactivity of mAbs with C3 fragments in K76COOH treated and non-treated sera. The goat anti-mouse C3 polyclonal antibody was used as positive control. Two mAbs—2/11 and 3/26—showed stronger reactivity with K76COOH-treated sera than the others.

3.2. Mapping of mAb recognized epitopes to mouse C3 chain

All eight mAbs that were found to recognize C3 by ELISA were also able to detect the entire C3 molecule under non-reducing conditions (Fig. 4). Some of the mAbs also recognized the β-chain of purified C3 under reducing conditions in Western blotting experiments (Fig. 4 and Table 1).

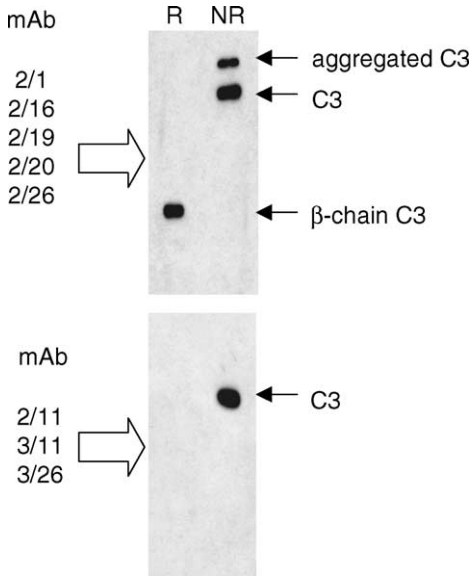

Fig. 4. Reactivity of monoclonal mAbs in Western blotting with purified C3 run under non-reducing (NR) or reducing (R) conditions.

3.3. Reactivity of mAbs with C3 fragments deposited on cells

The deposition of C3 fragments on the surface of B cells is the consequence of incubation of those cells with autologous serum and subsequent activation of the complement system via the alternative pathway (Kerekes et al.,

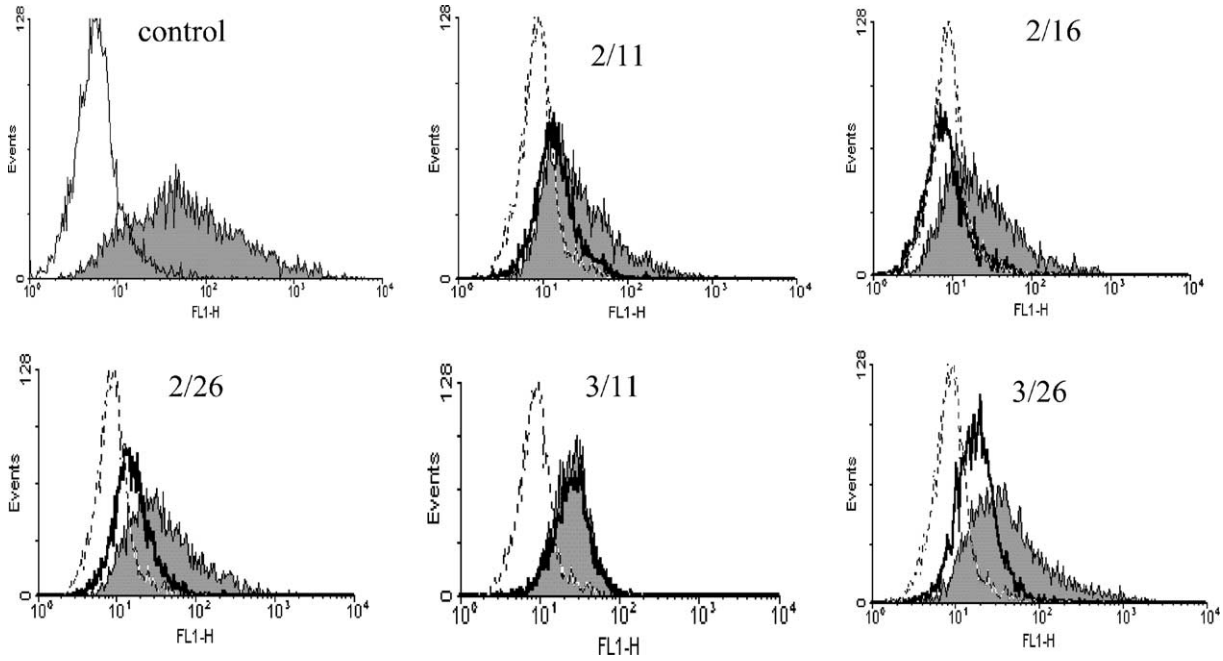

Fig. 5. Flow cytometric analysis of selected mAb binding to C3 fragments fixed on B cells. Splenocytes were incubated in autologous fresh mouse serum (shaded histogram) or EDTA-complemented serum (thick lines) to allow deposition of C3 on the cells, then the indicated monoclonal antibodies and FITC-conjugated goat anti-rat IgG were used to detect bound C3 fragments. Thin or dotted lines indicate fluorescence of unstained cells. Live B cells were gated on the basis of light scatter characteristics and Ig kappa chain positivity. Fluorescein conjugated polyclonal goat anti-mouse C3 antibody was used as a control.

1998). This model was applied in our experimental setting. Mouse splenocytes preincubated with serum from the same animal were stained with mAbs for the presence of C3 fragments. Kappa light chain of immunoglobulin positive cells (B cell lineage) were gated and analyzed for mAb binding. All mAbs except 3/11 showed positive reactivity in flow cytometry analysis. The most prominent increase in the fluorescence level was observed with the antibody 3/26 (Fig. 5). As a positive control for the detection of cell surface-bound C3, a polyclonal antibody (fluorescein

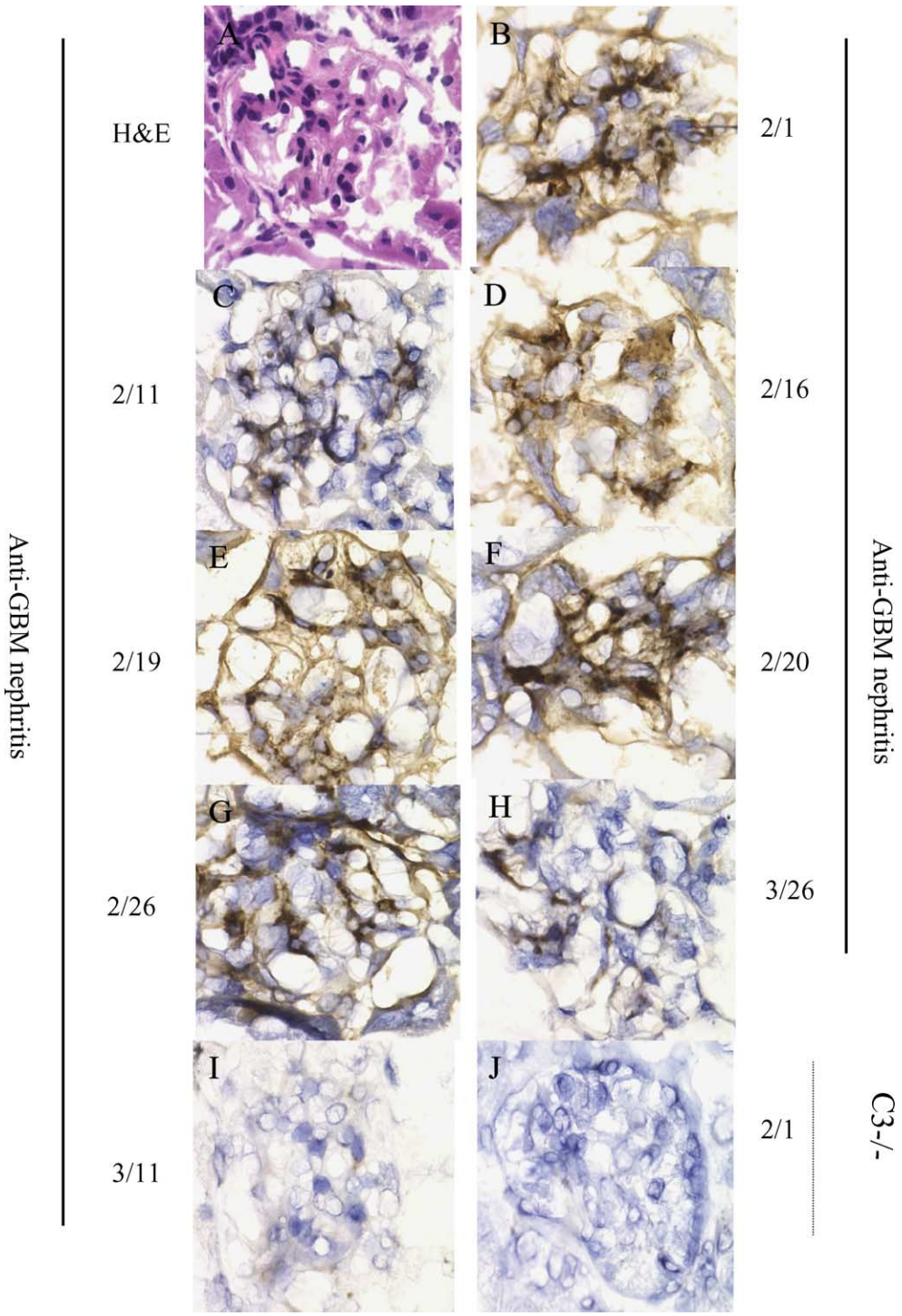

Fig. 6. Reactivity of anti-C3 mAbs with C3 deposited on glomerular basement membrane: (A) H and E staining and (B–I) immunohistochemistry of kidney sections presenting morphological features of GBM nephritis. Images B–I show immunohistochemical staining of the kidney glomeruli with mAbs 2/1, 2/11, 2/16, 2/19, 2/20, 2/26, 3/26, 3/11, respectively. (J) Histological section from a C3-deficient mouse stained with mAb 2/1 (magnification of all shown images, 600×).

conjugated goat F(ab′)$_2$ anti-mouse C3, Cappel, PA) was used.

3.4. Use of the mAbs for immunohistochemical detection of mouse C3

Histological sections from kidneys of mice treated with anti-glomerular basement antibody revealed morphological features of experimental anti-glomerular basement (anti-GBM) nephritis: segmental capillary lumen obliteration, an increase in segmental glomerular matrix and diffuse glomerular capillary wall thickening, which reflects the deposition of immune complexes and complement (Fig. 6A). All antibodies, except 3/11 (Fig. 6I), showed positive immunoreactivity, indicating C3b/iC3b deposition on the glomerular capillary walls (Fig. 6B–H). The diffuse linear pattern of C3 staining closely resembled that of immunofluorescence typical for anti-GBM nephritis. None of the mAbs reacted with kidney sections from C3-deficient mice. A representative example of this negative control is shown in Fig. 6J.

3.5. Effect of the mAbs on the hemolytic activity of mouse serum

The studies described above indicated that the mAbs could react with both native and degraded mouse C3. It is reasonable to hypothesize that they also might inhibit the formation of the C3 and C5 convertases. To verify this hypothesis, we assessed the ability of the mAbs to block complement activation and subsequent cell lysis. For this purpose we developed a sensitive hemolytic assay that allowed the detection of red blood cell lysis in the presence of culture medium. The amount of mouse serum required to achieve 70–100% hemolysis was initially determined in preliminary experiments. Culture supernatants containing the mAbs were mixed with freshly drawn mouse serum, and then added to the sensitized erythrocytes. Two of the mAbs, 2/11 and 3/26,

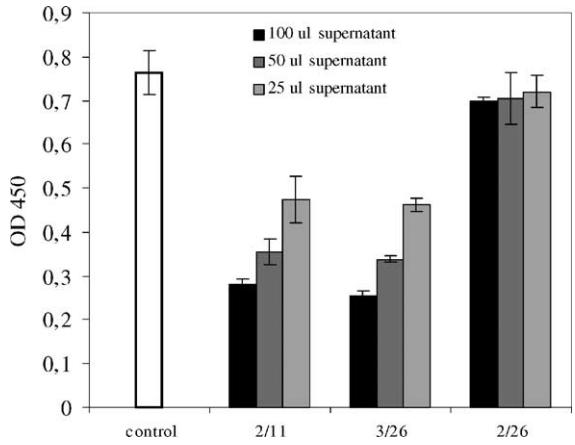

Fig. 7. Interference of mAbs 2/11, 3/26 and 2/26 with hemolytic activity of mouse serum. Fresh mouse serum was mixed with supernatants of the indicated hybridomas or with buffer as control before addition to sensitized rabbit erythrocytes. Hemolysis was measured on the basis of the peroxidase activity of the released hemoglobin using tetra-methyl-benzidine as substrate. Results are representative of at least three experiments, columns indicate mean optical densities, and error bars indicate S.D.

consistently inhibited hemolysis in a dose-dependent manner, while clone 2/26 had no effect in this system (Fig. 7).

3.6. mAb 3/26 enhances the deposition of C3 on B cells

We hypothesized that binding of the antibodies to C3 or its fragments could modify the extent of complement deposition on B cells. To test such an effect we incubated splenocytes with fresh mouse serum in the presence or absence of mAbs, and deposited C3 was detected by flow cytometry. As demonstrated in Fig. 8, mAb 3/26 facilitated C3 deposition onto B cells resulting in high C3 levels on the total B-cell population. The isotype-matched control mAb, 3/11 had no such effect. It has to be emphasized that in all experiments where mouse serum was the source of C3, sera drawn immediately before the experiment proved to be considerable more efficient than stored serum.

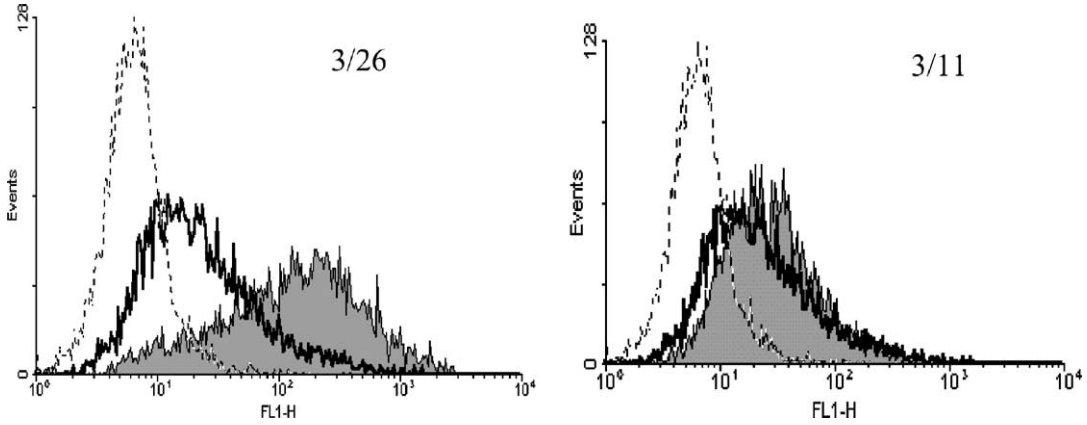

Fig. 8. Enhancement of C3 deposition on autologous B cells by mAb 3/26. Splenocytes were incubated in medium (thin line) or in fresh mouse serum without (thick line) or with 3/26 mAb (shaded histogram) for 1 h at 37 °C. As control, an isotype-matched mAb (clone 3/11) was used. Deposited C3 was stained with FITC conjugated goat anti-mouse C3. Live B cells were gated on the basis of light scatter and Ig kappa chain positivity.

4. Discussion

C3 and its activation products have been implicated in the pathophysiology of several diseases. In this respect, the advent of transgenic technology has enabled the investigation of C3-mediated functions in various pathophysiological settings, through the establishment of appropriate mouse models of disease. However, this field of complement research has long suffered from the lack of systematic reagents that can target mouse complement components and, in particular, from the lack of selective antibodies that can recognize C3 and its various bioactive degradation fragments. Despite the fact that previous studies have begun to address this problem by reporting the generation of rat monoclonal antibodies against mouse components C3 and C4 (Kremmer et al., 1990), this past effort has not yielded any antibodies that could differentially recognize fragments of C3 generated upon complement activation, or any antibodies that could effectively inhibit complement activation at various proteolytic steps of the cascade.

In the present study we report the generation and characterization of a panel of novel monoclonal antibodies that specifically recognize murine C3 as well as several of its degradation products. We have also demonstrated that these antibodies can be employed in various immunological assays to detect mouse C3 in both the fluid (serum) and solid phase (e.g., deposited on cells and tissues). Having developed a sensitive ELISA method for the detection of native (non-activated) versus activated C3, we were able to select those mAbs that preferentially reacted with the activation products of C3. Using a selective inhibitor of iC3b formation, we then showed that two of these mAbs, clones 2/11 and 3/26, selectively bind to C3b fragment of C3. The use of these mAbs will allow for the quantitation of complement activation levels in mouse fluids (e.g., serum) and provide a unique tool for the detection of C3 activation in mouse models of disease.

Our studies have also led to the identification of a single mAb (clone 3/11) that selectively binds the C3a anaphylatoxin. This antibody may prove to be a unique resource for developing sensitive ELISA schemes to quantitate complement activation in mice. It is generally accepted that ELISA assays detecting C3a generation constitute a particularly reliable and accurate means of measuring fluid-phase complement activation in various pathological conditions in humans. The identification of clone 3/11 may now provide a similar tool for monitoring acute complement activation (C3a generation) in experimental mouse models.

Studies using flow cytometric analysis and immunohistochemical staining revealed that all the mAbs, except clone 3/11, could detect C3b/iC3b fragments deposited on cells and tissues. These antibodies can serve as useful reagents for monitoring both acute and chronic complement activation as well as for detecting C3 deposition in disease mouse models.

In addition, our in vitro studies using a sensitive hemolytic assay demonstrated that mAbs 2/11 and 3/26 can also inhibit complement-mediated lysis. Interestingly clone 3/26 effectively enhanced C3 deposition on murine B cells, as well. This finding can be explained by the following main differences between the two experiments: on one hand hemolysis is initiated by the classical pathway, whereas deposition on B cells in this setting is alternative pathway mediated, on the other hand cells of different species are used in the assays. Since complement regulatory proteins function in a species specific manner, the quality and outcome of complement activation are different in the two experiments. Still, the apparently contradictory results can be resolved theoretically even for an identical system as follows. A possible mechanism of enhancement is that after recognizing cell-bound C3 (Fig. 5) 3/26 itself activates complement. This is in agreement with the finding that C3 deposition on T cells was not affected by 3/26 (K. Papp, unpublished observations). Enhancement of complement C3b(i) deposition by a monoclonal antibody against human C3b(i) has already been reported (Kennedy et al., 2003). Lysis of cells by complement requires the cleavage of C5 in order to generate the membrane attack complex. Since C3b is a constituent of the C5 convertase complex, 3/26 may bind to and inactivate these complexes, explaining its inhibitory effect in hemolysis.

The efficacy of these antibodies in interfering with complement activation in vitro will allow for the rational design of inhibition experiments targeting C3 and C3-derived fragments (C3a, C3b/iC3b) in vivo. Such studies will help clarify the mechanisms by which C3 and its bioactive fragments mediate their functions in various pathophysiological conditions. Experiments assessing the ability of these mAbs to interfere with complement activation in vivo are currently in progress in our laboratory.

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