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The role of complement in the induction and regulation of immune responses

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Introduction

The vertebrate host, constantly threatened and invaded by various pathogens, has evolved two mechanisms of resistance. The first and phylogenetically oldest mechanism is the non-specific protection provided by complement alone or in concert with phagocytes such as neutrophils and macrophages. The second and evolutionarily later mechanism is the specific immune response involving antibodies, lymphocytes and macrophages. Both mechanisms serve the same function: to protect the host against pathogens.

The complement system consists of over 20 serum proteins which operationally constitute the classical (C1q, C1r, C1s; C4, C2, C3) and alternative (Factors B, D and P; H, I, C3) pathways, the terminal components (C5, C6, C7, C8, C9), and several regulatory proteins. The classical pathway is activated by immune complexes containing IgM and IgG antibodies (Loos, 1982), the acute phase serum protein, C-reactive protein (Kaplan & Volanakis, 1974) and RNA tumour viruses (Muller-Eberhard, 1978; Hirsch, 1982). The alternative pathway does not require antibodies and is directly activated by bacteria, viruses, fungi, helminth and protozoan parasites, and lymphoblastoid cells (Santoro, 1982; Hirsch, 1982; Kazatchkine & Nydegger, 1982; Sundsmo, 1982). Thus in general, the alternative pathway constitutes the natural defence mechanism of the non-immune host and probably evolved earlier.

The activation of both pathways generate a C3 convertase which cleaves C3 into C3a and C3b. From C3b, a C5 convertase is assembled which cleaves C5 into C5a and C5b. The C5b then assembles either the chemotactic C5b67 or the membrane attack complex C5b6789 (C5b–9) by combining sequentially and non-enzymatically with the rest of the terminal components. The biologically active C3a, C3b and derivatives, C5a, C5b67 and C5b–9, as well as Ba and Bb constitute the formidable multifaceted armoury of the complement system against a wide variety of pathogenic organisms. The occurrence of severe recurrent bacterial infections in genetic deficiencies of properdin (Sjoholm, Braconier & Soderstrom, 1982), C3 (Alper *et al.*, 1972; Thompson & Lachmann, 1977), C5 and the terminal components (Peterson *et al.*, 1979; Snyderman *et al.*, 1979) underscore the singular importance of complement in host resistance.

In addition to its well established role in non-specific resistance which has been extensively reviewed (Muller-Eberhard, 1978; Minta & Movat, 1979; Santoro, 1982; Fearon & Austen, 1980; Muller-Eberhard & Schreiber, 1980; Hirsch, 1982), there is increasing evidence that complement plays an equally important role in specific immune responses. This aspect of complement function in host resistance has received little attention (Pepys, 1976). The object of this review is to evaluate critically the literature on the role of complement in immune responses. The implications of complement activation by pathogens, particularly helminth and protozoan parasites, on the immune response is discussed.

Cobra venom factor *in vivo* and immunological phenomena

Germinal observations

Between 1969 and 1975, several observations suggested that complement might play a role in immune responses to specific antigens. Firstly, murine lymphocytes were shown by a rosette-forming cell assay to be comprised of C3 receptor positive ($C3R^+$) and negative ($C3R^-$) populations; the $C3R^+$ cells were identified as B lymphocytes on the basis of surface immunoglobulin markers (Bianco, Patrick & Nussenzweig, 1970). Secondly, germinal centres of human and rabbit lymphoid follicles were shown by immunofluorescent studies to contain immune complexes containing complement (Gajl-Peczalska *et al.*, 1969). Germinal centres are cellular accumulations of lymphoblasts, macrophages and reticular cells which are currently regarded as sites of antigen retention and precursor B memory cells (Thorbecke, Romano & Lerman, 1974; Papamichail *et al.*, 1975; Klaus & Humphrey, 1977; Tew & Mandel, 1978; Humphrey, 1979). The presence of C3 at these sites therefore suggested that C3 might play a role in antigen localization, and the presence of C3 receptors on lymphocytes and macrophages suggested that C3 might be important in the induction of the immune response to the localized antigen.

Cobra venom factor as a probe for complement function

In vivo administration to animals of cobra venom factor (CVF) results in the generation of two complexes, CVF-Bb and CVF-Bb-C3b, which have C3 and C5 amplification convertase activities respectively (Muller-Eberhard & Schreiber, 1980). These convertases are resistant to mammalian regulatory proteins H and I (Alper & Balavitch, 1976) and therefore cause a sustained depletion of C3 as a result of persistent C3 activation (Muller-Eberhard & Schreiber, 1980). The CVF itself is a thymus-dependent immunogen which

very rapidly stimulates anti-CVF antibody in thymus 'intact' mice (Pryjma & Humphrey, 1975). Normal C3 levels return coincidentally with the appearance of anti-CVF antibody which, *in vitro*, can inhibit the C3 cleaving activity of CVF (Pryjma & Humphrey, 1975; Romball, Ulevitch & Weigle, 1980), In thymectomized mice, however, no antibody formation occurs and C3 depletion is prolonged for much longer than in intact mice (Pryjma & Humphrey, 1975; Klaus & Humphrey, 1977). Thus CVF has been utilized as a probe for complement function *in vivo* and *in vitro*. Working on the premise that C3 might play a role in the induction of immunological responses, various workers studied the effect of *in vivo* CVF treatments on mammalian antibody responses to a variety of antigens.

Cellular co-operation and antigen processing

CVF treatment of mice resulted in suppression of antibody responses to thymus-dependent antigens such as sheep red blood cells (SRBC), ovalbumin (OVA), and hapten-carrier conjugates such as dinitrophenylated keyhole limpet haemocyanin (DNP-KLH), ovalbumin (DNP-OVA) and bovine gamma globulin (DNP-BGG), but not to thymus-independent antigens such as polyvinylpyrrolidone, haptenated dextran, levan or Ficoll, and Type III pneumococcal polysaccharide (Pepys, 1972, 1976; Klaus & Humphrey, 1977; Lewis *et al.*, 1976). In addition, the thymus-dependent antibody classes IgA, IgE and IgG were depressed while the thymus-independent IgM response was not (Nielsen & White, 1974; Pepys *et al.*, 1976a, b). The immunosuppression of thymus-dependent responses was, according to these workers, causally associated with the C3 depletion induced by CVF. Thymus-dependent responses involve co-operation between B and T lymphocytes (Nelson, 1981). It was therefore proposed that C3 played a role in this cellular co-operation presumably by facilitating antigen processing and presentation (Pepys, 1972, 1976; Pepys & Butterworth, 1974; Pepys *et al.*, 1976a, c; Pepys, Wansbrough-Jones & Mirja, 1976b). Conversely, thymus-independent responses which do not involve similar cellular co-operation did not require C3, and hence were insensitive to C3 depletion.

Role of B lymphocyte C3 receptors

Several studies suggested that $C3R^+$ and $C3R^-$ B lymphocytes were involved in thymus-dependent and thymus-independent responses, respectively. Treatment of mice with CVF before priming or boosting with DNP-BGG or DNP-Ficoll suppressed the anti-DNP-BGG, but not the anti-DNP-Ficoll response (Lewis *et al.*, 1976). To probe the basis for the insensitivity of the anti-DNP-Ficoll (thymus-independent) response to suppression by CVF, $C3R^+$ and $C3R^-$ splenic B lymphocytes were challenged with DNP-Ficoll *in vitro*. Whereas $C3R^-$ cells showed a significant plaque-forming cell (PFC) response to DNP-Ficoll, $C3R^+$ cells did not. These observations suggested that $C3R^-$ B lymphocytes responded to thymus-independent antigens, while $C3R^+$ B lymphocytes responded to thymus-dependent antigens and required C3. These concepts were supported by the observation that depletion of $C3R^+$ cells from murine spleen completely abolished the thymus-dependent response to SRBC in irradiated murine recipients of splenic cells (Arnaiz-Villena, Playfair & Roitt, 1975). However, subsequent studies on the role of B lymphocyte C3 receptors in thymus-dependent and thymus-independent antibody responses have not confirmed the model engendered by the data of Arnaiz-Villena *et al.*, and Lewis *et al.* These studies revealed firstly, that it was the repeating determinant rather than thymus-dependent nature of the antigen that determined activation of $C3R^-$ B lymphocytes (Parish & Chilcott, 1975); secondly, that $C3R^+$ and $C3R^-$ B lymphocyte subpopulations showed quantitative rather than qualitative differences in response to thymus-dependent antigens (Simon & Hammerling, 1977); and finally, that even $C3R^+$ B lymphocytes were responsive to thymus-independent antigens (Nariucki & Kakiuchi, 1980).

Thus the role of C3 receptors on B lymphocytes remains unresolved. However, some limited data suggest that they may be involved in the modulation of B lymphocyte proliferation and differentiation. Lambris *et al.* (1982) reported that $F(ab)_2$ or Fab antibody fragments against CR2 (C3d) receptors suppressed proliferative responses induced by pokeweed mitogen, MLR and tetanus toxoid, but not by phytohaemagglutinin or concanavalin A (Con A). Purified soluble C3d fragments or C3d-coated erythrocyte membranes were similarly suppressive. The authors concluded that this probably reflected a control mechanism for complement-mediated lymphocyte activation. Furthermore, the addition of purified C3b to cultured human mononuclear cells suppressed pokeweed mitogen-induced polyclonal immunoglobulin synthesis (Berger & Fleisher, 1983). Although direct binding to the B cell C3b receptor was not demonstrated, the suppressive effect of C3b occurred at concentrations

comparable to the affinity constant of the human C3b receptor for monomeric C3b.

Functions of germinal centres

The role of complement in postulated functions of germinal centres such as antigen trapping and generation of memory B cells was supported by evidence from CVF-treated animals. Labelled immune complexes or aggregated human IgG (AHGG) localized within splenic germinal centres within 8 hr of injection (Papamichail *et al.*, 1975; Klaus & Humphrey, 1977). However, pretreatment with CVF prevented antigen or immune complex localization. Autoradiographic studies revealed that labelled antigen was only trapped in the spleens of mice with normal C3 levels (Klaus & Humphrey, 1977). Furthermore, follicular dendritic cells which are quintessential for antigen trapping and immunological memory (Klaus *et al.*, 1980) express C3 receptors (Gerdes & Stein, 1982). This observation is consistent with the role of C3 in the antigen-trapping mechanism.

Intact and thymectomized mice developed comparable B cell memory to thymus-dependent antigens (Roelants & Askonas, 1972; Diamantstein & Blitstein-Willinger, 1974; Klaus & Humphrey, 1977). As mentioned previously, CVF treatment of thymectomized mice results in a prolonged C3 depletion (Pryjma & Humphrey, 1975). These mice were therefore utilized to study the relationship between C3 and B cell memory. CVF-treated thymectomized mice did not only show inhibited antigen trapping in splenic germinal centres but also complete abrogation of B cell memory (Klaus & Humphrey, 1977). This suppression was attributed to inhibition of proliferation of B memory precursor cells after priming. In a subsequent study, Klaus (1978) reported that administration of preformed immune complexes was highly efficient in generating B memory cells. However, C3 deprivation by CVF treatment of thymectomized mice abrogated the development of B memory cells induced by antigen–antibody complexes. Furthermore, administration of CVF after immune complex localization had already occurred did not affect the development of memory. These observations provide strong evidence that the development of B memory cells in germinal centres depends on the C3-dependent localization of antibody–antigen–C3 or antigen–C3 (generated by the alternative pathway) complexes at these sites.

Further evidence for complement's role in antigen localization and in regulation of immune responses came from a rabbit model of cyclical antibody production. After a single immunization of rabbits with AHGG, three successive peaks of IgG PFC to HGG appear on days 5, 13 and 21, respectively (Romball & Weigle, 1973). The second peak was shown to be correlated with HGG retention in splenic germinal centres (Romball & Weigle, 1973; Romball *et al.*, 1980). Treatment of rabbits with CVF 1 day prior to AHGG injection suppressed the second peak and this suppression correlated with decreased antigen retention in the spleen (Romball *et al.*, 1980). A significant finding was that the first peak was not suppressed. This point will be discussed later. When CVF was given after antigen localization had already occurred, the second peak was not suppressed. This study essentially confirmed complement involvement in antigen retention as reported by others (Papamichail *et al.*, 1975; Klaus & Humphrey, 1977). However, it differed in that CVF (or C3 depletion) did not suppress the first peak of PFC response despite the fact that HGG is a thymus-dependent antigen. In this study, the role of C3 appeared to involve regulation of subsequent PFC responses by prolongation of antigen retention, rather than induction of the immune response. Thus, the role of C3 in immune responses appears to involve, in the rabbit model at least, mechanisms other than facilitation of cellular co-operation, at least for the first peak of IgG PFC.

Interpretation of CVF-induced immunosuppression

The foregoing observations bring into focus the interpretation of CVF-induced immunosuppression. The possibility that it resulted from non-specific effects of CVF or contaminants therein was rigorously pursued and excluded (Pepys, 1976; Pepys *et al.*, 1976a; Romball *et al.*, 1980), thus leaving two possible mechanisms namely, C3 depletion or C3 activation which results in the former. Many investigators focused only on C3 depletion *per se* as the mechanism of CVF-induced immunosuppression. Yet evidence suggests that this is not the case (Martinelli, Matsuda & Osler, 1978a; Martinelli *et al.*, 1978b; Matsuda, Martinelli & Osler, 1978). Martinelli *et al.*, reported that there was no congruence between lowered C3 levels and CVF-induced immunosuppression. These authors favour the notion that activated complement products modulate macrophage function, resulting in immunosuppression. Indeed, evidence from *in vitro* studies suggests that C3 activation modulates leucocyte functions (Sundsmo, 1982).

To recapitulate, *in vivo* CVF experiments in mice and rabbits have suggested a role for complement in

antigen processing and presentation, antigen trapping and retention in germinal centres, regulation of cyclical antibody responses, and generation of B cell memory.

Effects of complement and anti-complement antibodies on leucocyte immune functions *in vitro*

The role of complement in immune responses has been studied *in vitro* using purified complement fragments or antibody to specific complement proteins. These studies will be reviewed under four main categories of lymphocyte function, namely antibody production, lymphokine generation, antigen-, mitogen- or alloantigen-induced proliferation, and cytotoxic functions, all of which are influenced by complement proteins.

In vitro *antibody responses*

C3 has been reported as being necessary, suppressive or unnecessary for antibody responses. The suppressive activity of C3 was apparently due to induction of a suppressor T cell population by an unidentified fraction of C3. By contrast, C5a enhanced antibody production by stimulating macrophage production of interleukin-1 (IL-1) which directly activated B lymphocytes into antibody production. A critical appraisal of these findings follows.

Supernatants of murine spleen cells contained detectable C3 levels after 4 days of culture (Feldmann & Pepys, 1974). The addition of anti-murine C3 to cultures of spleen cells from mice primed with trinitrophenylated (TNP)-KLH suppressed the thymus-dependent response to TNP-KLH but not the thymus-independent response to dinitrophenylated polymeric flagellin (DNP-POL). Although this confirmed the *in vivo* observation that C3 depletion suppressed thymus-dependent responses, the possibility that C3–anti-C3 immune complexes inhibited the response was not ruled out. It is well established that the Fc fragment of antibodies can inhibit antibody production (La Via & La Via, 1978). In a subsequent study, pure IgG $F(ab)_2$ anti-mouse C3 (i.e. without the Fc piece) was also suppressive, suggesting that C3 was necessary for the TNP-KLH response (Pepys *et al.*, 1976b).

The addition of purified human C3 to murine spleen cells did not enhance antibody production (Waldmann & Lachmann, 1975), suggesting that C3 was not essential. However, this study employed an 'artifactual' heterologous system and murine lymphocytes do not bind human C3b (Bianco *et al.*, 1970; Pepys, 1976; Papamichail & Pepys, 1978). In contrast, the addition of purified human or rat C3 inhibited rat *in vitro* secondary antibody responses to SRBC; this inhibitory activity resided in a C3a-like fraction, Fr2, obtained when purified C3 was passed over a gel filtration column equilibrated with high ionic strength buffer (Hobbs *et al.*, 1981). However, further studies with anti-C3a antibody, and the ability of Fr2 but not C3a to inhibit Con A-induced human peripheral blood lymphocyte (PBL) blastogenesis, indicated that C3a and Fr2 were not identical (Ballas *et al.*, 1983).

The effects of purified C3a were studied in the specific SRBC and the nonspecific polyclonal antibody response of human PBL and murine spleen cells (Morgan, Weigle & Hugli, 1982; Weigle *et al.*, 1982). C3a suppressed both types of antibody responses but did not suppress B or T lymphocyte proliferation. The target cell for C3a-mediated suppression was an $Lyt-1^+2^-$ suppressor-inducer cell (Morgan *et al.*, 1982) which apparently matured to a T suppressor cell with the $Lyt\text{-}1^-2^+$ phenotype (Weigle *et al.*, 1982). The possibility that C3a activated a pre-existing suppressor population was ruled out. The cleavage product of C3a, C3a des Arg, had no suppressive activity.

In contrast to the suppressive effects of Fr2 and C3a, C5a and C5a des Arg enhanced the murine antibody response to SRBC and the mixed lymphocyte reaction (MLR) (Goodman, Chenoweth & Weigle, 1982a, b; Weigle *et al.*, 1982). The mechanism of this augmentation involved the interaction of C5a and C5a receptor positive ($C5R^+$), Ia^- macrophages and the latter released IL-1. The addition of C5a-pulsed, Ia^- $C5R^+$ macrophages, but not lymphocytes, to B lymphocyte cultures enhanced the antibody response, in the presence of antigen-presenting Ia^+ macrophages. Supernatants of C5a-pulsed, $C5R^+$ and Ia^-P388D1 macrophage line, but not of the closely related $C5R^-$ P388 line, possessed IL-1 activity and directly augmented the anti-SRBC response. Thus, the B lymphocyte is a target of the IL-1 and the C5a-induced immunopotentiation requires $C5R^+$, Ia^- macrophages. These findings have been confirmed and extended to a human system (Morgan *et al.*, 1983).

T and B lymphocyte proliferation

In response to antigenic stimulation, T and B lymphocytes proliferate and differentiate into effector cells which perform such specialized functions as antibody production (B lymphocytes), cytotoxic or regulatory activity (T lymphocytes), and immunological memory (both cell types). The proliferative response therefore

serves the teleological purpose of expanding the quantitative and qualitative scope of the host defence. The evidence reviewed below suggests that complement, particularly C3 and its activated fragments and C4 might play a role in this aspect of host defence.

Addition of purified human C3b, but not C3a or C3c, to murine spleen lymphocytes was directly mitogenic and enhanced B lymphocyte thymidine incorporation and blast formation (Hartmann & Bokisch, 1975). The relevance of the findings of this heterologous system to homologous systems remains unknown. It was also shown by two other groups that complement activation in the vicinity of B lymphocytes *in vivo* and *in vitro* resulted in the binding of C3, presumably C3b, iC3b or C3d, on B lymphocyte complement receptors (Dukor *et al.*, 1974; Stelzer, Filppi & Rheins, 1980). The binding of C3 was demonstrated by inhibition of rosette formation with antibody sensitized, complement-coated SRBC (EAC). The bound C3 conferred upon the B lymphocytes the ability to respond to putative T cell stimuli. These observations stimulated the thinking that bound C3 might act as a second signal, in addition to antigen, for B lymphocyte activation, and that it might replace T cells in thymus-dependent responses (Dukor *et al.*, 1974; Hartmann & Bokisch, 1975; Stelzer *et al.*, 1980). Although the concept of lymphocyte bound C3 as a second signal for B lymphocyte activation has not been popular, there is new evidence that complement activation on the surface of lymphocytes and macrophages is associated with the activation of these cells (Sundsmo, 1982).

Morgan *et al.* (1983) have reported that C5a potentiates antigen- and alloantigen-induced T cell proliferation. Addition of C5a to human PBL cultures enhanced the T cell proliferative response to tetanus toxoid and in a primary mixed lymphocyte reaction (MLR). However, C5a was unable to potentiate mitogen-induced T and B cell proliferative responses. The phenotype of the C5a-stimulated T cell was $OKT3^+$, 4^+, suggesting that it was a helper T cell.

The role of C5 in human MLR- or mitogen-induced blastogenesis has been confirmed by Sundsmo (1983) in two ways. Firstly, Fab′ antibody fragments and affinity-purified murine monoclonal antibody against C5 inhibited blastogenesis in a dose-dependent manner. The inhibitory effect of the anti-C5 Fab was absorbed with purified C5 but not C3 or human IgG. Secondly, purified C5a stimulated lymphocyte proliferation in serum-free medium; trypsin and factor Bb which cleave C5 also stimulated lymphocyte blastogenesis presumably by utilizing lymphocyte surface bound C5 as substrate. Sundsmo also reported that anti-C6 and anti-C7 Fab inhibited lymphocyte proliferation in the one-way MLR. These observations suggest that in addition to C5, C6, C7 and the terminal complement membrane attack complex probably play a role in triggering lymphocyte blastogenesis.

In contrast to the stimulatory activity discussed above, Fr2 (Needleman, Weiler & Feldbush, 1981), C3c (Pepys & Butterworth, 1974; Schenkein & Genco, 1979), C3d (Koopman *et al.*, 1975; Schenkein & Genco, 1979) and kallikrein-derived C3d fragment, C3d-K (Meuth *et al.*, 1983) suppressed antigen-, mitogen- or alloantigen-induced T cell proliferation. However, C3a did not suppress these responses (Morgan *et al.*, 1982; Payan, Trentham & Goetzl, 1982; Meuth *et al.*, 1983).

Monokine and lymphokine production

Soluble mediators such as the monokine IL-1 and various lymphokines integrate the various cellular components of the immune response. As already mentioned, C5a induced release of IL-1 by $C5R^+$ macrophages (Goodman *et al.*, 1982a; Weigle *et al.*, 1982). The capacity of human peripheral blood monocytes for antigen-induced release of IL-1 also depends on their expression of C3 receptors (Whisler, Newhouse & Lachman, 1982). $C3R^+$monocytes secreted four- to 12-fold greater amounts of IL-1 than $C3R^-$ monocytes. It is tempting to speculate that complement activation by antigen generated C3b which triggered IL-1 production by $C3R^+$ monocytes. The cultures were supplemented with 12% foetal bovine serum but it was not indicated whether the serum was intact or heat-inactivated. The role of C3b or iC3b in inducing IL-1 secretion therefore remains to be established. A major activity of IL-1 is to induce synthesis and secretion of the T cell-derived mitogenic lymphokine, interleukin-2 (IL-2). Thus, through IL-2, a primary macrophage-derived *maturational* signal may be converted into a secondary T cell-derived *proliferative* signal that results in amplification of specific immune responses (Mizel, 1982). Complement may also regulate specific immune responses by regulation of lymphokine production as reviewed briefly below.

When homologous C3b, purified or in the form EAC, was added to cultures of human and guinea-pig B lymphocytes, the supernatants of these cultures possessed a monocyte chemotactic factor (MCF) (Mackler *et al.*, 1974; Wahl, Iverson & Oppenheim,

1974; Koopman *et al.*, 1975; Sandberg, Wahl & Mergenhagen, 1975). Elaborate control studies established that B lymphocytes were the source of the MCF, and possible intrinsic MCF activity of C3b, or contaminating C5a was unequivocally ruled out.

Human peripheral blood T lymphocytes upon stimulation by antigens or mitogens released a leucocyte migration inhibitory factor (LIF) (Payan *et al.*, 1982). The addition of C3a or the synthetic octapeptide C3a (70–77) inhibited T lymphocyte LIF production without affecting proliferation. The possibility that C3a was interfering with LIF activity rather than LIF production was excluded. The evidence was presented, by the use of monoclonal OKT antibodies to T cell surface antigens and Sepharose–C3a column adsorption, that the C3a target was a LIF-producing, helper/inducer T cell subset ($OKT4^+$). Thus, C3a apparently interacts with and negatively regulates a distinct T cell inducer population, just as it induces a distinct T cell suppressor population discussed above (Morgan *et al.*, 1982; Weigle *et al.*, 1982). The relationship between the two C3a target T cell populations remains unclear.

Cytotoxic T lymphocyte and natural killer functions

Antigen-specific, MHC-restricted cytotoxic T lymphocytes (CTL) can be generated *in vitro* by incubation of responder cells with allogeneic stimulator cells (Cerottini & Brunner, 1974; Ballas, 1981) or syngeneic cells infected with viruses (Ada, Leung & Ertl, 1981) or or certain protozoan parasites (Eugui & Emery, 1981). Natural killer (NK) cells, by contrast, are non-specific, apparently occur spontaneously and do not express immunological memory (Herberman & Ortaldo, 1981). Addition of C3 or Fr2 at the onset, but not on day 4 or 5, of a mixed lymphocyte culture inhibited CTL generation (Ballas *et al.*, 1983). These results suggested that C3 preparations inhibited an early event such as proliferation but had no effect on differentiated CTL. Indeed, neither C3 not Fr2 suppressed the lytic function of differentiated CTL, spontaneous NK or interferon-induced NK cells.

In summary, *in vitro* studies have confirmed findings of *in vivo* CVF experiments that complement may play an important role in specific immune responses. Specifically, they demonstrate that activated or native complement protein influences antibody and lymphokine production, lymphocyte proliferation and differentiative responses. However, there appears to be continuing controversy regarding the precise portions of the C3 molecule that are responsible for the various immunoregulatory activities. This has stemmed probably from the use of poorly purified or chemically ill-defined reagents (Meuth *et al.*, 1983) and the fact that fragments of the C3 molecule produced as a result of activation and degradation are not yet well characterized (Ballas *et al.*, 1983).

Immune responses in C4 deficiency states

The role of C4 in immune responses has been studied in C4-deficient humans and guinea-pigs. That C4 might be involved in immune recognition was suggested by three observations. Firstly, it was discovered that C4 genes mapped within the major histocompatibility complex in the mouse and man (Hinzova, Demant & Ivanyi, 1972; Demant *et al.*, 1973; Rittner *et al.*, 1975; Shreffler, 1976) which controls the cell–cell recognition phenomena important in immune surveillance mechanisms. Secondly, C4 was demonstrated on the surface of human lymphoid cells by immunofluorescence and rosette cell-forming assays (Ferrone, Pellegrino & Cooper, 1976). Thirdly, Ferrone and his colleagues reported that IgG anti-C4 antibody inhibited mitogen- and MLR-stimulated proliferation. Although these authors did not rule out the possibility of Fc blockade, they concluded that C4 was required for these responses. However, C4 requirement was not demonstrated in guinea-pig MLR as $F(ab)_2$ anti-C4 did not inhibit the reaction (Burger & Shevack, 1979). It is not clear whether these conflicting results arose because of species or technique differences. Further support for the requirement of C4 in MLR comes from the report of diminished MLR reactions in two patients with C4 deficiency (Mascart-Lemone *et al.*, 1982).

The normal response to a standard dose of intravenously administered bacteriophage ΦX174, a T cell-dependent antigen, consists of a primary low titred IgM response and a secondary high titred IgG response (Ochs *et al.*, 1979, 1983). In humans and guinea-pigs with C4 and C3 deficiencies however, there was severe suppression of both responses and lack of the IgM–IgG switch. Primary immunization with concomitant administration of normal guinea-pig serum as a source of C4 restored the primary and secondary antibody responses to a level qualitatively and quantitatively comparable to those of normal controls.

The leucocyte complement system

It might be difficult to visualize how a serum com-

ponent could regulate lymphocyte function in solid lymphoid tissues not bathed in serum. However, recent findings suggest that there exists a distinct, functionally active leucocyte complement system (LCS) on the surface of macrophages and lymphocytes. These have been comprehensively reviewed elsewhere (Sundsmo, 1982) and will only be briefly mentioned here. Firstly, the complement proteins C1q, C3, C4, C5, C6, C7, C8, C9, factor B and properdin have been detected on the surface of leucocytes; some of these are actively synthesized but not secreted. Secondly, lymphocytes and lymphoblastoid cells activate complement on incubation in heterologous, homologous and autologous serum. Thirdly, lymphocyte activation is invariably associated with increased expression of complement activation-specific C5–9 neoantigens, specific for C5, C6, C7, C8, C9, and C5b–9 complex. Finally, activation of the LCS occurs even in serum-free medium. Clearly, a system which functions independently of serum and is susceptible to activation by various antigens, in uniquely suited for a role in induction and regulation of antigen-specific immune responses.

Complement activation of macrophages and mast cells

Activated complement products stimulate macrophages and mast cells to release important immunoregulatory mediators. Rutherford & Schenkein (1983) have reported that C3b, iC3b and C3c stimulate human monocytes to release prostaglandins, the class of which was not determined. Hadding *et al.* (1982) similarly reported that C3b stimulated peritoneal macrophages to release prostaglandin E. There is abundant evidence that prostaglandins regulate immune responses (Goodwin & Webb, 1980; Gemsa, 1981), particularly prostaglandins of the E class which have been shown *in vitro* to inhibit T lymphocyte proliferation and lymphokine secretion (Smith, Steiner & Parker, 1971; Goodwin, Bankhurst & Messner, 1977; Goodwin & Webb, 1980; Gemsa, 1981; and Gordon, Bray & Morley, 1976). Walker *et al.* (1983) have reported that prostaglandin E_2 inhibits T lymphocyte proliferation by inhibiting IL-2 production.

The anaphylatoxins C3a and C5a induce mast cell degranulation which results in the release of histamine and other inflammatory mediators (Hugli & Muller-Eberhard, 1978). Histamine affects a wide spectrum of immunological functions ranging from cytotoxicity (Plaut *et al.*, 1973; Schwartz, Askenase & Gershon, 1980), lymphocyte proliferation (Ogden & Hill, 1980; Thomas, Huchet & Granjon, 1981) and antibody production (Herbert *et al.*, 1981) to lymphokine release (Rocklin, 1976) and delayed hypersensitivity skin reactions (Avella *et al.*, 1978; Ambanelli *et al.*, 1979). A possible control mechanism for these histamine-mediated effects is the C3-induced granulocyte release of histaminase recently reported by Melamed *et al.* (1983).

It is clear from the above discussion that activation of complement may indirectly influence a number of immune functions by activation of macrophages, mast cells, and possibly other cell types to release immunoregulatory mediators. The leukotrienes constitute another important group of mediators and are discussed separately below.

Leukotrienes and complement

There is increasing evidence that 5- or 15-lipoxygenation metabolites of arachidonic acid, particularly the leukotrienes B_4, D_4 and E_4, and 15-hydroxy-eicosatetraenoic acid (15-HETE) modulate several lymphocyte functions. These include suppression of mitogen-induced T lymphocyte proliferation (Bailey *et al.*, 1982; Webb *et al.*, 1982; Payan & Goetzl, 1983), induction of T suppressor cells which suppress lymphocyte proliferative responses (Rola-Pleszczynski, Borgeat & Sirois, 1982), and modulation of T lymphocyte migration *in vitro* (Payan & Goetzl, 1981). Although lymphocytes generate substantial quantities of these metabolites (Parker *et al.*, 1979; Goetzl, 1981; Bailey *et al.*, 1982), macrophages, eosinophils and neutrophils constitute the major source (Borgeat & Samuelsson, 1979; Bach *et al.*, 1980; Rouzer *et al.*, 1980; Jorg *et al.*, 1982). These cells possess receptors for various activated complement fragments (Ross, 1982) and it is possible that the binding of activated complement fragments present in the microenvironment may stimulate the release of 15-HETE and leukotrienes. Indeed, Claesson, Lundberg & Malmsten (1981) have reported that serum-treated zymosan, which is coated with C3b, stimulates the synthesis and release of leukotriene B_4 from human neutrophils. Thus a role for C3 receptors in triggering leukotriene release is a distinct possibility. The binding of these receptors by activated complement fragments may also trigger the oxidative respiratory burst of phagocytes which results in the generation of several reactive oxygen metabolites such as the superoxide anion, hydrogen peroxide (H_2O_2), and hydroxyl

radicals (Babior, 1978). There is evidence that these oxygen metabolites oxidatively inactivate leukotrienes (Goetzl, 1982; Lee *et al.*, 1982; Henderson, Jorg & Klebanoff, 1982; Henderson & Klebanoff, 1983). Furthermore, C3b stimulates prostaglandin release as already discussed, and a recent report has implicated prostaglandins in the suppression of the release of leukotriene B_4 from neutrophils (Ham *et al.*, 1983). We therefore speculate that complement activation may play a role in determining the local availability and half-lives of leukotrienes, and in this manner therefore indirectly influences several lymphocyte functions.

Immune complexes and complement

There is abundant evidence that immune complexes (IC) suppress or potentiate humoral and cellular immune responses (Theofilopoulous & Dixon, 1979). Immunoregulation by IC is probably mediated by the Fc regions of antibody which suppress antibody responses (La Via & La Via, 1978); augment antigen-induced (T cell) proliferative, mixed lymphocyte culture, and cell-mediated lympholysis responses (Morgan, Thoman & Weigle, 1981a, b); induce B cell proliferation (Weigle & Berman, 1979) and polyclonal antibody secretion (Morgan & Weigle, 1980); and possess potent adjuvant effects (Morgan *et al.*, 1980). Immune complexes and complement have a two-sided relationship in that IC activate complement and complement may be involved in IC degradation and clearance (Theofilopoulous & Dixon, 1979).

Immune complex-induced activation of complement may generate biologically active complement fragments which account for the various immunoregulatory effects of IC. However, evidence for complement involvement in IC-mediated lymphocyte proliferation is not currently clear. Bloch-Shtacher, Hirschhorn & Uhr (1968) reported that in cultures of human lymphocytes and IC, fresh normal rabbit serum supported a significantly greater proliferative response compared with aged serum. Supernatants of normal serum and IC possessed stimulatory activity, which the authors attributed to an unidentified C3 fragment. In another study, fresh, but not heated or zymosan-treated rabbit serum supported IC-induced proliferation of normal rabbit lymphocytes (Soderberg & Coons, 1978). In contrast, Moller (1969) reported that supplementation of cultures of human lymphocytes and IC with fresh guinea-pig serum did not enhance DNA synthesis. In fact DNA synthesis was greater in such cultures supplemented with heat-inactivated serum. Morgan & Weigle (1983) recently reported that IC stimulated murine splenic B lymphocyte proliferation and polyclonal antibody secretion in cultures supplemented with heat-inactivated foetal calf serum. The role of complement in the immunoregulatory properties of ICs therefore remains equivocal.

The role of complement in the clearance of IC is also equivocal. *In vitro* studies have shown that C3 and C3 receptors are important for binding, internalization and degradation of immune complexes (van Snick & Masson, 1978; Kijlstra, van Es & Daha, 1979a, b, 1981; Abrahamson & Fearon, 1983). In a rat model, impairment of Kupffer cell Fc and C3 receptor function by circulating IC correlated with impaired clearance of labelled IC (Nishi *et al.*, 1981), thus implicating these receptors in IC clearance. However, assessment of the relative importance of Fc or C3 receptors was not possible. Wilson *et al.* (1982) reported an hereditory deficiency of C3b receptors in cells of patients with systemic lupus erythematosus (SLE). The paucity of C3b receptors in SLE patients has also been documented by another group (Iida, Mornaghi & Nussenzweig, 1982). In these patients there is marked impairment of IC clearance, thus supporting the notion that complement and C3 receptors are important for eliminating IC. However, in one study, depletion of complement by CVF as assessed by a haemolytic assay, did not affect the clearance of soluble IgG-containing complexes in rabbits (Arend & Mannik, 1971). These observations have been confirmed by others (Bockow & Mannik, 1981; Harkiss & Brown, 1981, 1983; Brown & Harkiss, 1981) and suggest, contrarily, that complement is not important for IC clearance. However, this interpretation should be tempered with the caveat that C3 depletion by CVF is not absolute and that residual, albeit small amounts, of C3 may be activated by the classical pathway convertase whose generation is not affected by CVF. Furthermore, C3 synthesis by macrophages and monocytes may provide sufficient complement for IC clearance.

Thus evidence, albeit equivocal, exists that complement mediates IC immunoregulation, and probably influences the longevity of this immunoregulation by facilitating IC clearance.

Synthesis and catabolism of complement

The evidence presented in the preceding sections highlight documented and possible immunoregula-

tory activities of several complement proteins. A consideration of complement synthesis, regulation of complement activation, and the degradation of activation products is therefore warranted, since these events ultimately determine local availability of the various complement fragments within the microenvironment of immunocompetent cells. The synthesis of C3 and other complement proteins has been extensively reviewed (Colten & Einstein, 1976). The liver has been implicated as an important site of C3 synthesis. In addition, there is abundant evidence that macrophages and monocytes synthesize and secrete many complement proteins such as C1, C2, C3, C4, C5, C6, C7, C8 and factors D, B and P. These have been referenced extensively in Sundsmo (1982) and Zimmer *et al.* (1982). A significant observation is that a local inflammatory stimulus results in macrophage activation with concomitant augmented synthesis and secretion of C4, C2 and C3 (Cole *et al.*, 1980; Zimmer *et al.*, 1982). Thus, leucocytes possess the synthetic capacity to augment the local availability of certain complement proteins, which may be secreted or remain surface bound (Sundsmo, 1982).

The regulation of complement activation and the degradation of the activation products have been reviewed elsewhere (Muller-Eberhard & Schrieber, 1980; Loos, 1982; Kazatchkine & Nydegger, 1982). We will therefore very briefly discuss the fate of the activated complement fragments such as C3a, C3b, iC3b, C3c and C5a. Surface-bound C3b on leucocytes is almost invariably converted to surface-bound iC3b by factor I in the presence of factor H which serves as a cofactor. Recently, a membrane-bound protease specific for C3b, but distinct from the C3b receptor (CR1) has been reported for human erythrocytes (Charriaut, Barel & Frade, 1982). It is not known at present whether lymphocytes and macrophages possess a similar membrane C3b-ase activity. The C3b on the surface of activators of the alternative pathway such as zymosan resist factor I and factor H cleavage and remain in this form. The surface-bound iC3b may be cleaved to release C3c into the fluid phase, leaving C3d bound to the surface, by two mechanisms. Firstly, several endoproteases have been implicated *in vitro* but the presence of a physiologically relevant protease *in vivo* remains to be established. Secondly, recent evidence implicates the CR1 as a cofactor in factor I-mediated cleavage of iC3b (Medof *et al.*, 1982; Ross *et al.*, 1982; Medicus, Melamed & Arnaout, 1983). The presence of a membrane-bound C3b-ase and CR1 cofactor requirement, if established for leucocytes, would therefore confer upon leucocytes the capacity to regulate C3b- and iC3b-mediated cellular processes through ligand degradation. However, information about the half-lives of these ligands is sparse.

Tryptic degradation of C3c produces the leucocytosis-inducing C3e. However, the physiological counterpart of the enzyme remains to be established. In contrast, human C3a and C5a are rapidly inactivated within 2 min at 20° by a serum anaphylatoxin inhibitor (Bokisch & Muller-Eberhard, 1970). This inhibitor is present in human, guinea-pig, rabbit and rat sera and has been identified as a carboxypeptidase B which cleaves and releases the C-terminal arginine residue. The evidence reviewed in a preceding section showed that C5a des Arg retains, but C3a des Arg loses immunoregulatory activity (Goodman, Chenoweth & Weigle, 1982a, b; Weigle *et al.*,1982; Morgan *et al.*, 1982). In light of the potency of the serum carboxypeptidase B activity, it is possible that under more physiologically relevant conditions *in vivo*, C5a may be more important than C3a since the latter loses immunoregulatory activity following degradation.

To recapitulate, leucocytes appear to have the capacity to augment the local availability of certain complement proteins. Several mechanisms for the degradation of C3b, iC3b, C3a, C3c and C5a have been described *in vitro* and could potentially be important for determining the half-lives of these ligands. However, the physiological relevance of some mechanisms remain unknown.

The role of complement in immune dysfunction during parasitic infections

Many helminth and protozoan parasites activate complement via the alternative pathway in the absence of antibody and via the classical pathway in the presence of antibody (Santoro, 1982). Host infection by these parasites is invariably accompanied by marked modulation of various immunological functions (Terry, 1977; Mitchell, 1979; Bancroft & Askonas, 1982). Given the diversity of the effects of immunologically active complement fragments on the immune response as reviewed above, it is reasonable to speculate that parasite-induced activation of complement constitutes a probable mechanism of the immune dysfunction commonly seen in parasitic diseases. Some pertinent observations from several host–parasite models will be briefly highlighted to support this speculation.

In the murine host, *Nippostrongylus brasiliensis*

(helminth) infection results in either suppression or potentiation of humoral antibody responses to heterologous antigens. IgE, IgG1 and mercaptoethanol resistant agglutinating antibody responses to DNP were suppressed, with the IgM response being the least affected, if mice were immunized 2–7 days after infection with *N. brasiliensis* (Haig, Lima & Mota, 1980). A similar observation was made in this laboratory by McElroy, Szewczuk & Befus (1983) who reported that IgG, IgM and IgA antibody responses to TNP-BGG (thymus-dependent antigen) were suppressed, whereas the humoral responses to DNP-Ficoll (thymus-independent) were not. Furthermore, memory to TNP-BGG was also abrogated in infected mice. In the *N. brasiliensis*-murine system, suppression occurred only if immunization took place during the time when most of the infective inoculum was still present in the hosts and probably activating complement. *N. brasiliensis* L3 activate complement via the alternative pathway (Mackenzie *et al.*, 1980; Egwang, Gauldie & Befus, 1984). The pattern of this immunosuppression and the critical requirement that parasite infection precede antigen presentation are strikingly similar to CVF-induced immunosuppression: humoral antibody responses and development of B memory cells were suppressed only if CVF administration preceded antigen presentation or immune complex administration (Klaus & Humphrey, 1977, Klaus, 1978). This similarity between the immunosuppression in the *N. brasiliensis*-murine and CVF-murine systems strongly suggest a common mechanism such as the ability of both *N. brasiliensis* and CVF to activate complement via the alternative pathway. It is interesting that in the *N. brasiliensis*-murine system, potentiation of IgE responses to heterologous antigens occurred if immunization followed infection (Kojima & Ovary, 1975). Suppression or potentiation may therefore reflect whether complement-induced suppressor or helper mechanisms predominate.

In murine malaria, *Plasmodium berghei* and *Plasmodium yoelii yoelii* infections result in suppression of not only primary antibody responses but also induction and expression of memory to heterologous infection or antigen (Salaman, Wedderburn & Bruce-Chwatt, 1969; McBride & Micklem, 1977, 1979). In this system, the striking thing is that the immunosuppression occurs only if immunization occurs during patent parasitaemia, at which time complement activation is probably taking place as shown by depressed native C3 levels during malaria (Greenwood & Brueton, 1974).

During murine and bovine trypanosomiasis, generalized suppression of lymphocyte responses and the appearance of suppressor cells in the spleen parallel the appearance of living trypanosomes in peripheral blood. At this time infected mice show poor germinal centre activity. However, upon clearance of trypanosomes by the trypanocidal drug, Berenil, there is rapid restoration of normal responsiveness and loss of suppressor cell activity, and treated mice show extremely active germinal centres (Murray *et al.*, 1974; Roelants *et al.*, 1979; Rurangirwa *et al.*, 1979, 1980).

In all of the parasitic infections mentioned above, the presence of living parasites in the host during the time of heterologous antigen presentation was essential for the generation of immunosuppression. The following scenario is therefore possible. Parasite activation of complement results in two important consequences. Firstly, depletion of native C3 occurs. This results in insufficient opsonization of antigen or immune complexes and therefore impaired antigen trapping and generation of memory in germinal centres, as shown in the CVF-murine systems. Additionally, the requisite cellular cooperation for thymus-dependent responses do not occur, resulting in impaired thymus-dependent responses but intact thymus-independent responses, as shown by McElroy *et al.* (1983). Secondly, 'immunologically' active complement fragments are generated which exert a diversity of effects on lymphocyte function directly or indirectly by the stimulation of mediator release from macrophages or granulocytes (IL-1, prostaglandins, leukotrienes, etc.) and mast cells (histamine) (Table 1). Thus, C3a may induce suppressor cells directly as shown by Morgan *et al.*, (1982, 1983) or indirectly through stimulation of mast cell histamine release. Clearance of the parasites by self-cure as in some helminth infections, or by drug treatment, results in the cessation of complement activation with concomitant removal of the immunologically active complement fragments and the return of native C3 levels to normal. The clearance of parasites from the host would therefore result in the restoration of normal immune responsiveness.

Rurangirwa *et al.* (1980) observing that following therapy of bovine trypanosomiasis, the restoration of normal C3 levels rather lagged behind the more rapid restoration of normal immune responses, remarked that the role of complement in the immunosuppression during bovine trypanosomiasis remains equivocal. These authors assumed that it was lowered native C3 levels *per se* that was associated with immunosuppression. However, according to the scenario postu-

Table 1. The role of complement in various immunologic functions*

1. Antigen trapping, processing and presentation
2. Thymus-dependent antibody responses
3. Regulation of splenic cyclical antibody responses
4. Recognition in the MLR
5. Regulation of IgM–IgG switch
6. Modulation of T and B lymphocyte proliferation and differentiation
7. Activation and differentiation of B lymphocytes (second signal)
8. Induction of T suppressor or helper cells
9. Involvement in immunomodulation by immune complexes
10. Modulation of monokine or lymphokine release
11. Stimulation of secretion of mediators (e.g. histamine, IL-1, prostaglandins, leukotrienes, etc.)

* References, which can be found in the text, have been intentionally left out for spatial reasons.

lated above, the lagging of normal C3 levels behind the restoration of normal immune responses is to be expected. Synthesis of more C3 and its equilibration in various tissues and blood would naturally require several days to occur and this explains the lag period.

In conclusion, the findings from several host–parasite systems suggest, but do not prove, that complement activation by metazoan and protozoan parasites constitutes one underlying mechanism of immune dysfunction in parasitic disease. At the other end of the spectrum, complement activation by parasites may trigger afferent and efferent arms of anti-parasite immune responses. However, there is currently a paucity of information on the effect of infection by various pathogens including bacteria, viruses, metazoan and protozoan parasites on synthesis and catabolism of complement components and the regulatory proteins C1 inhibitor, C4 binding protein, and factors H, I and P. It would be surprising if some infections did not have a major effect on the synthesis and/or catabolism of complement components and regulatory proteins. Recent results have established that interleukin-2 production can be markedly depressed by *Leishmania donovani* (Reiner & Finke, 1983) and *Trypanosoma cruzi* (Harel-Bellan *et al.*, 1983) infections. Future studies along these lines would not only provide useful insights into the role of complement in the host–parasite relationship but contribute additional information on the survival strategies of these pathogens in vertebrate hosts.

Conclusions

The complement system plays an important role in host resistance and individuals deficient in certain components suffer recurrent and sometimes fatal infections. Although complement function has been traditionally viewed in the context of a predominantly non-specific resistance mechanism, there is accumulating evidence that complement is involved in the induction and regulation of specific immune responses. Specifically, complement activation appears to be intrinsically associated with the activation of macrophages and lymphocytes, the localization and retention of antigens in germinal centres, the generation of B cell memory, cellular co-operation, immunosuppression, immunopotentiation, and the regulation of cyclical antibody production. A critical appraisal of the literature on immune dysfunction in some parasitic diseases suggest that complement may be involved in the genesis of impaired immune responses. Thus the current view of complement function as a non-specific resistance mechanism must be broadened to embrace the concept that complement plays an important role in immunoregulation.

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