Clin. exp. Immunol. (1983) **53**, 208–216.

The role of complement in the induction of antibody responses

H. D. OCHS, R. J. WEDGWOOD, M. M. FRANK, S. R. HELLER & S. W. HOSEA
Department of Pediatrics, University of Washington School of Medicine, Seattle, Washington and Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, Maryland, USA

(Accepted for publication 4 February 1983)

SUMMARY

To determine the effect of complement on the normal antibody response to T cell-dependent antigens, we immunized normal and C4 deficient guinea-pigs with bacteriophage ΦX 174. Following primary immunization with a standard dose (2×10^9 PFU/Kg) given intravenously, C4 deficient guinea-pigs produced less antibody than normal guinea-pigs and were unable to maintain measurable antibody levels. Following secondary immunization, antigen clearance of C4 deficient guinea-pigs was delayed and the subsequent antibody response was identical to their primary response without amplification or isotype switch. Increased antigen dose and administration of antigen in adjuvants into footpads improved the responses but did not make them normal. The primary and secondary responses became essentially normal, however, when small amounts of normal guinea-pig serum were given to the deficient animals at the time of the primary (but not the secondary) immunization. We postulate that the contribution of complement to the mature humoral immune response is related to activation of C3. Our data show that antigen initiates a primary immune response. The resultant antigen–antibody complexes interact with complement and are then non-specifically trapped by C3 receptors on dendritic cells, B cells and macrophages. Thus, antigen is selectively accumulated within the lymphoid organs and in turn 'captures' antigen specific B cells by interaction of the trapped antigen with antigen specific sIg. The approximation of specific lymphoid cells, macrophages and antigen permits generation of specific memory cells and ensures prompt, mature antibody response on subsequent antigen exposure.

Keywords complement antibody responses ΦX 174

INTRODUCTION

Induction and maintenance of the antibody response to a T cell-dependent antigen requires not only interactions between B lymphocytes, T lymphocytes and macrophages (Katz & Benacerraf, 1972), but also the localization and persistence of antigen in the lymphoid organs (Tew & Mandel, 1978). Complement appears to be required for the process: mice depleted of complement with cobra venom (CoF) fail to localize antigen (Papamichail *et al.*, 1975) and do not make normal antibody responses to T dependent antigens (Pepys, 1972, 1974; Dukor *et al.*, 1974; Lewis, Ranken & Goodman, 1977; Pepys, Wansbrough-Jones & Mirjah, 1976; Pepys *et al.*, 1977; Martinelli, Matsuda & Osler, 1978; Matsuda, Martinelli & Osler, 1978). The mechanisms by which CoF might effect the immune response are complex. In addition to *in vivo* complement depletion (Pepys, 1976) CoF might have a direct effect on the immune system through antigen competition (Pepys, 1972) or through the effect of complement split products (e.g. C3a, C3b, C5a) on macrophage function

Correspondence: H. D. Ochs, Department of Pediatrics RD-20, School of Medicine, University of Washington, Seattle, Washington 98195, USA.

(Martinelli *et al.*, 1978; Matsuda *et al.*, 1978; Goodman, Chenoweth & Weigle, 1982). The naturally occurring defects of complement provide an opportunity to study the role of complement on antibody responses directly. Following the serendipitous observation (Jackson, Ochs & Wedgwood, 1979) of an aberrant immune response in a young boy with a deficiency of the fourth component of complement we initiated a series of studies of the analogous animal model, the C4 deficient guinea-pig (Ellman, Green & Frank, 1970; Ellman *et al.*, 1971), using bacteriophage ΦX 174 which permits an unusually sensitive assessment of the immune response (Uhr, Finkelstein & Baumann, 1962; Wedgwood, Ochs & Davis, 1975; Jackson *et al.*, 1977).

MATERIALS AND METHODS

Experimental animals. A breeding colony of C4 deficient guinea-pigs was obtained from the National Institutes of Health animal production section, and young healthy C4 deficient female animals were selected for the experiments. Control animals were either normal female 'NIH multipurpose' guinea-pigs of the strain from which the C4 deficient guinea-pigs were derived, or outbred female guinea-pigs obtained from a local supplier. The animals' sera were tested for the presence or absence of C4 by a double diffusion (Ouchterlony) technique or by determining total haemolytic complement activity (CH_{50}).

Immunization of experimental animals. C4 deficient and control animals were approximately 3 months old and weighed between 400 and 500 g at the beginning of the experiments. The various groups of animals studied are listed in Table 1. Blood was obtained by cardiac puncture or from the retro-orbital plexus. Intravenous injections were either by heart puncture, using a 23 gauge scalp vein needle, or by injection into the hind footpad vein using a 26 gauge needle. Bacteriophage ΦX 174 antigen was grown, harvested and purified as described previously (Wedgwood *et al.*, 1975). Preparations of bacteriophage, containing 1×10^{11} plaque forming units (PFU)/ml were stored in small aliquots at −70°C until thawed for use. The phage is stable under these conditions for at least 6 months. Antibody was assayed as neutralizing activity, and expressed as rate of phage inactivation (Kv) from a standard formula (Wedgwood *et al.*, 1975). Susceptibility of the antibody to 2-mercaptoethanol was used to differentiate IgM from IgG antibody (Grubb & Swahn, 1958). The

Table 1. Groups of guinea-pigs (GP) studied for 1° and 2° antibody responses to bacteriophage ΦX 174

Group No.	Number of GP		Dose of ΦX 174 (PFU/Kg)*	Route of injection	Injection of normal serum (days when given)
	C4D	Normal			
1	7	7	2×10^9 (1 × STD)†	i.v.	—
2	14	7	1×10^{10} (5 × STD)	i.v.	—
3	3	4	1×10^{11} (50 × STD)	i.v.	—
4	7	7	1×10^{10} (5 × STD, given in FCA)‡	footpad	—
5	6		1×10^{10} (5 × STD)	i.v.	+ (0, 2, 4)§
6	4		1×10^{10} (5 × STD)	i.v.	+ (0)§
7	6		1×10^{10} (5 × STD)	i.v.	+ (2 or 4)§

* PFU = plaque forming units.

† STD = Standard dose.

‡ FCA = Freund's complete adjuvant.

§ Normal guinea pig serum (3 ml/kg body weight) was given intravenously after 1° immunization with bacteriophage on the days indicated.

amount of antigen was adjusted to body weight and was given intravenously twice, 6 weeks apart. One group of animals received a single dose of antigen in Freund's complete adjuvant (FCA, Bacto H37 Ra, DIFCO Lab, Detroit, Michigan, USA) into both footpads. In the experiments on complement replacement, fresh frozen pooled normal guinea-pig serum, thawed immediately before use, was given slowly by intracardiac infusion at a dose of 3 ml/kg. All cardiac punctures were done on anesthetized (ether) animals.

RESULTS

Antibody responses to standard intravenous antigen injection

C4 deficient and control guinea-pigs were immunized intravenously with a standard dose (2×10^9 PFU/kg) of bacteriophage. Following primary immunization blood was drawn at days 1, 2, 3, 5, and 7 and then at weekly intervals. Phage could be detected for 48 h in all animals except one (a C4 deficient guinea-pig with a low antibody titre prior to phage injection that cleared the antigen in 2 days). Three to five days after immunization, infective phage could no longer be demonstrated in sera from any of the guinea-pigs, indicating phage neutralization and/or antigen clearance. Thereafter normal guinea-pigs, both individually and as a group, showed rapid antibody production (Fig. 1). Serum antibody showed a characteristic biphasic response with peaks at 1 and 4 weeks, the latter concurrent with the appearance of antibody of the IgG class. Titres then decreased slowly. In contrast, C4 deficient guinea-pigs produced significantly less antibody. Serum antibody peaked at 5 days and thereafter decreased rapidly. Four weeks after primary immunization antibody could no longer be demonstrated.

A second phage injection was given 6 weeks after primary immunization (Fig. 1). All normal control guinea-pigs, but only two of seven C4 deficient guinea-pigs, cleared phage within 24 h ($\chi^2 = 6{\cdot}9$, $P < 0{\cdot}01$). By 48 h all animals had cleared bacteriophage. The secondary antibody responses of normal guinea-pigs were brisk with a peak antibody titre at 1 week (geometric mean Kv = 194). Subsequently, the antibody activity decreased slowly (at 6 weeks, geometric mean Kv = 14·8). At the peak response most of the antibody (67%) was of the IgG class. Again, in sharp contrast, the secondary antibody responses of the C4 deficient guinea-pigs were markedly depressed being almost identical to their primary responses. The peak response was reached at 1 week and then rapidly decreased. Antibody was no longer detectable 4 weeks following the second phage

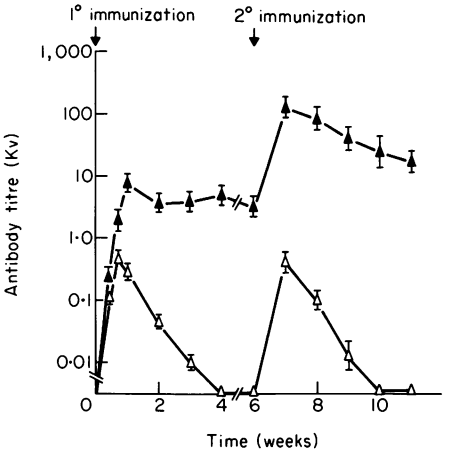

Fig. 1. Antibody responses of normal guinea-pigs (▲—▲) and C4 deficient guinea-pigs (△——△). Phage was injected intravenously at a dose of 2×10^9 PFU/kg body weight (1 × standard dose) as indicated (↓). Antibody titres, expressed as rate of phage inactivation (Kv) were determined at weekly intervals. Each point represents the geometric mean of individual animals; the bars indicate s.e. (mean).

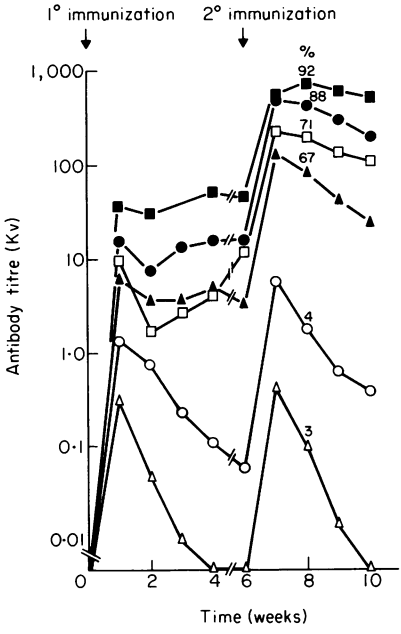

Fig. 2. Antibody responses of normal guinea-pigs (▲, ●, ■) and C4 deficient guinea-pigs (△, ○, □) to various doses (1 × [▲, △], 5 × [●, ○] and 50 × [■, □] standard dose) of bacteriophage ΦX 174, given intravenously at the time indicated (↓). Antibody titres were determined at weekly intervals. Percentage (%) IgG of the anti-phage antibody is given for the 2 week sample during the 2° response.

injection. The antibody of the C4 deficient guinea-pigs was entirely susceptible to 2-mercapto-ethanol, indicating that it was of the IgM class.

Antigen dose and antibody response

The effect of antigen dose on the antibody response was studied by giving C4 deficient and normal guinea-pigs either 2×10^9 PFU/kg (standard dose), 1×10^{10} PFU/kg (5 × standard dose) or 1×10^{11}

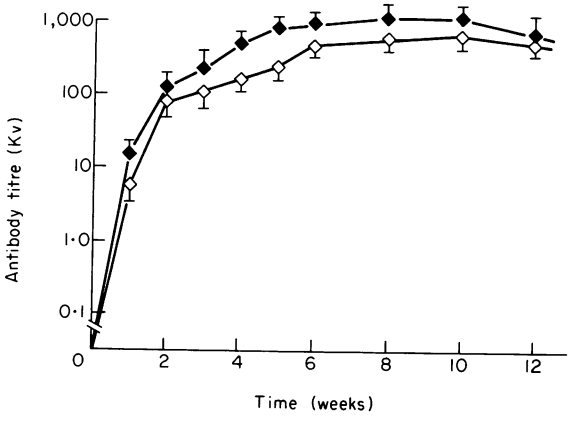

Fig. 3. Antibody responses of normal (♦) and C4 deficient (◊) guinea-pigs injected at time 0 into the footpads of both hind legs with bacteriophage ΦX 174 in FCA at a dose of 1×10^{10} PFU/kg weight. The differences in antibody titres are significant for the 4 week ($P < 0{\cdot}05$) and 5 week ($P < 0{\cdot}01$) values. During the first 2 weeks following antigen injection antibody is IgM, and subsequently becomes predominantly IgG in both groups of animals.

PFU/kg (50 × standard dose) at each antigen injection. Results of these studies are shown in Fig. 2. There was a dose-dependent increase of antibody production in both normal and C4 deficient guinea-pigs. Normal guinea-pigs at all antigen doses showed a typical primary followed by a characteristic secondary antibody response with amplification and isotype switch from IgM to IgG. By injecting more antigen both the amount of antibody and the proportion of IgG antibody increased. In contrast, C4 deficient guinea-pigs responded to the low antigen doses (1 × standard dose and 5 × standard dose) with very low antibody titres, lacking both amplification and effective isotype switch. With the highest dose of antigen (50 × standard dose), a nearly normal antibody response was achieved with amplification and isotype switch from IgM to IgG. However, the antibody titres and the proportion of IgG antibody during the secondary response observed in the C4 deficient guinea-pigs were at all antigen doses consistently less than the responses in normal guinea-pigs immunized with a similar antigen dose.

Antibody responses to antigen in FCA (Fig. 3)

Bacteriophage ΦX 174 mixed in FCA was injected into the footpads of normal and C4 deficient guinea-pigs in a single dose (1×10^{10} PFU/Kg weight). Both groups of animals promptly responded with antibody titres that at 2 weeks were equivalent to titres seen during the secondary response of normal guinea-pigs receiving antigen without adjuvant. However, the C4 deficient guinea-pigs had a consistently lesser response than the normal control, the difference being significant at 4 weeks

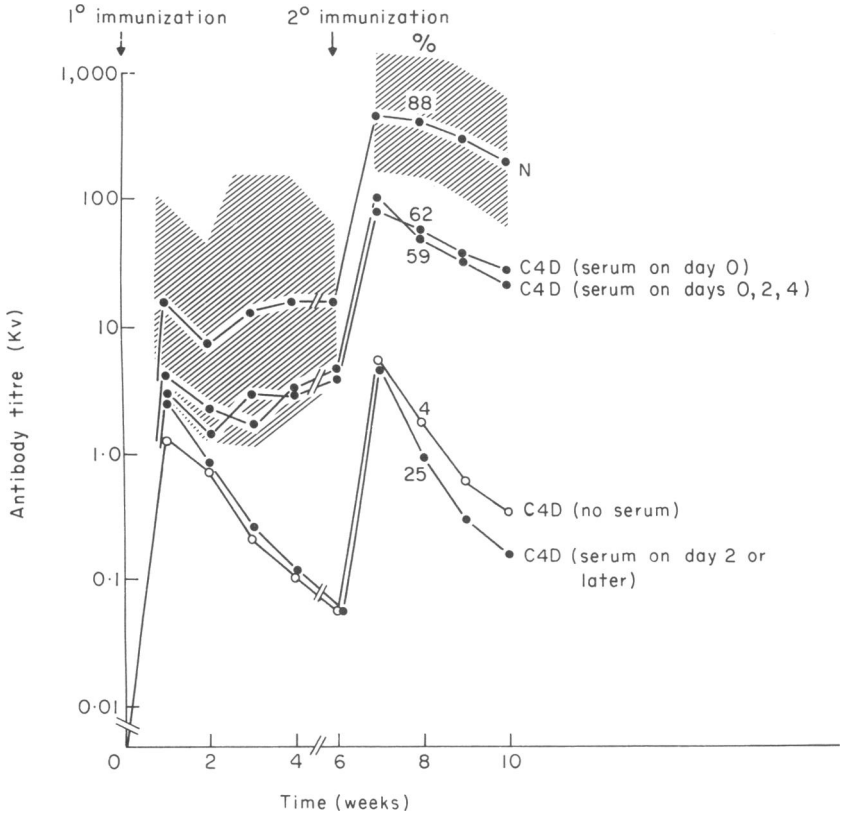

Fig. 4. Antibody responses of normal (N) and C4 deficient (C4D) guinea-pigs; bacteriophage ΦX 174 was injected intravenously at a dose of 1×10^{10} PFU/kg weight (↓). Fresh frozen serum (3 ml/kg body weight) was given at the time indicated. Percentage (%) IgG of the antiphage antibody is shown for the 2 week sample during the 2° response.

($P < 0{\cdot}05$), and 5 weeks ($P < 0{\cdot}01$). In both normal and C4 deficient guinea-pigs the class of antibody was predominantly IgM during the first 2 weeks following antigen injection and subsequently became predominantly IgG.

Effect of replacement C4 on the antibody response

A group of C4 deficient guinea-pigs was injected on day 0 with bacteriophage ΦX 174 (1×10^{10} PFU/kg) and 3 ml of fresh frozen normal guinea-pig serum. Some of these animals were, in addition, given 3 ml of fresh frozen normal guinea-pig serum at 48 h (day 2) and 96 h (day 4). Other groups of animals were given the primary injection of bacteriophage ΦX 174 alone, with the injection of fresh frozen normal guinea-pig serum either at 48 or 96 h (see Table 1). No further injections of normal serum were given during the remainder of the primary immune response nor during the secondary immunization with bacteriophage. As shown in Fig. 4, a typical biphasic primary, followed by a typical secondary response with isotype switch was observed in the group of C4 deficient guinea-pigs given normal serum at the time of the primary immunization. The antibody titres and the proportion of IgG in the secondary response of the C4 deficient animals were, however, still significantly lower than in normal guinea-pigs immunized similarly. If the normal serum was not given until 48 h or more after the primary immunization the antibody response was indistinguishable from that of C4 deficient guinea-pigs receiving no normal serum (Fig. 4). Thus the provision of a small amount of normal serum at the time of the primary immunization appeared to restore the deficit, permitting the establishment of an intact secondary response some 6 weeks later.

DISCUSSION

Deficiency of the fourth component of complement in guinea-pigs is an autosomal recessive trait and affected animals, though healthy and with normal fertility, completely lack C4 in the serum, both histochemically and functionally (Ellman *et al.*, 1970, 1971). Impaired antibody production has been described in such animals (Ellman *et al.*, 1971) suggesting that complement may play a role in antibody formation. This hypothesis is supported by studies of antibody formation in animals 'decomplemented' by cobra venom factor (CoF) (Pepys, 1972, 1974; Dukor *et al.*, 1974; Lewis *et al.*, 1977; Pepys *et al.*, 1976, 1977; Martinelli *et al.*, 1978; Matsuda *et al.*, 1978; Cochrane, Müller-Eberhard & Aikin, 1970). Our studies on the antibody responses of C4 deficient guinea-pigs to the T-dependent neoantigen bacteriophage ΦX 174 also support the proposition.

Antibody responses to bacteriophage ΦX 174 have been studied extensively in experimental animals (Uhr *et al.*, 1962; Ching & Wedgwood, 1967; Ochs *et al.*, 1974; Lopez, Davis & Smith, 1972) and man (Wedgwood *et al.*, 1975; Uhr *et al.*, 1962; Ochs, Davis & Wedgwood, 1971; Lopez *et al.*, 1975; Noel *et al.*, 1978). Natural exposure to this antigen is rare, and the first immunization results in a true primary response allowing measurement of antigen clearance and subsequent IgM antibody production. In normal controls the secondary response is prompt; memory, amplification and isotype switch from IgM to IgG can be clearly defined (Uhr *et al.*, 1962; Wedgwood *et al.*, 1975; Ching & Wedgwood, 1967; Ochs *et al.*, 1974; Lopez *et al.*, 1972); T-dependency has been shown (Jackson *et al.*, 1977). Abnormal antibody responses of various degrees and severity have been observed in experimental animals and in patients with primary (Wedgwood *et al.*,1975; Jackson *et al.*, 1977; Ochs *et al.*, 1971) and secondary (Ochs *et al.*, 1974; Lopez *et al.*, 1972, 1975; Noel *et al.*, 1978) immunodeficiency states.

Compared with normal guinea-pigs, C4 deficient pigs produced less antibody to bacteriophage ΦX 174 and were unable to maintain good antibody levels; following secondary exposure antigen clearance was delayed and the secondary response was identical to the pirmary without amplification or isotype switch. Although the differences were reduced by increased antigen dosage or adjuvant, the responses of the C4 deficient animals remained abnormal. If fresh serum was given to the C4 deficient guinea-pigs at the same time as the primary antigen dose near normal antibody production occurred including a normal secondary response some 6 weeks later showing memory, amplification and isotype switch.

The abnormal antibody response of C4 deficient guinea-pigs might not be related to the C4

deficiency alone. The genes coding for the C4 protein are located within the major histocompatibility complex (MHC) (Ochs *et al.*, 1977; Meo, Krasteff & Shreffler, 1975; Shevach, Frank & Green, 1976). Thus, the abnormal response could result from an associated defect in the antigen recognition region of the MHC unrelated to C4 deficiency but segregating with it. Alternatively, the depressed antibody response might be the consequence of active suppression. However, the fact that fresh serum from normal guinea-pigs will restore the antibody response indicates that the cellular network of the C4 deficient guinea-pigs is functionally intact. Thus the abnormal antibody response observed is most probably directly related to the absence of C4.

The fact that the restoration of the antibody response could only be induced when the fresh normal serum was given together with the antigen during the primary immunization suggests that the stage for the secondary response 6 weeks later is set during the early primary response. It is in agreement with the observations by others (Martinelli *et al.*, 1978; Klaus & Humphrey, 1977) who reported that CoF suppresses the *in vivo* antibody response only if decomplementation was achieved during primary but not during secondary immunization.

It seems probable that activation of C3 is central to the requirement for complement in the induction of the humoral response. A series of *in vitro* and *in vivo* experiments are consistent with this hypothesis. Anti-mouse C3 antibody added to cultures of primed mouse spleen cells suppresses the *in vitro* antibody response (Dukor *et al.*, 1974; Feldman & Pepys, 1974; Lewis *et al.*, 1976; Pryjma & Humphrey, 1975). C3b receptors on macrophages (Lay & Nussenzweig, 1968) and B lymphocytes (Pryjma & Pryjma, 1978) provide a mechanism for interaction of these cells with immune complexes and for modulation of their function (Pepys, 1976). Following immunization, lymph nodes and spleen show deposits of antigen, antibody (IgM, IgG) and complement (C3) which are trapped within the germinal centers (Tew & Mandel, 1978; Gajl-Peczalska *et al.*, 1969; Humphrey & Frank, 1967; Brown *et al.*, 1973); this localization is antibody- and complement-dependent (Papamichail *et al.*, 1975; Dukor *et al.*, 1974; Klaus & Humphrey, 1977; Romball, Ulevitch & Weigle, 1980; White *et al.*, 1975). Antigen–antibody complexes are much more effective than soluble antigens in priming B cells and in initiating germinal centers (Klaus, 1978). The cumulated evidence suggests that antigen, given intravenously, initiates a primary IgM response; the resultant antigen–antibody interaction initiates the formation of complexes consisting of antigen, antibody and complement (AgAbC1423b). These complexes would bind to C3 receptor positive cells including dendritic cells, B cells, and macrophages (Dukor *et al.*, 1974). Complexes non-specifically trapped in this manner could then in turn entrap antigen specific B cells by interaction of the trapped antigen with antigen specific sIg. The resultant structure would provide a focal interactive network of specific and non-specific cells, allowing generation of specific memory cells and ensuring a prompt, mature antibody response on subsequent exposure to the antigen.

Supported in part by grants from the National Institutes of Health (AI-07073); the March of Dimes Birth Defects Foundation (6-273 and 1-771); and the Thrasher Research Foundation.

REFERENCES

BROWN, J.C., HARRIS, G., PAPAMICHAIL, M., SLIJIVIC, V.S. & HOLBOROW, E.J. (1973) The localization of aggregated human γ-globulin in the spleens of normal mice. *Immunology*, **24,** 955.

CHING, Y.C. & WEDGWOOD, R.J. (1967) Immunologic responses in the axolotl, *Siredon mexicanum*. *J. Immunol.* **99,** 191.

COCHRANE, C.G., MÜLLER-EBERHARD, H.J. & AIKIN, B.S. (1970) Depletion of plasma complement *in vivo* by a protein of cobra venom: its effect on various immunologic reactions. *J. Immunol.* **105,** 55.

DUKOR, P., DIETRICH, F.M., GISLER, R.H., SCHUMANN, G. & BITTER-SUERMANN, D. (1974) Possible targets of complement action in B-cell triggering. *Prog. Immunol. II*, **3,** 99.

ELLMAN, L., GREEN, I. & FRANK, M. (1970) Genetically controlled total deficiency of the fourth component of complement in the guinea pig. *Science*, **170,** 74.

ELLMAN, L., GREEN, I., JUDGE, F. & FRANK, M.M. (1971) *In vivo* studies in C4-deficient guinea pigs. *J. exp. Med.* **134,** 162.

FELDMAN, M. & PEPYS, M.B. (1974) Role of C3 in *in vitro* lymphocyte cooperation. *Nature*, **249,** 159.

GAJL- PECZALSKA, K.J., FISH, A.J., MEUWISSEN, H.J., FROMMEL, D. & GOOD, R.A. (1969) Localization of immunological complexes fixing β_{1C} (C3) in germinal centers of lymph nodes. *J. exp. Med.* **130,** 1367.

GOODMAN, M.G., CHENOWETH, D.E. & WEIGLE, W.O.

(1982) Potentiation of the primary humoral immune response *in vitro* by C5a anaphylatoxin. *J. Immunol.* **129,** 70.

GRUBB, R. & SWAHN, B. (1958) Destruction of some agglutinins but not of others by two sulfhydryl compounds. *Acta Pathol. Microbiol. Scand.* **43,** 305.

HUMPHREY, J.H. & FRANK, M.M. (1967) The localization of non-microbial antigens in the draining lymph nodes of tolerant, normal and primed rabbits. *Immunology*, **13,** 87.

JACKSON, C.G., OCHS, H.D., SIEBER, O.F., JONES, J.F. & WEDGWOOD, R.J. (1977) Interaction of T- and B-lymphocytes in the immune response to a thymus-dependent antigen. *Clin. Res.* **25,** 155A.

JACKSON, C.G., OCHS, H.D. & WEDGWOOD, R.J. (1979) The immune response of a patient with deficiency of the fourth component of complement and systemic lupus erythematosus. *N. Engl. J. Med.* **300,** 1124.

KATZ, D.H. & BENACERRAF, B. (1972) Regulatory influence of activated T-cells. *Adv. Immunol.* **15,** 1.

KLAUS, G.G.B. (1978) The generation of memory cells. II. Generation of B memory cells with preformed antigen–antibody complexes. *Immunology*, **34,** 643.

KLAUS, G.G.B. & HUMPHREY, J.H. (1977) The generation of memory cells. I. The role of C3 in the generation of B memory cells. *Immunology*, **33,** 31.

LAY, W.H. & NUSSENZWEIG, V. (1968) Receptors for complement of leukocytes. *J. exp. Med.* **128,** 991.

LEWIS, G.K., RANKEN, R. & GOODMAN, J.W. (1977) Complement-dependent and -independent pathways of T cell–B cell cooperation. *J. Immunol.* **118,** 1744.

LEWIS, G.K., RANKEN, R., NITECKI, D.E. & GOODMAN, J.W. (1976) Murine B-cell subpopulations responsive to T-dependent and T-independent antigens. *J. exp. Med.* **144,** 382.

LOPEZ, V., DAVIS, S.D. & SMITH, N.J. (1972) Studies in infantile marasmus. IV. Impairment of immunologic responses in the marasmic pig. *Pediatr. Res.* **6,** 779.

LOPEZ, V., OCHS, H.D., THULINE, H.C., DAVIS, S.D. & WEDGWOOD, R.J. (1975) Defective antibody response to bacteriophage ΦX 174 in Down syndrome. *J. Pediair.* **86,** 207.

MARTINELLI, G.P., MATSUDA, T. & OSLER, A.G. (1978) Studies of immunosuppression by cobra venom factor. I. On early IgG and IgM responses to sheep erythrocytes and DNP-protein conjugates. *J. Immunol.* **121,** 2043.

MATSUDA, T., MARTINELLI, G.P. & OSLER, A.G. (1978) Studies on immunosuppression by cobra venom factor. II. On responses to DNP·Ficoll and DNP·polyacrylamide. *J. Immunol.* **121,** 2048.

MEO, T., KRASTEFF, T. & SHREFFLER, D.C. (1975) Immunochemical characterization of murine H-2 controlled Ss (serum substance) protein through identification of its human homologue as the fourth component of complement. *Proc. Natl. Acad. Sci. USA.* **72,** 4536.

NOEL, D.R., WITHERSPOON, R.P., STORB, R., ATKINSON, K., DONEY, K., MICKELSON, E.M., OCHS, H.D., WARREN, R.P., WEIDEN, P.L. & THOMAS, E.D. (1978) Does graft-versus-host influence the tempo of immunologic recovery after allogeneic human marrow transplantation? An observation on 56 long-term survivors. *Blood*, **51,** 1087.

OCHS, H.D., DAVIS, S.D. & WEDGWOOD, R.J. (1971) Immunologic responses to bacteriophage ΦX 174 in immunodeficiency diseases. *J. clin. Invest.* **50,** 2559.

OCHS, H.D., ROSENFELD, S.I., THOMAS, E.D., GIBLETT, E.R., ALPER, C.A., DUPONT, B., SCHALLER, J.G., GILLILAND, B.C., HANSEN, J.A. & WEDGWOOD, R.J. (1977) Linkage between the gene (or genes) controlling synthesis of the fourth component of complement and the major histocompatibility complex. *N. Engl. J. Med.* **296,** 470.

OCHS, H.D., STORB, R., THOMAS, E.D., KOLB, H.-J., GRAHAM, T.C., MICKELSON, E., PARR, M. & RUDOLPH, R.H. (1974) Immunologic reactivity in canine marrow graft recipients. *J. Immunol.* **113,** 1039.

PAPAMICHAIL, M., GUTIERREZ, C., EMBLING, P., JOHNSON, P., HOLBOROW, E.J. & PEPYS, M.B. (1975) Complement dependence of localisation of aggregated IgG in germinal centres. *Scand. J. Immunol.* **4,** 343.

PEPYS, M.B. (1972) Role of complement in induction of the allergic response. *Nature, New Biology*, **237,** 157.

PEPYS, M.B. (1974) 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. *J. exp. Med.* **140,** 126.

PEPYS, M.B. (1976) Role of complement in the induction of immunological responses. *Transplant. Rev.* **32,** 93.

PEPYS, M.B., BRIGHTON, W.D., HEWITT, B.E., BRYANT, D.E.W. & PEPYS, J. (1977) Complement in the induction of IgE antibody formation. *Clin. exp. Immunol.* **27,** 397.

PEPYS, M.B., WANSBROUGH-JONES, M.H. & MIRJAH, D.D. (1976) Suppression of IgA antibody production by *in vivo* complement depletion. *Clin. exp. Immunol.* **23,** 378.

PRYJMA, J. & HUMPHREY, J.H. (1975) Prolonged C3 depletion by cobra venom factor in thymus-deprived mice and its implication for the role of C3 as an essential second signal for B-cell triggering. *Immunology*, **28,** 569.

PRYJMA, J. & PRYJMA, K. (1978) Attempts to evaluate the role of the third complement component (C3) and complement receptor lymphocytes (CRL'+') in the induction of the humoral immune response. *Arch. Immunol. Ther. Exp.* **26,** 295.

ROMBALL, C.G., ULEVITCH, R.J. & WEIGLE, W.O. (1980) Role of C3 in the regulation of a splenic PFC response in rabbits. *J. Immunol.* **124,** 151.

SHEVACH, E.M., FRANK, M.M. & GREEN, I. (1976) Linkage of the gene controlling the synthesis of the fourth component of complement to the major histocompatibility complex of the guinea pig. *Immunogenetics*, **3,** 595.

TEW, J.G. & MANDEL, T. (1978) The maintenance and regulation of serum antibody levels: evidence indicating a role for antigen retained in lymphoid follicles. *J. Immunol.* **120,** 1063.

UHR, J.W., DANCIS, J., FRANKLIN, E.C., FINKELSTEIN, M.S. & LEWIS, E.W. (1962) The antibody response

to bacteriophage ΦX 174 in newborn premature infants. *J. clin. Invest.* **41,** 1509.

UHR, J.W., FINKELSTEIN, M.S. & BAUMANN, J.B. (1962) Antibody formation. III. The primary and secondary antibody response to bacteriophage ΦX 174 in guinea pigs. *J. exp. Med.* **115,** 655.

WEDGWOOD, R.J., OCHS, H.D. & DAVIS, S.D. (1975) The recognition and classification of immunodeficiency diseases with bacteriophage ΦX 174. In *Immunodeficiency in Man and Animals* (ed. by D. Bergsma), Vol XI, No. 1. p. 331. The National Foundation-March of Dimes Birth Defects: Original Article Series. Sinauer Associates, Inc., Sunderland, Massachusetts.

WHITE, R.G., HENDERSON, D.C., ESLAMI, M.B. & NIELSEN, K.H. (1975) Localization of a protein antigen in the chicken spleen. Effect of various manipulative procedures on the morphogenesis of the germinal centre. *Immunology*, **28,** 1.