ROLE OF COMPLEMENT IN INDUCTION OF ANTIBODY PRODUCTION IN VIVO*

EFFECT OF COBRA FACTOR AND OTHER C3-REACTIVE AGENTS ON THYMUS-DEPENDENT AND THYMUS-INDEPENDENT ANTIBODY RESPONSES

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There has been considerable recent progress in understanding of the mechanisms involved in cooperation between T and B lymphocytes in the induction of humoral antibody formation (see for example 1), but much still remains to be learned. The discovery of an interaction between lymphocytes and the fixed C3 component of complement (2–5), and the demonstration by Nussenzweig et al. that C3 receptors are a feature of B-cell populations (6–10), raise the possibility that C3 might play a part in this process (11). Germinal centers of lymphoid tissue, in which the induction of antibody production may occur, contain B cells, fixed C3, and complexes capable of fixing C3 (12). I have previously shown that treatment of mice in vivo with the C3-cleaving protein of cobra venom (CoF)[1] suppresses thymus-dependent but not thymus-independent antibody production, suggesting a possible role for C3 in lymphocyte cooperation (11). Analogous results have been obtained in vitro (13). These results are extended in the present paper.

Materials and Methods

Animals.—Balb/c mice, bred in the Department of Pathology, University of Cambridge, 7–12 wk of age and weighing 18–28 g were used throughout.

Antisera.—Antimouse C3 serum was raised in a Clun Forest sheep by a modification of the method of Mardiney and Müller-Eberhard (14, 15). It was monospecific for C3. Anti-CoF sera were raised in Balb/c mice by injection of purified CoF emulsified in complete Freund's adjuvant, followed by booster doses of CoF in incomplete adjuvant. The sera were inactivated

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[1] *Abbreviations used in this paper:* CoF, C3-cleaving protein of cobra venom; HGG, human IgG; OA, ovalbumin; PBS, phosphate-buffered 0.15 M saline pH 7.2; PBS/BSA, PBS containing 0.2% bovine serum albumin; PVP, polyvinylpyrrolidone; PVP 360 and PVP 10, PVP of average mol wt of 360,000 and 10,000, respectively; RFC, rosette-forming cells; SSSIII, type III pneumococcal polysaccharide.

(56°C, 30 min), absorbed with SRBC, and then concentrated 10-fold by salt precipitation before use in vivo.

Measurement of Serum C3.—C3 was measured by electroimmunodiffusion (16). Conversion of C3 was assayed by antigen-antibody-crossed electrophoresis using anti-C3 in the second stage gel (17).

Isolation of CoF.—CoF was purified from *Naja naja* venom (Sigma Chemical Co., St. Louis, Mo.) by the method of Ballow and Cochrane (18). Average yield from three different batches of crude venom was 6.75 mg of purified CoF/g of venom. In each case the CoF had an anticomplementary activity of 1 U/4.0-5.0 μg protein, was homogenous on analytical poly-acrylamide gel electrophoresis at an applied sample concentration of 250 μg/ml, and gave a single line on gel diffusion testing with antisera raised against it in mice and rabbits.

Other C3-Reactive Agents.—Yeast cell walls were prepared from baker's yeast as described by Lachmann et al. (19). Human IgG (HGG) was isolated from normal serum by salt precipitation and exclusion from DEAE-cellulose in 0.01 M phosphate buffer pH 8.0. It was aggregated by incubating a solution in phosphate-buffered saline (PBS) of 4.0 mg/ml for 10 min at 63°C. IgG1 was isolated from monospecific sheep antimouse C3 serum by salt precipitation and chromatography on DEAE-cellulose in 0.01 M Tris-phosphate buffer pH 8.0. Purified type III pneumococcal polysaccharide (SSSIII) was obtained from Welcome Reagents Ltd., Beckenham, Kent, England.

Antigens.—SRBC from the same sheep were used throughout. Blood was drawn weekly into acidified citrate dextrose, stored at 4°C, and washed three times with PBS before use. Ovalbumin (OA) (twice crystallized) (Koch-Light Laboratories Ltd., Colnbrook, England) was absorbed onto nascent alumina at pH 6.5. HGG solution (Batch C-717, American Cyanamide Co., Lederle Laboratories Div., Pear River, N. Y.). A saline was emulsified in Freund's incomplete adjuvant. Polyvinylpyrrolidone with an average mol wt of 360,000 (PVP 360) (Sigma Chemical Co., St. Louis, Mo.) was used in saline solution.

Serum Antibody Titration.—Antibodies were detected by direct agglutination of SRBC or passive agglutination of antigen-coated red cells. Sera were titrated in microtiter plates (Cooke Engineering Co., Alexander, Va.) in vol of 25 μl. PBS, or PBS containing 0.2% bovine serum albumin (PBS/BSA) when tanned cells were in use, was used as a diluent. 25 μl of indicator cells were added to each well and, after thorough mixing on a "Micromixer" (Taiyo Bussan Co., Tokyo, Japan), allowed to settle at room temperature in a moist chamber. All sera from each experiment were tested on the same occasion; the agglutination patterns and end-points were read "blind" by the same person throughout. The end-points in replicate titrations of the same sample did not differ from each other by more than one tube and were usually identical.

Mercaptoethanol-Resistant Antibody.—The direct method of Scott and Gershon was used (20). Filtration of sera on Sephadex G200 (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden) and antiglobulin testing of different fractions using specific antimouse μ and γ sera were undertaken to confirm that mercaptoethanol-sensitive and resistant antibody did correspond to IgM and IgG, respectively.

Preparation of Indicator Cells.—SRBC were used at 1% in PBS. OA was coated onto tanned human 0 cells by incubation of equal volumes of 2% tanned cells and 5 mg/ml OA in PBS for 30 min at room temperature. After washing and resuspension in PBS/BSA the cells were used at 2%. Tanned fresh human group O red cells were coated with PVP by the method of Andersson (21). Equal volumes of 2% tanned cells and 0.2 mg/ml polyvinylpyrrolidine with an average mol wt of 10,000 (PVP 10) (Sigma Chemical Co., St. Louis, Mo.) were incubated together at room temperature for 10 min. After washing they were used at 1%.

Immune Elimination (22).—The antibody response to HGG was evaluated by following the whole body clearance of [^{125}I]HGG as described elsewhere (23).

Antigen-Specific Rosette-Forming Cells (RFC).—RFC in the spleens of mice were detected

by a modification of the method of McConnell et al. (24), which detects only B-cell RFC especially in Balb/c mice (reference 25 and footnote 2).

Experimental Design.—The pattern of most of the experiments designed to test the possible effects of C3-reactive agents on antibody production in vivo was as follows. Balb/c mice all of the same age and sex were subdivided into control and different test groups numbering from 8 to 12 in each. After being weighed they were all bled for control serum C3 levels and test groups were given CoF or other agents at different times before or after injection of antigen on day 0; controls received PBS at the same time. A total dose of 200 U of CoF/kg/animal in four equally divided i.p. injections over 24 h was given (11, 26). In most experiments the mice were also bled again immediately before injection of antigen to obtain control serum antibody levels. After receiving antigen all the mice were bled at intervals during the period of the antibody response.

Presentation of Data.—The results of antibody titrations are presented either in tabular form or graphically, each point respresenting the arithmetic mean, with 95% confidence points, of the $-\log_2$ titers of all the animals in each group on each day.

Statistical Methods.—Data from test and control groups in each experiment were examined by analysis of variance and Bartlett's test for homogeneity of variance. Significant contrasts between groups were detected by t tests, the value of t being computed from the residual variation, and are indicated by P values.

RESULTS

Primary Anti-SRBC Response.—The primary hemagglutinating antibody response to SRBC, especially its IgG component, was significantly inhibited by pretreatment of mice with CoF, particularly when the period of maximal plasma C3 depletion produced by CoF was between days 2–4 after injection of the antigen (11) (Fig. 1). This effect was the same irrespective of the dose of CoF, between 100–500 U/kg/animal, or the route of administration, i.v. or i.p., of the SRBC (14).

Secondary Anti-SRBC Response.—The secondary response to SRBC was suppressed by treatment of primed mice with CoF before the second injection of SRBC, though in this case most of the antibody detected was IgG in both control and CoF-treated mice (Fig. 2). The secondary response in mice whose primary response had been suppressed by CoF was delayed and poorly sustained by comparison with control mice primed without CoF treatment (Fig. 3).

SRBC-RFC Response.—After i.p. injection of SRBC on day 0 there is a rapid increase in the number of specific SRBC-RFC in the spleen between days 2–4. Pretreatment of mice with CoF producing maximal systemic C3 depletion during this period suppressed the proliferation of RFC (Fig. 4). None of the CoF-treated animals immunized with 2.5×10^7 SRBC produced any IgG antibody, but the relative proportion of RFC inhibited by class-specific antimouse μ and γ sera was the same as in control mice (14).

Effect of Passive Anti-CoF Serum.—Injection of anti-CoF serum into mice depleted of C3 by CoF caused a prompt rise in plasma C3. After an optimal dose of anti-CoF, plasma C3 rose from less than 1% to 50% of normal in 15 h, 75% at 24 h, and 100% or more by 48 h (14). The effects of such a dose of anti-CoF on

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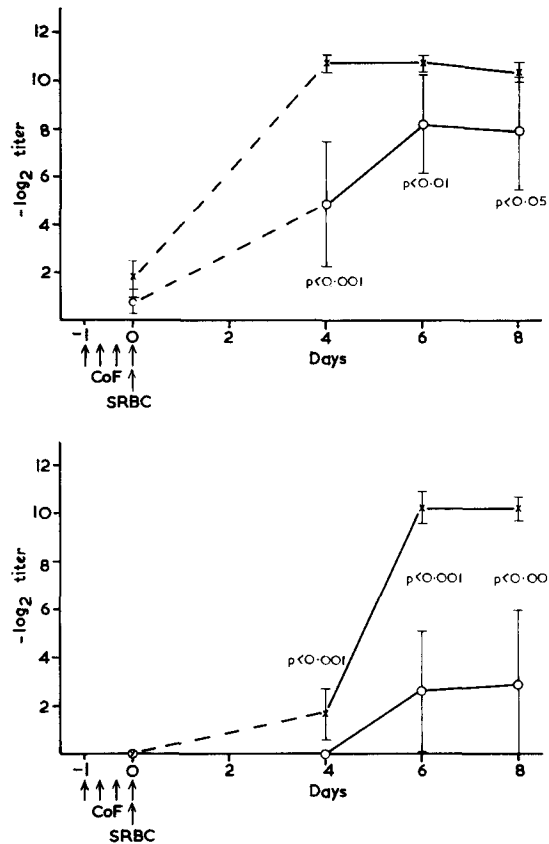
[2] M. F. Greaves, personal communication.

FIG. 1. Effect of CoF on the total and mercaptoethanol-resistant anti-SRBC response. CoF was given to the test animals so as to produce maximal depletion of circulating C3 between 2–4 days after i.p. injection of 2.5×10^8 SRBC. Above, total antibody titers; below, mercaptoethanol-resistant antibody titers. ×, controls treated with PBS; O, CoF-treated mice; ↑, i.p. injection.

the suppressive action of CoF are shown in Fig. 5. Injection of anti-CoF on either day 0 or day 2 which restored to normal, or at least appreciably raised C3 levels between days 2 and 4, permitted a normal anti-SRBC response to be mounted. When anti-CoF was given on day 4 the response was suppressed until then, but subsequently reached normal levels with a delay of 2 days. Anti-CoF enabled CoF-treated mice to produce IgG antibody. Furthermore, in the day 8 sera of mice given anti-CoF on day 4 virtually all the antibody was IgG, mean titer ± SD equals 6.8 ± 0.6, whereas in the normal control mice the IgG anti-SRBC titer was only 3.6 ± 2.6 ($P < 0.001$).

Anti-CoF serum had no effect on the anti-SRBC response in mice not treated with CoF. As a further control, normal serum which had been inactivated, absorbed, and concentrated in parallel with the anti-CoF serum had no effect on
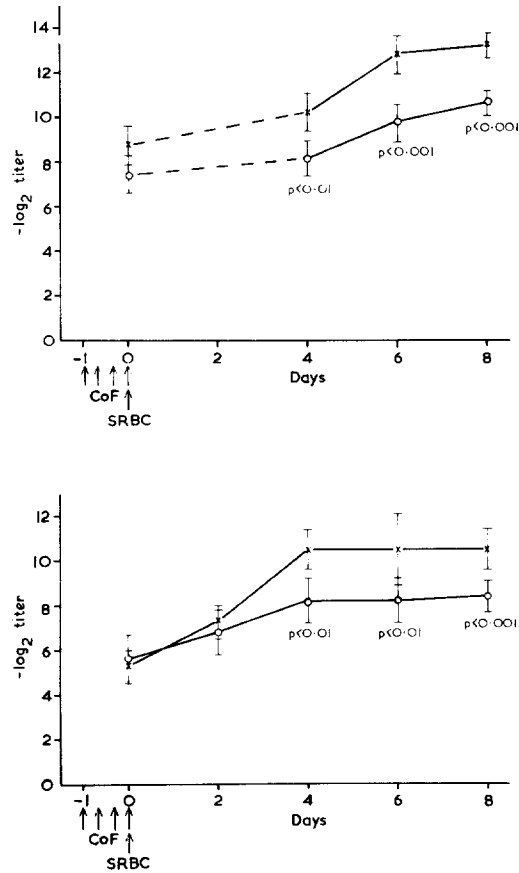
FIG. 2. Effect of CoF on the secondary anti-SRBC response. Groups of mice were primed by i.p. injection of either 2.5 × 10⁸ SRBC 6 mo previously (above) or 2.5 × 10⁷ SRBC 3 wk previously (below). CoF was given so as to produce maximal depletion of circulating C3 2–4 days after a secondary i.p. injection of 2.5 × 10⁸ SRBC. ×, controls treated with PBS; O, CoF-treated mice; and ↑, i.p. injection.

either the control anti-SRBC response or the suppression of that response by CoF.

A low dose of anti-CoF, which enabled plasma C3 levels in CoF-treated mice to rise almost as rapidly as an optimal dose, was, however, less effective in abrogating the suppressive effect of CoF on the anti-SRBC response (14). This may be due to the fact that although even trace amounts of anti-CoF antibody in vivo can clear circulating CoF and permit plasma C3 levels to rise (14), relatively high concentrations of anti-CoF antibody are required to inhibit C3 cleavage by CoF (Fig. 6).

Effect of Active Immunity to CoF.—The sera of mice actively immunized with
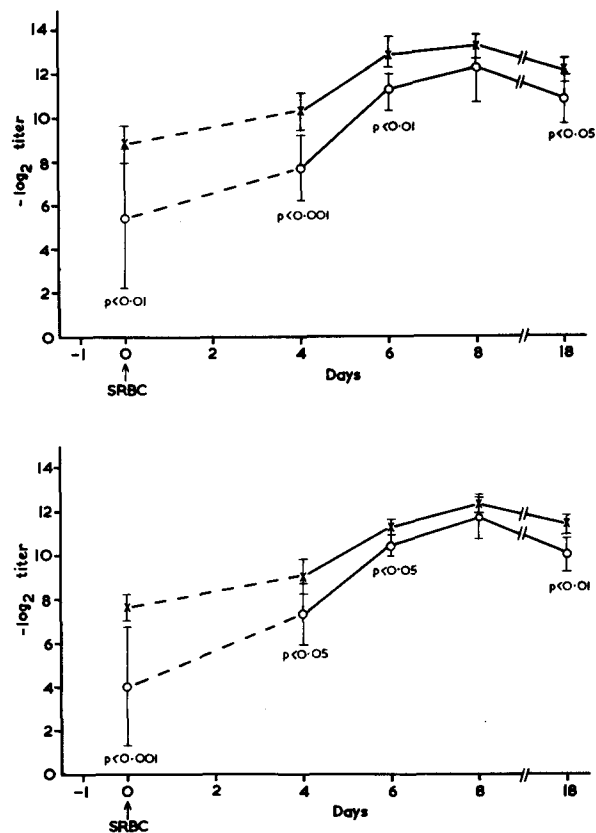
Fig. 3. Effect of CoF on induction of immunological memory. Two groups of mice were each given an i.p. injection of 2.5 × 10⁸ SRBC 6 mo after priming with 2.5 × 10⁸ SRBC. ×, control mice treated with PBS before priming; O, test mice treated with CoF so as to produce maximal depletion of circulating C3 2–4 days after priming 6 mo previously; and ↑, i.p. injection. Above, total antibody titers; below, mercaptoethanol-resistant antibody titers.

CoF in adjuvant contained high levels of anti-CoF antibodies which blocked in vitro C3 cleavage by CoF, whereas the sera of mice immunized with CoF in saline did not (Fig. 6). In neither group did CoF lower plasma C3 in vivo. The anti-SRBC response was unaffected by CoF in mice whose sera inhibited CoF in vitro, while in mice whose sera failed to inhibit it the anti-SRBC response was suppressed by CoF (Table I).

Effect of Other C3-Reactive Agents.—Zymosan (yeast cell walls), heat-aggregated HGG, the IgG1 fraction of monospecific sheep antimouse C3 serum and SSSIII all interact with C3 in various ways (27, 28). None of them produced more than a transient lowering of plasma C3 in vivo, but they all caused sig-
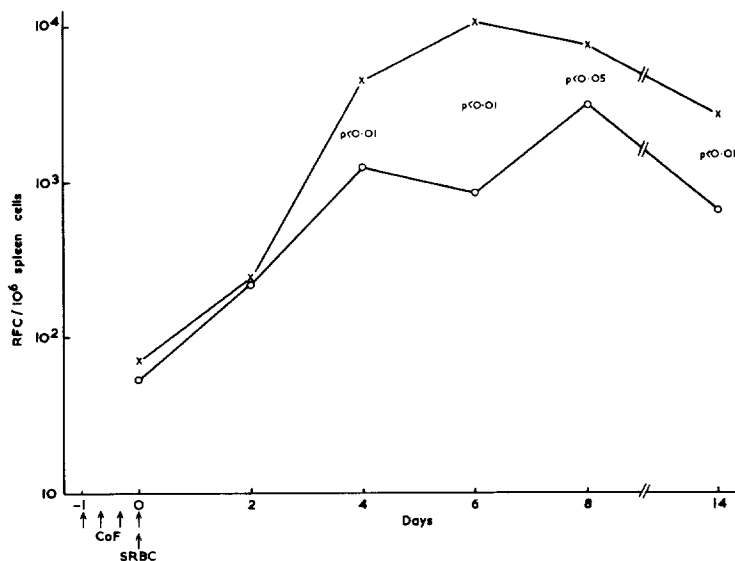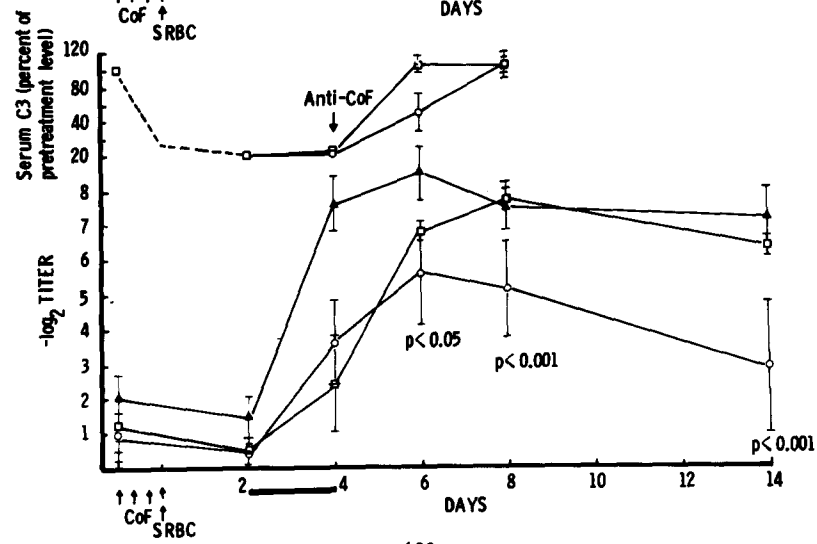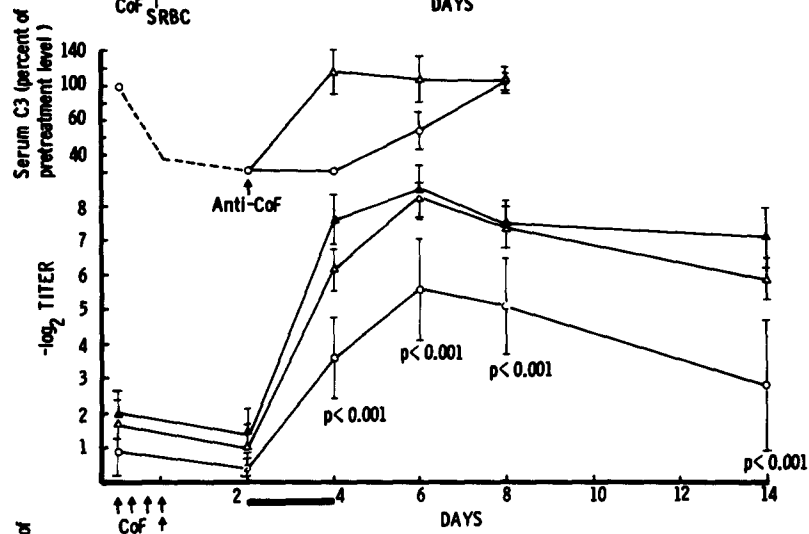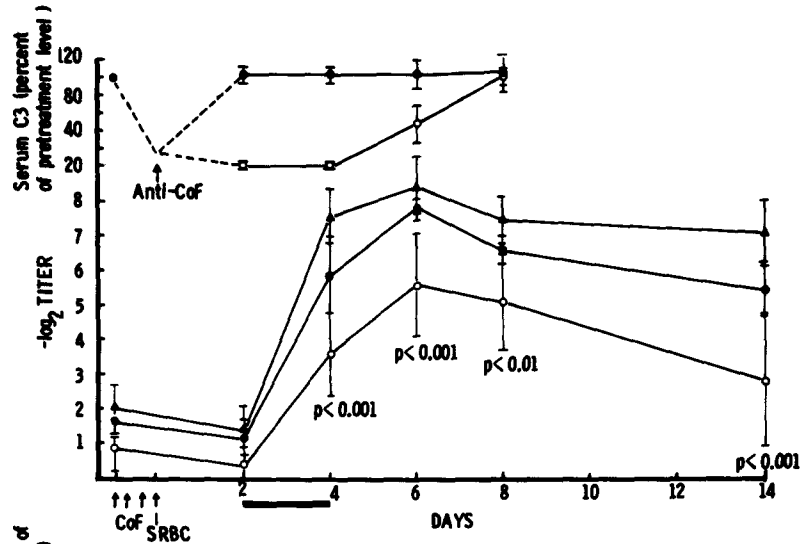
Fig. 4. Effect of CoF on the RFC response to SRBC. Mice were given an i.p. injection of 2.5 × 10⁷ SRBC on day 0. Each point represents the geometric mean of four mice on each day. ×, controls treated with PBS; and O, CoF-treated mice (confidence limits not shown).

nificant suppression of the anti-SRBC response (Table II). PVP 360 resembles SSSIII in being a high molecular weight, nonbiodegradable polymer which is itself a thymus-independent antigen (29); but unlike SSSIII it had no effect either on C3 in normal murine serum in vitro (Fig. 7) or on the anti-SRBC response in vivo (Table III).

Primary Response to Protein Antigens.—The onset of antibody production after immunization with OA was delayed for about 2 days by CoF, irrespective of whether it was given so as to maximally deplete C3 between days 0–2, 2–4, or 4–6 (Fig. 8). The immune elimination of [¹²⁵I]HGG was significantly re-

Fig. 5. Effect of passive anti-CoF serum on suppression of the anti-SRBC response by CoF. Mice were given either CoF or PBS before i.p. injection of 2.5 × 10⁷ SRBC on day 0. Different groups of CoF-treated mice were then given 100 μl of mouse anti-CoF serum (10 × concentrated, absorbed with SRBC) on day 0 (above), day 2 (middle), or day 4 (below). The effects on C3 levels (above in each section) and antibody titers (below in each section) are shown. Each point represents the mean with 95% confidence limits of either the serum C3 level or the antibody titer. ▲, controls, treated with PBS; O, CoF-treated mice; ●, CoF-treated mice given anti-CoF on day 0; △, CoF-treated mice given anti-CoF on day 2; □, CoF-treated mice given anti-CoF on day 4; ↑, i.p. injection; and ▬, time during which serum C3 was <5% of normal in mice treated with CoF but not anti-CoF. Significant differences between the groups which received CoF alone and those which received CoF and anti-CoF are indicated by *P* values.
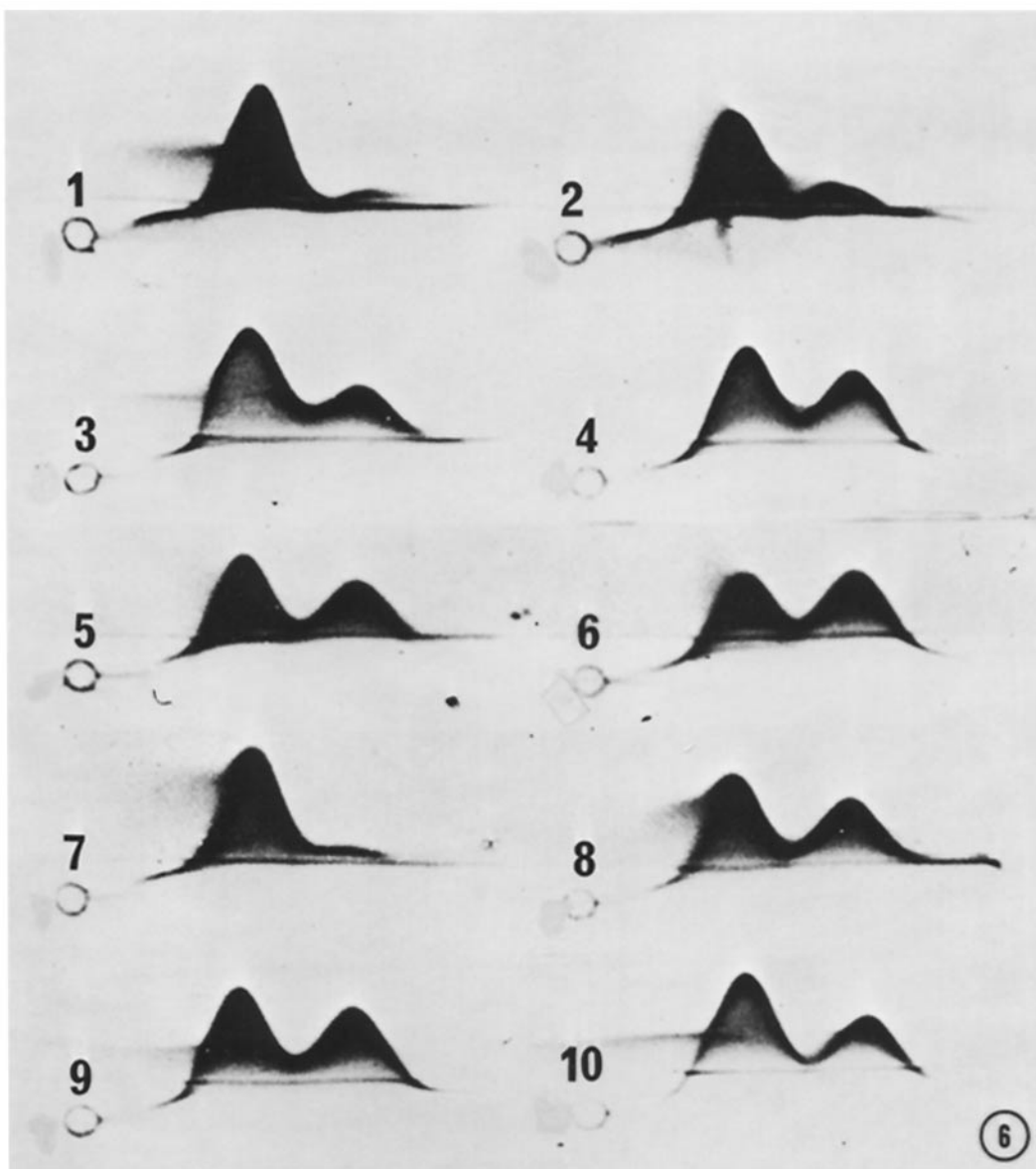
Serum C3 (percent of pretreatment level)

Anti-CoF

-log₂ TITER

p< 0.001

p< 0.001

p< 0.01

p< 0.001

CoF SRBC

DAYS

Serum C3 (percent of pretreatment level)

Anti-CoF

-log₂ TITER

p< 0.001

p< 0.001

p< 0.001

p< 0.001

CoF SRBC

DAYS

Serum C3 (percent of pretreatment level)

Anti-CoF

-log₂ TITER

p< 0.05

p< 0.001

p< 0.001

CoF SRBC

DAYS

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Fig. 6. Effect of anti-CoF antibodies on C3 cleavage by CoF in vitro. CoF (5.0 μg) or PBS were preincubated with 50 μl vol of fresh neat mouse anti-CoF sera, dilutions of these antisera in fresh normal mouse serum or normal mouse serum alone, before addition of EDTA to 0.01 M and an excess of native murine C3 (20 μl of 10 $\times$ concentrated serum euglobulins). After incubation at 37°C the C3 profile was analyzed by antigen-antibody-crossed electrophoresis. The two anti-CoF sera tested were pooled from mice immunized with either CoF in PBS ("weak" anti-CoF) or CoF in adjuvant ("strong" anti-CoF). 1–5, strong Anti-CoF; 7–10, weak anti-CoF; 1 and 7, no CoF, PBS controls; 6, no anti-CoF, normal serum control; 2 and 8, neat anti-CoF; 3 and 9, ½ anti-CoF; 4 and 10, ¼ anti-CoF; and 5, ⅛ anti-CoF.

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TABLE I

Effect of Active Immunity to CoF on Suppression of the Anti-SRBC Response by CoF

Immunization*	Pretreat-ment‡	Agglutination titer§					
		Day 4		Day 6		Day 8	
		Total	IgG	Total	IgG	Total	IgG
2 × 10 µg CoF in PBS	PBS	7.6 ± 0.5	3.6 ± 0.8	7.3 ± 0.5	6.1 ± 0.4	7.4 ± 0.5	6.1 ± 0.4
2 × 10 µg CoF in PBS	CoF	0.4 ± 0.7 $P < 0.001$	0 $P < 0.001$	5.5 ± 1.1 $P < 0.001$	1.1 ± 2.1 $P < 0.001$	4.6 ± 1.3 $P < 0.001$	1.0 ± 1.9 $P < 0.001$
20 µg CoF in FCA 20 µg CoF in FICA	CoF	7.6 ± 0.5	5.7 ± 0.8	7.6 ± 0.8	6.3 ± 1.0	8.3 ± 1.0	6.9 ± 0.7

* Groups of eight mice were each immunized by i.p. injections of CoF in the doses shown, the last injection being 4 wk before the start of this experiment.
‡ 4 i.p. injections of either 5.0 µg each of CoF or of PBS alone were given during the 24 h preceding i.p. injection of 2.5 × 10⁸ SRBC on day 0.
§ Mean $-\log_2$ titer ± SD of all the mice in each group. Significant differences from the control, PBS pretreated, group are shown by P values.

TABLE II

Effect of C3-Reactive Agents on the Anti-SRBC Response

Pretreatment*	Agglutination titer‡			
	Day 4		Day 6	
	Total	IgG	Total	IgG
PBS	7.6 ± 0.5	3.8 ± 1.4	7.9 ± 0.6	7.5 ± 0.5
CoF	1.9 ± 1.1 $P < 0.001$	0.1 ± 0.4 $P < 0.001$	3.8 ± 2.7 $P < 0.001$	1.4 ± 2.6 $P < 0.001$
Zymosan	6.2 ± 1.0 $P < 0.05$	2.2 ± 0.7 $P < 0.001$	7.1 ± 0.9	5.9 ± 2.4
Aggregated HGG	4.1 ± 1.5 $P < 0.001$	1.8 ± 1.0 $P < 0.001$	6.6 ± 0.5 $P < 0.05$	5.9 ± 2.3
Anti-C3 IgG	4.0 ± 1.4 $P < 0.001$	1.3 ± 0.9 $P < 0.001$	6.3 ± 0.7 $P < 0.05$	5.0 ± 2.6 $P < 0.05$
SSSIII	2.4 ± 1.5 $P < 0.001$	0.4 ± 0.5 $P < 0.001$	6.0 ± 1.1 $P < 0.01$	3.6 ± 3.2 $P < 0.01$

* Groups of eight mice were each given four i.p. injections during the 24 h preceding i.p. injection of 2.5 × 10⁸ SRBC on day 0. The doses of each agent were as follows: CoF, 4 × 5.0 µg; zymosan, 4 × 0.75 mg; aggregated HGG, 4 × 10 mg; anti-C3 IgG, 4 × 0.5 mg; and SSSIII, 4 × 5.0 µg.

‡ Mean $-\log_2$ agglutination titers ± SD of all the animals in each group. Significant differences from the control, PBS pretreated, group are shown by P values.
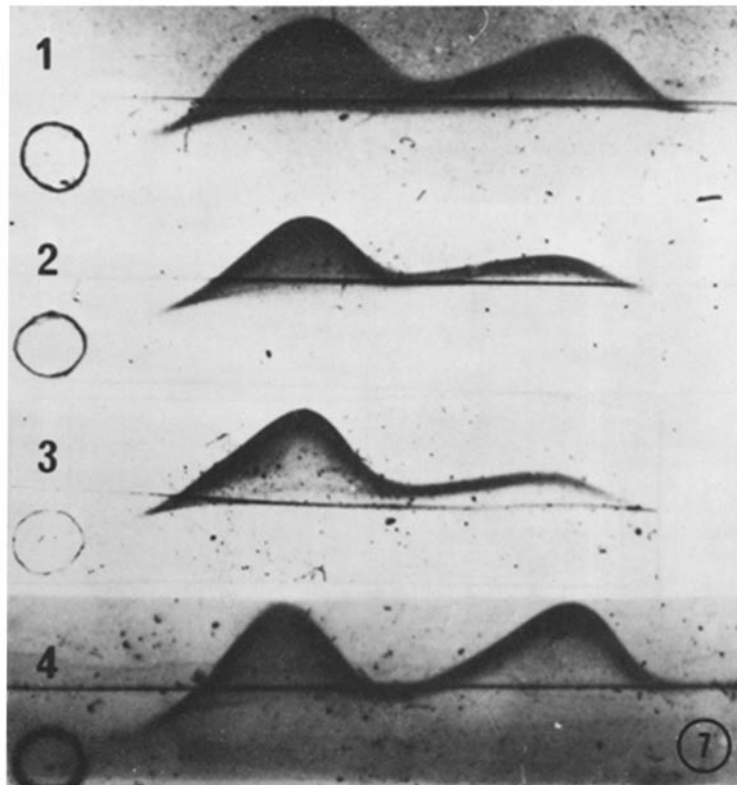
FIG. 7. Effect of thymus-independent antigens on C3 in normal mouse serum. SSSIII and PVP 360 in a concentration of 5 μg/ml were incubated for 1 h at 37°C with fresh normal mouse serum. The C3 profiles were then analyzed by antigen-antibody-crossed electrophoresis. From above downwards; 1, SSSIII; 2, PVP 360; 3, PBS control; and 4, yeast cell walls control. The extent of C3 conversion by PVP 360 did not differ from that in PBS control, while SSSIII had the same effect as the yeast cell walls.

tarded in mice treated with CoF before immunization with HGG (Fig. 9). OA and HGG are both thymus-dependent antigens (30, 31).

Primary Response to PVP.—CoF had no effect on the response to the thymus-independent antigen PVP 360 (29), irrespective of the relative timing of injections of CoF and antigen (Fig. 10).

DISCUSSION

The present observations confirm that CoF suppresses thymus-dependent antibody production, particularly the relatively more thymus-dependent IgG component of the anti-SRBC response (32, 33), but not thymus-independent responses (11). The inhibition by CoF of proliferation of specific RFC (B cells) suggests that it acts at an early phase of the allergic response.

TABLE III

Effects of SSSIII and PVP on the Anti-SRBC Response

Pretreatment*	Agglutination titer‡					
	Day 4		Day 6		Day 8	
	Total	IgG	Total	IgG	Total	IgG
PBS	5.9 ± 1.7	0	5.4 ± 1.3	4.3 ± 3.0	4.7 ± 2.1	3.7 ± 2.8
PVP 360	6.3 ± 1.4	0	5.9 ± 1.0	3.4 ± 2.9	5.4 ± 1.3	2.8 ± 3.0
SSSIII	2.6 ± 2.1	0	4.0 ± 1.9	0.1 ± 0.4	2.6 ± 2.4	0
	$P < 0.001$			$P < 0.01$		$P < 0.01$

* Groups of eight mice were each given four i.p. injections during the 24 h preceding i.p. injection of 2.5×10^8 SRBC on day 0. The doses of each agent were as follows: PVP 360, 4×5.0 μg; and SSSIII, 4×5.0 μg.

‡ Mean $-\log_2$ agglutination titer ± SD of all the animals in each group. Significant differences from the control, PBS pretreated, group are shown by P values.

The possibility that the effects of CoF on induction of antibody production were due to some other mechanism than complement depletion must be considered. In the doses used CoF was nontoxic in vivo (14, 26) and it had no cytotoxic action on cultured murine spleen cells during 48 h in vitro.[3] That CoF suppressed secondary as well as primary responses argues against antigenic competition as a possible mode of action, since competition is a feature only of primary responses (34, 35). In mice suitably preimmunized with CoF its suppressive effect on antibody production was prevented, whereas previous active immunization with the dominant antigen of a competing pair generally enhances rather than abolishes subsequent competition (35). Thymus-independent antigens do not cause antigenic competition of the thymus-dependent sequential type under consideration here (35), but SSSIII, which activates C3, inhibited particularly the IgG component of the anti-SRBC response. In contrast another thymus-independent antigen, PVP, which does not activate C3, had no effect.

Diverse agents which all interact with C3: yeast cell walls, aggregated HGG, SSSIII, and anti-C3 antibodies, also suppressed the anti-SRBC response. None of these agents produced sustained systemic C3 depletion, and CoF itself is still able to suppress antibody production in animals protected from plasma C3 depletion by low levels of anti-CoF antibodies which do not inhibit its C3-cleaving action. It is, therefore, likely that if C3 does have a role in the induction of antibody production then this is played out in the peripheral lymphoid organs. This suggestion is supported by the specific localization of [125I]CoF in the spleens of mice with circulating anti-CoF antibodies (14).

The effects on antibody responses might result from complement activation

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[3] G. Janossy, personal communication.
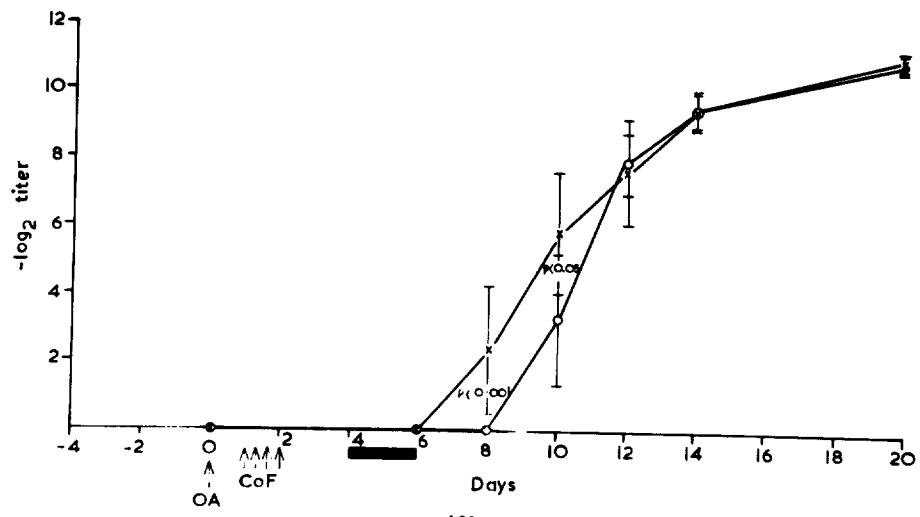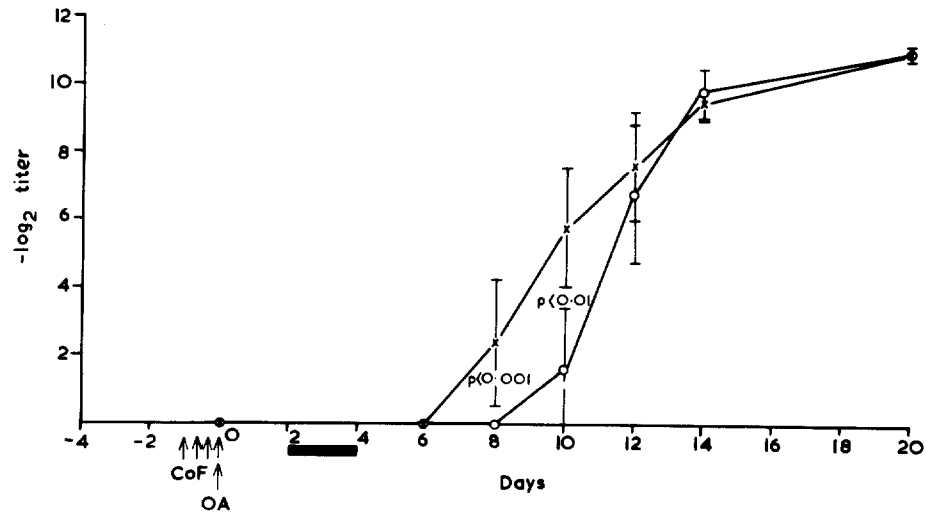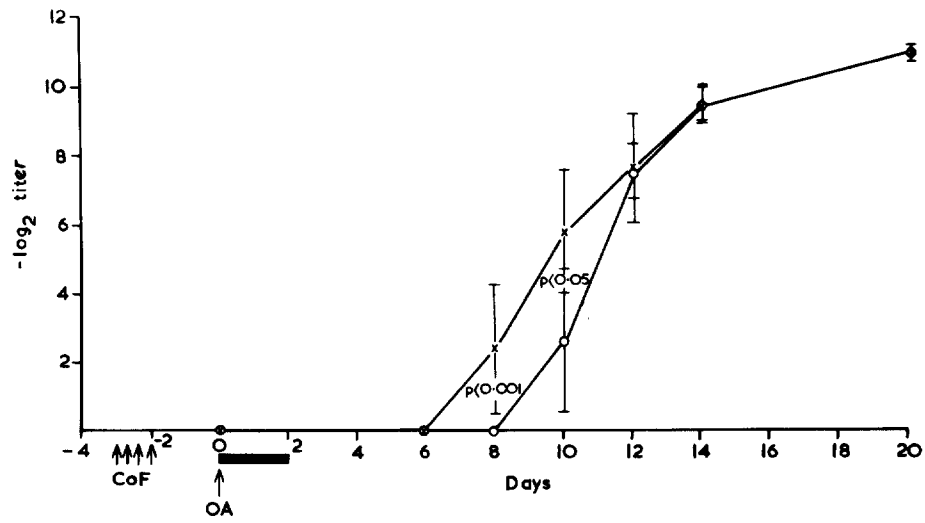
rather than interference with direct participation of complement in induction. For example C3 fragments can suppress in vitro antigen-induced lymphocyte transformation.[4] However, the inhibition by isolated anti-C3 antibodies of thymus-dependent antibody responses which has been observed in vitro (13) cannot be attributed to the generation of C3 fragments. It seems more likely that the various C3-reactive agents act by reducing functional complement at the site of induction of antibody production.

C5- and C6-deficient animals mount normal antibody responses (36), which, in conjunction with the observed effects of anti-C3 antibodies, indicates that C3 is the important component. C4-deficient guinea pigs, although they can still fix C3 by the alternate pathway, have impaired responses to certain antigens (37).

The present in vivo results, and other in vitro data (13), suggest that C3 plays a part in induction of thymus-dependent antibody production, and may, therefore, participate in lymphocyte cooperation. Thymus-dependent presentation of antigen to B cells at the surface of macrophages has been suggested in several hypotheses (38, 39) and there is evidence that B cells undergoing activation to transform and secrete antibody, both in vivo and in vitro, tend to be aligned on the surface of, or clustered around macrophages (40–43). In in vitro studies with separated cell populations Feldmann has shown that activated T cells are stimulated by antigen to secrete a specific antigen-binding product (44). It is apparently a form of IgM which, in complexes with antigen, adheres to the surface of macrophages and leads to triggering of specific B cells to antibody formation (45, 46).

It is postulated here that C3 may have a role in presentation of antigen to B cells, by enhancing approximation of the different cell types involved in cooperation. Complexes of antigen, either with the putative specific T-cell product or with other immunoglobulin, could fix C3 and then bind to the surface of macrophages which bear a membrane receptor for fixed C3 (4). B cells would, by virtue of their C3 receptor, tend to adhere nonspecifically to macrophages bearing C3-coated complexes, and if a B cell had the appropriate specific antigen receptors it might then recognize the presented antigen and be triggered. It is relevant that while both IgG and IgM-antigen-C3 complexes bind to macrophages, only the IgM complexes are held on the cell surface and are not

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[4] Pepys, M. B., and A. E. Butterworth. 1974. Inhibition by C3 fragments of C3-dependent rosette formation and antigen-induced lymphocyte transformation. *Clin. Exp. Immunol.* In press.

FIG. 8. Effect of CoF on the antibody response to OA. CoF was given to different groups of mice so as to produce maximal depletion of circulating C3 between 0–2 (above), 2–4 (middle), and 4–6 (below) days after i.p. injection of 0.75 mg of alum-precipitated ovalbumin. x, controls treated with PBS; O, CoF-treated mice; ↑, i.p. injection; and ▬, time during which serum C3 was <5% of normal in CoF-treated mice.

phagocytosed (47). Also passively administered IgM antibody, as opposed to IgG antibody, can enhance active antibody production in vivo to the specific antigen (48).

Such a concept implies that fixed C3 provides a nonspecific "amplification" mechanism facilitating T-B cooperation by increasing the likelihood of rare specific B cells encountering their appropriate antigen. On the other hand when cells are aggregated only by C3, without any specific antigen-receptor binding, this would be transient. The action of the C3b inactivator on fixed C3 inhibits its adherence to macrophages (49), and binding to the B-cell C3 receptor is also a reversible process (50). The recent observation of spontaneously reversible, complement-mediated, C3-dependent mixed adherence of macrophages, lymphocytes, and other cells in vitro shows that this model is feasible (51).

An alternative recent hypothesis is that C3 binding to B-cell C3 receptors constitutes an obligatory second signal for triggering which B cells must receive in addition to specific recognition of T-dependent or T-independent antigens (52, 53). It has also been suggested that the interaction of "activated" C3 with B cells is mitogenic per se (52, 53). Direct testing has, however, demonstrated the complement independence of B-cell triggering by mitogens (54). Furthermore, the present in vivo results and analogous in vitro observations (13) provide no evidence for a connection between the complement system and antibody responses to thymus-independent antigens.

SUMMARY

In an in vivo study in mice, suppression by the C3-cleaving protein of cobra venom (CoF), and other C3-reactive agents (zymosan, aggregated IgG, anti-C3 antibodies, and type III pneumococcal polysaccharide) of the thymus-dependent antibody responses to sheep erythrocytes, ovalbumin, and human IgG was demonstrated. The thymus-independent antibody response to polyvinyl-pyrrolidone was however unaffected by CoF.

These and other published observations suggest that there may be a requirement for functional C3 in induction of thymus-dependent but not thymus-independent antibody production. A model for the role of C3 in lymphocyte cooperation is proposed based on these data analyzed in the light of existing knowledge of this process. It is postulated that fixed C3 interacting with macro-

Fig. 9. Effect of CoF on the antibody response to HGG. CoF was given to different groups of mice so as to produce maximal depletion of circulating C3 between 0–2 (above) and 2–4 (below) days after subcutaneous injection of 100 μg of HGG emulsified in incomplete adjuvant. On day 2 and day 11 100 μg of [^{125}I]HGG were injected i.p. and its rate of elimination measured by whole body counting. Each point represents the geometric mean with 95% confidence limits, of all the animals in each group. ×, controls treated with PBS; O, CoF-treated mice; ↑, i.p. injection; and ■, time during which serum C3 was <5% of normal in CoF-treated mice.
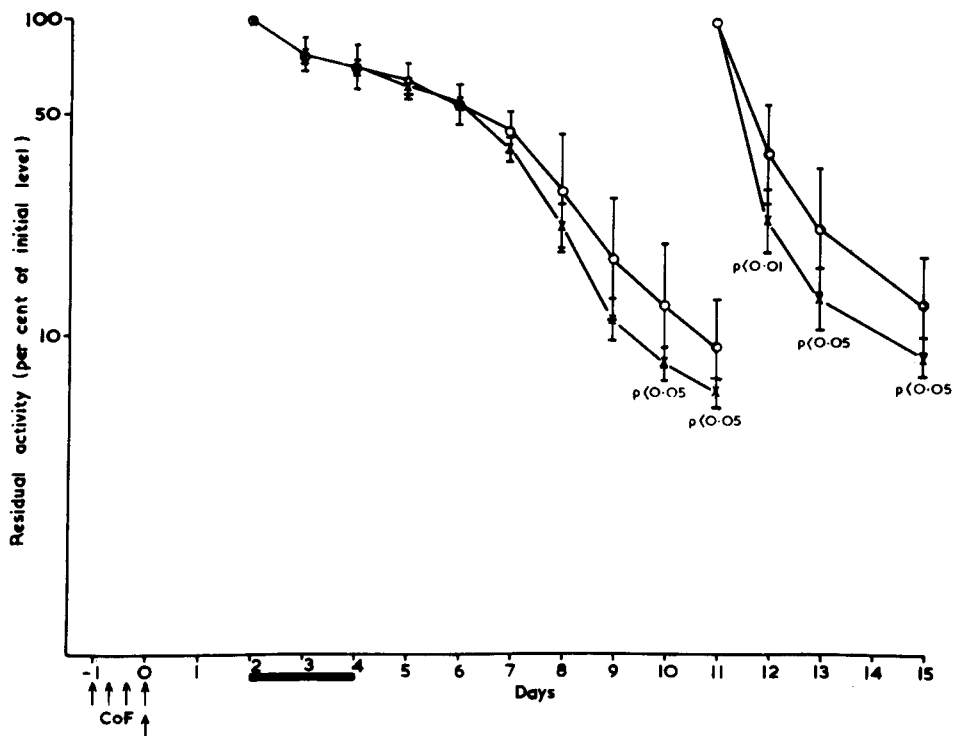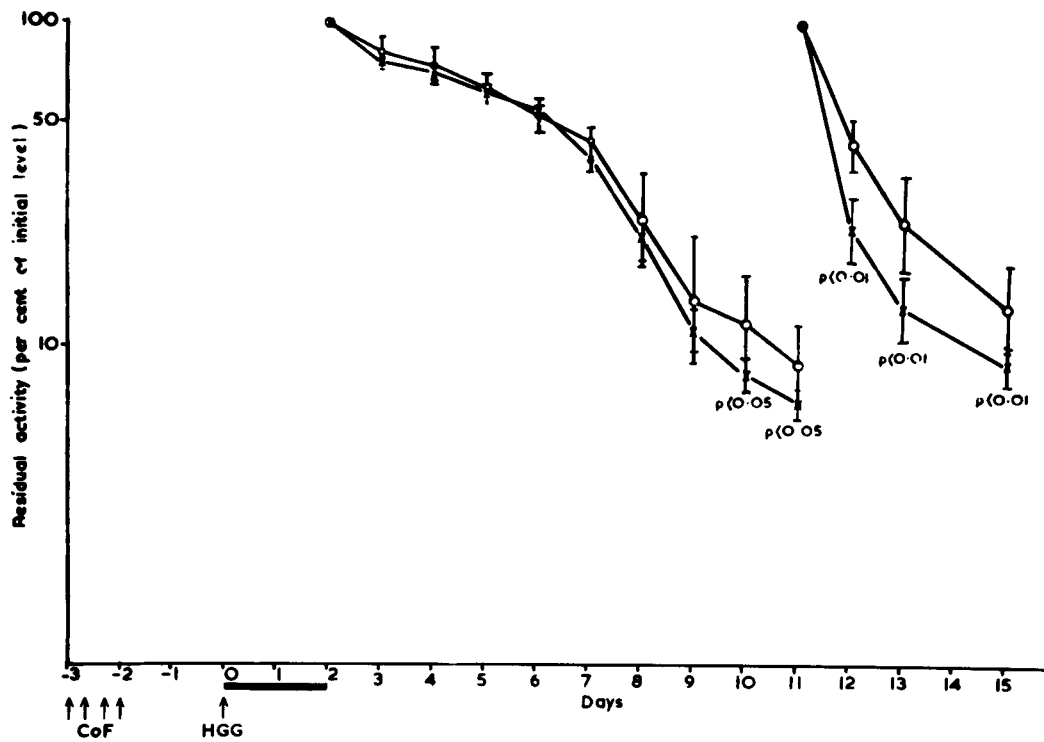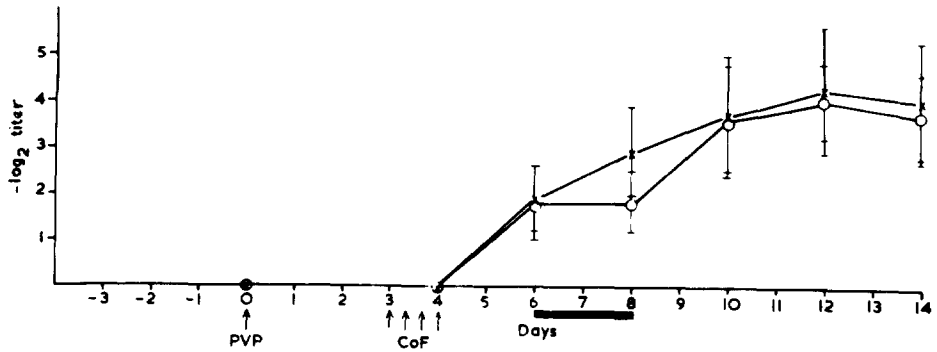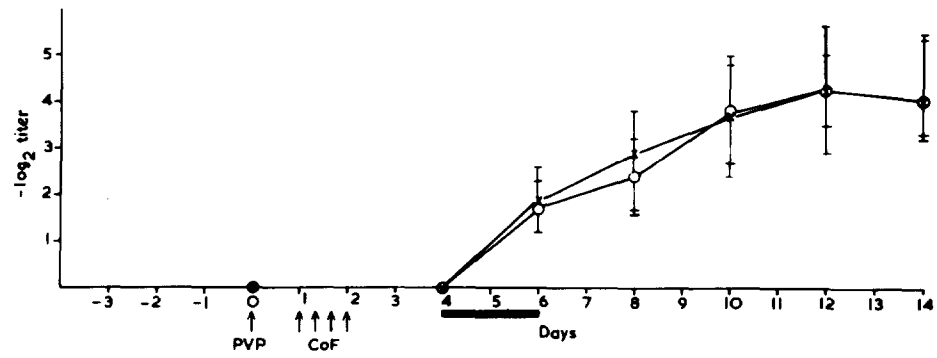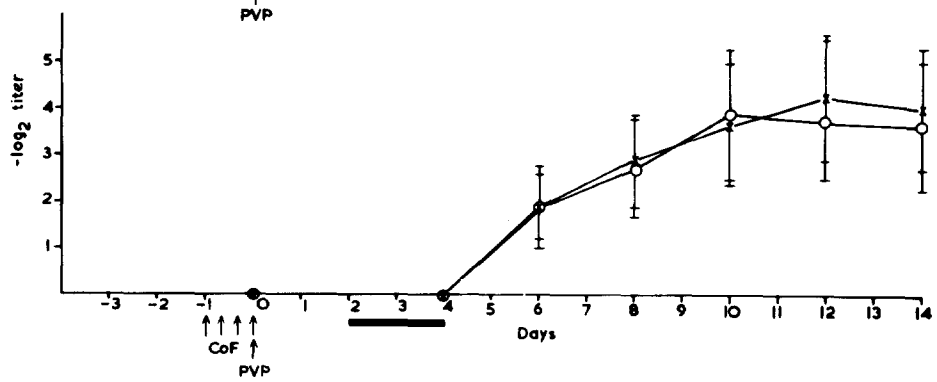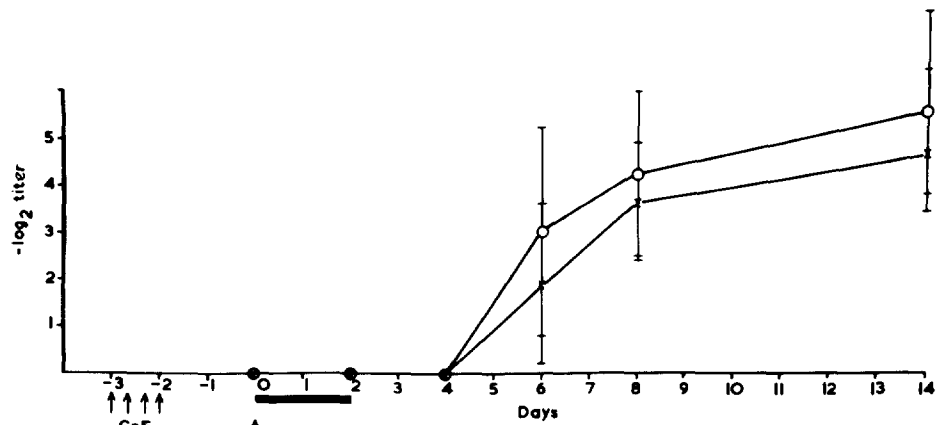
phage and B-cell C3 receptors might enhance or facilitate T-dependent presentation of antigen to B cells.

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FIG. 10. Effect of CoF on the antibody response to PVP. CoF was given to different groups of mice either before or after i.p. injection of 1.0 μg of PVP 360. x, controls treated with PBS; ○, CoF-treated mice; ↑, i.p. injection; and ▬, time during which serum C3 was <5% of normal in CoF-treated mice.

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