Brief Definitive Report

In Vivo Inhibition of the Antibody Response by a Complement Receptor-Specific Monoclonal Antibody

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Summary

BALB/c mice were injected intravenously with three different monoclonal antibodies (mAbs) specific for complement receptor 1 (CR1). Two of the mAbs crossreacted with CR2. 24 h later, the mice were immunized with horse erythrocytes or keyhole limpet hemocyanin (KLH), and the primary antibody response was measured. One of the anti-CR antibodies, 7G6, suppressed >99% of the direct plaque-forming cell response against horse red blood cells (HRBC). The same antibody markedly suppressed the serum antibody responses to both HRBC and KLH. To be optimally suppressive, the mAb had to be injected before suboptimal concentrations of antigen. The other two complement receptor-specific antibodies had very moderate, if any, effects on the antibody response. 7G6 was able to downregulate CR1 and CR2 on the surface of B cells and, in addition, to inhibit rosette formation with C3d-coated sheep erythrocytes (EC3d). One of the antibodies with a weak effect downregulated only CR1. The other downregulated both CR1 and CR2, although not as efficiently as 7G6, and was unable to inhibit EC3d rosette formation. We conclude that the reason 7G6 is outstanding in its suppressive capacity is that it is the only mAb tested that functionally blocks CR2. The data suggest that CR2 is of crucial importance in the initiation of a normal antibody response to physiological concentrations of antigen.

The importance of the complement system for the ability of animals to mount a normal antibody response has been demonstrated in a number of different ways (reviewed in references 1 and 2). Treatment of animals with cobra venom factor (CVF), which leads to complement depletion by activating the alternative pathway, suppresses the antibody response to both thymus-dependent (3–5) and thymus-independent antigens (6). Hereditary deficiencies in complement factors C1–C4, described in man, guinea pigs, and dogs, generally lead to a low antibody response and poor induction of immunological memory (7–9). The enhancing effect of specific Ig on the antibody response is abrogated when mice are treated with CVF before immunization, or when mutant, non-complement-activating IgM is used (10, 11). Moreover, several in vitro studies have demonstrated that complement receptor type 2 (CR2) is involved in B cell proliferation and differentiation (12–15).

We have produced mAbs, specific for murine complement receptors, that enabled us to design an in vivo experimental system where CR1, or both CR1 and CR2, are functionally blocked. This approach does not have the disadvantages of in vitro systems and avoids the massive complement activation in CVF-treated mice. Our results demonstrate that blocking of CR2 suppressed >99% of the primary in vivo antibody response in mice to thymus-dependent antigens.

Materials and Methods

Mice. Female BALB/c mice, aged 8–16 wk, from Bomholdtgard, Ry, Denmark, were used throughout.

Antigens. HRBC (National Veterinary Institute, Hâtunaholm, Sweden) were stored in sterile Alsever's solution at 4°C. Before use, erythrocytes were washed three times in PBS. Keyhole limpet hemocyanine (KLH) (Calbiochem-Behring Corp., La Jolla, CA) was dialyzed against PBS before use.

Antibodies. Three different rat mAbs, 8C12 (IgG2c), 7E9 (IgG2a), and 7G6 (IgG2b), with specificity for murine complement receptors were used (16, 17). The antibodies were purified from ascites fluids of nude mice by precipitation in ammonium sulfate at 40% saturation followed by ion exchange chromatography on carboxymethyl-cellulose for 8C12 and DEAE-cellulose for 7E9 and 7G6. After dialysis against PBS, antibodies were sterile filtered and stored at −20°C.

Immunization. Anti-CR antibodies (200 μg/mouse) and antigen in 0.1 ml PBS were injected in the tail vein as described in the legends of Table 1 and Figure 1.

Plaque-forming Cell (PFC) Assay. A modified version of the Jerne direct haemolytic plaque assay was used (18).

ELISA. HRBC-specific IgG antibodies were assayed in an ELISA, using alkaline phosphatase–conjugated protein A (Pharmacia Fine Chemicals, Uppsala, Sweden) to detect bound antibody, as described elsewhere (19). KLH-specific IgG was assayed in a similar manner, but by using 50 μg/ml of KLH as a coating agent and detecting bound antibodies with alkaline phosphatase–conjugated

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goat anti-mouse IgG (H+L) (BioRad Laboratories, Richmond, CA). The data are expressed as absorbance values at 405 nm.

Results and Discussion

The anti-CR-antibodies 7G6, 7E9, and 8C12 are specific for different epitopes on CR1. 7G6 and 7E9 crossreact extensively with CR2, whereas 8C12 is monospecific for CR1 (16, 17). 7G6 inhibits EC3d rosette formation and 8C12 inhibits EC3b rosette formation, whereas 7E9 is unable to inhibit rosette formation. Upon intravenous injection of 200 μg of the antibodies in mice, 7G6 and 7E9 downregulate the expression of CR1 and CR2 on murine B cells for 7 d, whereas injection of 8C12 leads to downregulation of CR1 alone (17).

To investigate the effect of CR on the antibody response, groups of mice were injected intravenously with 200 μg of individual CR-specific mAb, followed after 24 h by an intravenous injection of HRBC. The results of representative experiments are shown in Table 1. 7G6 is able to inhibit 99.6–99.7% of the direct PFC response (Exp. 1–3). The serum IgG antibody levels are concomitantly suppressed. The nearly complete inhibition of the antibody response required that 7G6 was administered before the antigen. When 7G6 was injected 24 h after antigen, only 86% of the PFC response was suppressed (Exp. 2, last group). Although this suppression is highly significant, the number of PFC is 42 times higher than when 7G6 was injected 24 h before antigen in the same experiment (8,370:200). Another requirement for complete suppression was that suboptimal doses of HRBC ($3–4 \times 10^5$) were used. 7G6 injected before a high dose (1.8×10^8) of HRBC caused only a slight reduction of the PFC response and no reduction in serum IgG levels (Exp. 5).

The other CR-specific antibodies, 8C12 and 7E9, were compared with 7G6 for ability to induce immunosuppression. All antibodies were injected in the concentrations shown to downregulate CR during at least 5 d (17). In an experiment where 7G6 reduced the direct PFC response to 0.3% of the control, 7E9 suppressed to 72% and 8C12 to 47% of the control (Table 1, Exp. 3). Also, the suppression of the IgG response was much more pronounced in the 7G6 group than in the 8C12 and 7E9 groups (Exp. 3 and 4).

To examine the effect of anti-CR antibodies over a longer time period, and to assess whether the suppressive effect on

Table 1. *Antibody Response in Mice Treated with CR-specific Antibodies*

Exp.	Immunization*	PFC-anti-HRBC‡	Geometric mean§	Percent of c‖	p vs. c¶	IgG-anti-HRBC**	p vs. c¶
1	3×10^5 HRBC	4.43 (0.42)‡‡	26,873	100		0.34 (0.17)‡‡	
	7G6 + 3×10^5 HRBC	2.03 (0.41)	107	0.4	.001	0.06 (0.03)	0.025
2	4×10^5 HRBC	4.77 (0.09)	59,387	100		0.57 (0.08)	
	7G6 + 4×10^5 HRBC	2.30 (0.85)	200	0.3	.001	0 (0)	0.001
	7G6§§ + 4×10^5 HRBC	3.92 (0.15)	8,370	14	.001	0.15 (0.13)	0.005
3	4×10^5 HRBC	4.23 (0.07)	16,888	100		0.73 (0.11)	
	7G6 + 4×10^5 HRBC	1.70 (0.30)	50	0.3	.001	0.03 (0.02)	0.001
	8C12 + 4×10^5 HRBC	3.90 (0.39)	7,866	47	NS‖‖	0.32 (0.20)	0.005
	7E9 + 4×10^5 HRBC	4.08 (0.10)	12,121	72	.05	0.49 (0.13)	0.025
4	4×10^5 HRBC	ND				1.41 (0.05)	
	7G6 + 4×10^5 HRBC	ND				0.37 (0.29)	0.001
	8C12 + 4×10^5 HRBC	ND				0.94 (0.12)	0.001
	7E9 + 4×10^5 HRBC	ND				1.21 (0.18)	NS
5	1.8×10^8 HRBC	4.91 (0.05)	81,549	100		0.82 (0.12)	
	7G6 + 1.8×10^8 HRBC	4.56 (0.10)	36,411	45	.001	0.91 (0.14)	NS

* BALB/c mice (three to six per group) were immunized intravenously with the indicated amounts of HRBC. To some mice in each experiment, 200 μg of anti-CR antibody was injected intravenously 24 h previously (except in Exp. 3, last group that received 7G6 24 h after antigen).

‡ 5 d after antigen immunization, direct PFC/spleen were assayed and expressed as $\log_{10}$/spleen (SD).

§ Geometrical mean (antilog of the $\log_{10}$ value).

‖ Percent of control value (the group immunized with antigen alone).

¶ p value compared with the control group, as determined by student's t test.

** Absorbance at 405 nm (SD) of sera tested in ELISA.

‡‡ Numbers in parentheses are SD.

§§ In this group, 7G6 was injected 24 h after HRBC.

‖‖ $p > 0.05$.

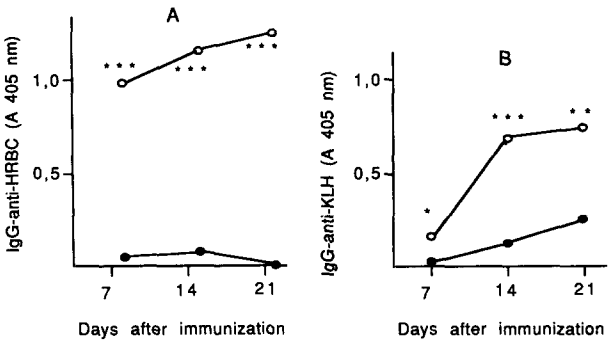

Figure 1. Groups of four to five mice were immunized intravenously with 4 × 10^5 HRBC (*A*) or 10 μg KLH (*B*) in PBS 24 h after the intravenous administration of 200 μg 7G6 (●). Control groups received antigen without prior 7G6 (○). Sera were tested in ELISA. (***) $p < 0.001$; (**) $p < 0.01$; (*) $p < 0.05$.

the antibody response affected also soluble protein antigens, mice were bled 7, 14, and 21 d after immunization with 7G6 and HRBC or KLH (Fig. 1, *A* and *B*). The IgG antibody levels for both antigens were significantly suppressed during the whole time period.

Thus, of the three anti-CR antibodies tested, 7G6 had the strongest effect on the anti-HRBC response. This antibody significantly suppressed the IgM and IgG responses to suboptimal concentrations of two different thymus-dependent antigens. We suggest that the reason 7G6 is outstanding in its suppressive capacity is that it is the only mAb that functionally inhibits CR2, as demonstrated by its ability to downregulate the surface expression of CR1 and CR2, and, in addition, to inhibit EC3d rosetting. The rosette-inhibiting capacity is probably important in blocking complement receptors still remaining on the cell surface, and explains why 7E9 (which can only downregulate the surface expression, but not inhibit rosetting) is not suppressive. The reason 8C12 is not suppressive is probably that it does not downregulate CR2 but is monospecific for CR1.

It is unlikely that the almost complete suppression of the antibody response by 7G6 is due to toxic effects on cells involved in the generation of an antibody response. First, it was shown that the number of spleen cells with IgM, Ia, and B220 on the surface was unchanged in 7G6-treated mice (17). Second, the response to an optimal dose of antigen was close to normal (Table 1, Exp. 5) and, third, LPS-induced proliferation was not inhibited by the mAbs (not shown). Negative effects mediated via crosslinking of the Fc and complement receptor by 7G6 were not responsible for the immunosuppression, as these should also have been observed with 7E9, and were not. The possibility that the CR-specific antibodies crossreacted with Ig, and thereby influenced the antibody response, was excluded by experiments that showed that surface IgM expression was intact in treated mice (17).

Our data confirm earlier studies showing the importance of C3 in antibody responses (1–15), and, in addition, demonstrate, in an in vivo system, that signals via CR2 are of crucial importance. Carter et al. (12) has shown that crosslinking of surface Ig and CR2, but not CR1, led to increased Ca^{2+} influx in human B cells in vitro. The data presented here suggest that crosslinking of CR2 and Ig also have dramatic effects in vivo. This crosslinking could take place in several ways, the simplest being that surface Ig on a B cell, after binding to antigen, activates complement leading to ligand binding to nearby CR2. Alternatively, immune complexes containing preformed, complement-activating antibody and antigen could crosslink CR2 and Ig on B cells. Binding to surface Ig by an antigen that is able to activate complement via the alternative pathway would be a third way to crosslink Ig and CR.

Another, not mutually exclusive, model for explaining the complement dependence of the antibody response is that complement mediates uptake of antigen by follicular dendritic cells that also carry CR2. This model has received experimental support by data showing that localization of antigen and immune complexes in the spleen is abolished after treatment of mice with CVF (10, 20). However, since localization of erythrocytes in the spleen is known to take place within hours (21, 22), this mechanism cannot fully explain our findings. When 7G6 was injected 24 h after HRBC, 86% of the PFC response was still suppressed (Table 1, Exp. 3). Further experimentation is required to distinguish between direct effects of complement on B cells and its effects on antigen localization.

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