The role of complement in the acquired immune response

C. H. NIELSEN,*† E. M. FISCHER† & R. G. Q. LESLIE *Department of Immunology and Microbiology, University of Southern Denmark, Odense, Denmark, and †INSERM U430, Hôpital Broussais, Paris, France

SUMMARY

Studies over the past three decades have clearly established a central role for complement in the promotion of a humoral immune response. The primary function of complement, in this regard, is to opsonize antigen or immune complexes for uptake by complement receptor type 2 (CR2, CD21) expressed on B cells, follicular dendritic cells (FDC) and some T cells. A variety of mechanisms appear to be involved in complement-mediated promotion of the humoral response. These include: enhancement of antigen (Ag) uptake and processing by both Ag-specific and non-specific B cells for presentation to specific T cells; the activation of a CD21/CD19 complex-mediated signalling pathway in B cells, which provides a stimulus synergistic to that induced by antigen interaction with the B-cell receptor (BCR); and promotion of the interaction between B cells and FDC, where C3d-bearing immune complexes participate in intercellular bridging. Finally, current studies suggest that CR2 may also play a role in the determination of B-cell tolerance towards self-antigens and thereby hold the key to the previously observed correlation between deficiencies of the early complement components and autoimmune disease.

IN VIVO EVIDENCE FOR THE ROLE OF COMPLEMENT IN THE ACQUIRED IMMUNE RESPONSE

While it is only in recent years that the role of complement in the induction and regulation of acquired immunity has come to be fully appreciated, the first indication that this might be the case dates back to the mid-1970s to the pioneering studies of Mark Pepys, who showed that depleting mice of complement by injection of cobra venom factor (CVF) markedly impaired their humoral responses to primary antigen[1,2] (see Table 1 for a historical overview). Evidence for involvement of the classical pathway (CP) of complement activation was derived from the observation that C2 or C4 deficiency[3–5] results in similar impairment in immune responsiveness to that seen with C3-depleted mice, or with C3-deficient guinea-pigs[6] and dogs.[7] Support for this view was also provided by the finding that the enhancement of a humoral response achieved by administering immunoglobulin M (IgM) class antibodies of appropriate specificity, concurrently with the immunizing antigen, failed to occur when a mutant IgM monoclonal antibody (mAb) with impaired complement-activating ability was employed.[8] Somewhat paradoxically, CP component deficiencies in humans[4] (reviewed in reference 9) and guinea-pigs[10] were also found to be predisposing for autoimmune conditions, suggesting that the complement system may additionally be involved in the induction and/or maintenance of tolerance at the humoral level.

Initial *in vivo* studies of the mechanism(s) underlying the contribution of complement to acquired immunity, focused attention on its role in ensuring the retention of antigen by the follicular dendritic cells (FDC) in germinal centres,[11–15] thereby providing a constant source of antigenic stimulus to activated, antigen-specific B cells. Concomitant with subsequent *in vitro* studies, revealing that the C3-fragment- (iC3b and C3dg) binding complement receptor type 2 (CR2/CD21) can act synergistically with the B-cell antigen receptor (BCR) in B-cell activation (see below), a range of *in vivo* investigations provided clear evidence that CR2 was also involved in the induction of a primary humoral response (Table 1). These included:

(1) Studies showing that blockade of murine CR2 with a mAb which interfered with ligand binding, abrogated the primary immune response to both T-dependent[16] and T-independent antigens[17] without impairing T helper (Th) cell induction.[18]

(2) The demonstration that whereas neutralization of CR2 function by competing soluble CR2 diminished the humoral response,[19] ligation of C3d fragments to the immunizing antigen markedly enhanced the response. Thus, immunization of mice with the engineered chimeric protein, hen egg lysozyme-(HEL-)$C3d_3$, resulted in a 100-fold and 10 000-fold enhancement in response, respectively, compared to immunization with HEL in Freund's complete adjuvant or with HEL alone.[20]

(3) Studies with CR2-knockout mice.[21,22] These, in common with their C3- and C4-knockout counterparts,[23] displayed marked inhibition in the production of Abs arising

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Correspondence: R. G. Q. Leslie, Department of Immunology and Microbiology, University of Southern Denmark, Winsløwparken 21, DK-5000, Odense C, Denmark.

from class switching (i.e. immunoglobulin G [IgG]2a, IgG2b and IgG3), as well as the generation of fewer, and smaller, germinal centres.

(With regard to the aforementioned observations, it should be noted that complement receptor type 1 (CR1/CD35) and CR2 in mice are encoded from the same gene, Cr2, where CR1 is expressed as a longer isoform of CR2.[24] Thus, the effects of a lack of one or other of these receptors cannot readily be distinguished.)

While these studies demonstrated unequivocally that CR2 is implicated in the induction of a humoral response, they did not shed any light on the relative contributions to this process made by CR2 expressed on B cells and FDC, respectively. This was achieved using two reconstitution mouse models. In the first model, RAG-2 mice were reconstituted with B cells from CR2-knockout mice to provide CR2-negative B cells against a CR2-positive FDC background[24] In the second model, $Cr2^{-/-}$ mice were implanted with $Cr2^{+/+}$ bone marrow to create the reverse situation[26] (Table 2). In the former case of B-cell CR2 deficiency, the phenotype resembled that of the total CR2 knockouts (namely, an impaired initial response and failure of class-switch). By contrast, mice with selective CR2 deficiency on their FDC displayed a normal initial response, although the long-term IgG antibody response was depressed and there was a lack of memory induction.

It should be noted that an observation common to many of the aforementioned studies was that the contribution of complement to induction of a humoral response was most apparent upon immunization with low doses of antigen and was diminished or even abolished by administering the antigen in higher doses. However, it can be argued that immunization with low doses of antigen may well reflect the type of challenge that arises from many naturally occurring infections, and thus be closely representative of the physiological state.

COMPLEMENT OPSONIZATION OF ANTIGENS

The observations that C2, C4, C3 and CR2 deficiencies result in impairment of the humoral immune response whereas C5 deficiency does not,[8] all point to C3 fragment engagement of the CR2 receptor as being the pivotal interaction in the induction process. However, equally decisive is the physical association of the C3 fragment with the immunizing antigen.[20] Under normal physiological conditions, this association may arise by covalent deposition of C3 fragments on antigens capable of activating the alternative pathway (AP), classical pathway (CP) or lectin pathway (LP) directly. Alternatively, the formation of immune complexes (IC) with circulating antibodies, which are then capable of activating complement via the CP, would result in C3 fragment attachment to the antigen and/or the adjacent antibodies. The latter mechanism may be operative, even in the primary immune response, owing to the existence of natural antibodies, which are polyreactive immunoglobulins of IgM, IgG or immunoglobulin A (IgA) isotypes. These Abs recognize a great variety of self- and foreign antigens with low affinity, and are present in the serum of all individuals without previous deliberate immunization (reviewed in references 27–29). Natural antibodies express heavy chain variable region (V_H) genes in virtually non-mutated configuration[30] and are often directed against public epitopes and antigens that are well conserved during evolution. They are thought to play an important role in the first line of defence during the period necessary to mount a specific antibody response, and may be involved in immune regulation by facilitating formation of complement-activating IC with antigen and subsequent binding to antigen-presenting cells (APC) via complement receptors (see below).

MECHANISMS WHEREBY COMPLEMENT ENHANCES THE IMMUNE RESPONSE

The attachment of C3 to an antigen has been demonstrated to affect various phases of the acquired immune response. With respect to the function of B cells, it appears to be involved in:

(1) The promotion of antigen uptake, processing and presentation by B cells to antigen-specific T cells;
(2) Direct activation of B cells; and
(3) Facilitation of B-cell interactions with FDC.

Promotion of antigen uptake, processing and presentation by B cells

In 1988, Arvieux *et al*. demonstrated that coupling of C3b or C4b to an antigen, tetanus toxin (TT), enhanced the proliferative and cytotoxic responses of antigen-specific Th cell clones.[31] The role of complement, in this connection, was to

Table 1. Milestones in the study of the role of complement in the immune response

	In vivo studies	*In vitro* studies
1970–1980	C3 depletion abrogates the HIR[1,2]	
1980–1990	Classical pathway deficiencies result in impaired HIR[3–7]	Identification of CR2 on B cells[108] and FDC[67] *In vitro* demonstration of synergy in BCR and CR2 signalling[41–44]
1990–2000	Blockade of CR2 receptor function abrogates the primary HIR[16–19]	Identification of the CR2/CD19 signalling complex[47,48]
	Coupling of C3d fragments to antigen ehnahces the HIR[20]	Opsonized immune complexes of Ag and natural Ab are shown to induce the HIR[32,36]
	Knockout mice define the roles of CR2 on B cells and FDC in the HIR[25,26,77] Cr2 and CD19 shown to regulate B-cell self-tolerance[106,107]	Characterization of BCR, CR2/CD19 and FcγRIIb signalling pathways[51–66]

Ab, antibody; Ag, antigen; BCR, B-cell receptor; FDC, follicular dendritic cells; HIR, humoral immune response.

target the antigen to CR2 and CR1 on Epstein–Barr virus (EBV)-transformed B cells employed for antigen presentation to the T cells. More recently, it has been observed that the binding, processing and presentation of antigens, in the form of IC, are substantially enhanced by incorporation of C3 fragments into the complexes.[32,33] Furthermore, CR1 and, particularly, CR2 on the B cells were shown to be of key importance for the binding of the opsonized IC.[32–34] Notably, studies with both C3b-TT[35] and opsonized IC[32,33,36] have indicated that the interaction of C3 fragments with CR2 (and CR1) allows non-specific B cells to participate in antigen presentation to specific T cells – albeit less efficiently than antigen-specific B cells[32] – thereby greatly enhancing the efficiency of antigen presentation. Subsequently it was shown that only the antigen-specific B cells were reciprocally stimulated for antibody synthesis upon culture of peripheral blood mononuclear cells (PBMC) with IC, indicating that regulatory mechanisms exist to prevent polyclonal B-cell activation.[36]

Recent studies indicate that attachment of C3 to antigens not only enhances the antigen uptake by B cells but also modulates downstream events, such as endosomal targeting of antigen, as well as the processing and binding of peptides to major histocompatibility complex (MHC) class II molecules. Thus, covalent attachment of C3b to TT results in enhanced and prolonged stimulation of specific T cells by both non-specific and TT-specific B-cell clones, presumably owing to delayed proteolysis of C3b–TT by an endosomal enzyme, cathepsin D.[37] It has been suggested that the delayed endosomal proteolysis results in an improved peptide loading on MHC class II molecules and an increased stability of these molecules in the lysosomes.[38] Consistent with this observation is the finding that attachment of C3b to the heavy chain of murine IgG results in a 100-fold reduction in the amount of IgG required for human B-cell lines to stimulate heavy chain-specific T-cell clones, without enhancing antigen presentation to light chain-specific T-cell clones.[39]

Apart from facilitating antigen presentation, IC may play an additional enhancing role by inducing the expression of costimulatory molecules. Thus, the costimulatory molecule CD80,[32] which binds to CD28 on T cells, has been demonstrated to be up-regulated by the ligation of IC-associated IgG to B-cell FcγRII (CD32), with CR2 playing a synergistic role. Likewise, IC ligation to either CR2 or FcγRII can activate another costimulatory molecule, lymphocyte function-associated antigen-1 (LFA-1; CD11a/CD18), that binds to intercellular adhesion molecule-1 (ICAM-1, CD54) on T cells.[36] (Note: this enhancing role of FcγRII in immune regulation is in contrast to the down-regulatory signalling through this receptor seen in relation to B-cell activation – see below.) It has also been reported that the expression of CD86, which also binds CD28, could be induced by cross-linking CR2,[40] although, according to another study, T-cell factors are required for the up-regulation of CD86 on B cells by IC.[33]

As mentioned above, natural antibodies may be involved in the formation of complement-activating IC, which could play a decisive role in mediating immune responses to primary antigens. Indeed, it has been reported that incubation of keyhole limpet haemocyanin (KLH) with normal human serum results in the formation of complement-opsonized IC with the aforementioned effects on B-cell antigen binding and processing.[32] Furthermore, we have shown recently that natural autoantibodies and complement, under normal conditions may, likewise, mediate the binding of human thyroglobulin to peripheral B cells and subsequently elicit Th cell proliferation (C.H. Nielsen, R.G.Q. Leslie, M.D. Kazatchkine, S. Kaveri, E.M. Fischer, unpublished).

Direct B-cell stimulation

The first indication that complement may be directly involved in B-cell activation was derived from *in vitro* studies showing that cross-linking of CR2 with the BCR enhanced calcium mobilization and the T cell-independent proliferative response of B cells induced by BCR aggregation,[41,42] and that polyvalent ligands for CR2 were capable of priming B cells for enhanced response to stimulation via the BCR,[43,44] whereas monovalent ligands proved to be inhibitory.[44] The crucial observation, regarding the mechanism for this enhancement, was that CR2, which possesses only a short 34 amino acid (aa) or 35 aa cytoplasmic tail, in the case of human and murine CR2, respectively,[45,46] associates non-covalently with the CD19/TAPA(CD81)/Leu-13 signalling complex.[47,48] CD19, when co-ligated directly with the BCR, was found to lower markedly the threshold for stimulation via the BCR.[49,50]

Synergy between the BCR and CD19 appears to be achieved through both mutual stimulation and the parallel activation of signalling pathways. Thus, aggregation of the

Table 2. The role of complement receptor type 2 (CR2) on B cells and follicular dendritic cells (FDC) in the development of a humoral immune response

Chimera construction memory	Cell phenotype	Immune response			
		Primary Ab response	Class switch response	Long-term response	IgG response
$Cr2^{-/-}$ es cells in RAG-$2^{-/-}$ blastocytes	$CR2^-$ B cells + $CR2^+$ FDC	↓	↓	↓	↓
$Cr2^{+/+}$ BM cells in irradiated $Cr2^{-/-}$ mice	$CR2^+$ B cells + $CR2^-$ FDC	Normal	Normal	↓	↓

BM, bone marrow, es cells, embryonic stem cells; FDC, follicular dendritic cells; RAG-2, recombination-activating gene-2; ↓, decreased.

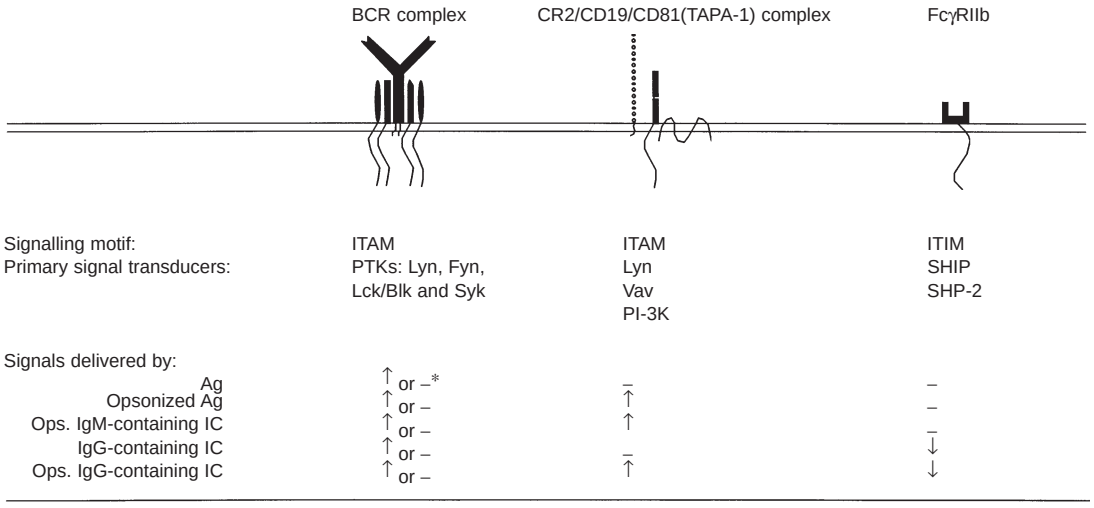

Figure 1. Signalling processes induced by ligation of B-cell receptor (BCR), complement receptor type 2 (CR2) and FcγRII with opsonized antigen and immune complexes. Ag, antigen; IC, immune complexes; IgG, immunoglobulin G; IgM, immunoglobulin M; ITAM, immunoreceptor tyrosine-based activation motif; ITIM, immunoreceptor tyrosine-based inhibition motif; Ops. opsonized; PI-3K, phosphatidylinositol-3 kinase; PTK, protein tyrosine kinase; SHIP, inositol phosphate 5-phosphatase; SHP-2, protein tyrosine phosphatase, SHP-2; Vav, guanidine nucleotide exchange factor, Vav. ↑, activation; ↓, inhibition; –, no effect; *, for Ag-specific or non-specific B cells, respectively.

BCR activates constitutively associated protein tyrosine kinases (PTK) of the Src-family (i.e. Lyn, Fyn, Blk and/or Lck) (see Fig. 1), which then phosphorylate immunoreceptor tyrosine-based activation motifs (ITAMs) in the cytoplasmic tails of the immunoglobulin-α (CD79a) and immunoglobulin-β (CD79b) components of the BCR complex (reviewed in reference 51). This enables recruitment of the cytosolic PTK, Syk, which plays a key role in the activation of a wide range of downstream pathways. These include phosphatidylinositol cleavage and mobilization of intracellular free calcium ions as well as activation of protein kinase C and the mitogen-activated protein kinases (MAP kinases), which are responsible for the regulation of nuclear transcription.[51] Central to the activation process is the recruitment to the BCR complex of two adaptor molecules, BLNK/SLP-65 and Shc, which are responsible for the binding of essential downstream signalling elements such as phospholipase C-γ (PLC-γ), the guanidine nucleotide exchange factor, Vav, and phosphatidylinositol-3 kinase (PI-3K). While CD19 is also constitutively associated with Lyn[52] and, possibly, the nucleotide exchange factor, Vav,[53] phosphorylation of tyrosyl residues in the ITAM of its cytoplasmic tail by BCR-associated PTK[54] results in enhanced binding of Vav[53,55] and recruitment of the Src-family PTK, Fyn, and PI-3K[56] (Fig. 1). Thus, CD19 functions as an adaptor protein, participating co-operatively with BLNK/SLP-65 and Shc in BCR signalling.

The role of CR2 is apparently to amplify association of the CD19/CD81/Leu-13 with the activated BCR, via cross-linking with complement-opsonized Ag, where the CD19 molecule itself provides the synergistic signalling, whilst CD81 is involved in promoting homotypic cellular interactions essential for the activation process.[57] The role of Leu-13 remains, as yet, undefined. It has also been reported that the CR2 cytoplasmic domain, when phosphorylated, is capable of inducing signal transduction independently of CD19, apparently through the binding of a p53 anti-oncoprotein, a p68 calcium-binding protein and the nuclear p120 ribonucleoprotein as well as by activation of PI-3K.[58,59]

In the case of B-cell stimulation by IC containing IgG antibodies, a down-regulatory signalling pathway is also drawn into the picture. The B-cell Fcγ receptors (CD32, FcγRIIb1 and FcγRIIb2), in contrast to their myeloid cell counterpart, FcγRIIa, bear immunoreceptor tyrosine-based inhibitory motifs[60] (ITIMs) in their cytoplasmic domains. These motifs, upon phosphorylation by the BCR-associated PTK, recruit the SH2 domain-containing inositol polyphosphate 5-phosphatase, SHIP[61] and, in the case of humans, the protein tyrosine phosphatase, SHP-2,[62] (Fig. 1). When cross-linked to the BCR by IgG-containing IC, FcγRIIb acts to down-regulate antigen-mediated stimulation through a variety of mechanisms, including inhibition of calcium influx,[61] dephosphorylation of the PTK, Syk,[63] and inhibition of the MAP kinase cascade.[64,65]

B-cell activation by complement-opsonized IC can thus be described as being under the control of a signalling triad, consisting of the BCR and CR2–CD19 complex, as stimulatory components, and FcγRIIb as a negative regulator of the activation process (Fig. 1). In the case of antigen-specific B cells, the binding of IgG-containing opsonized IC will result in engagement of the full triad, whereas non-specific B cells will be engaged only via CR2 and FcγRIIb. IgM-containing complexes, on the other hand, will, when opsonized, engage either CR2 alone (in the case of non-specific B cells) or both the BCR and CR2. The, as yet, limited *in vitro* data available on the dynamics of signalling via the triad suggests that whereas engagement of FcγRIIb markedly inhibits signalling via either BCR or CR2 alone, triple engagement results in a similar degree of activation to that attained by single stimulation via the BCR.[66] However, it is probable that the nature of the B-cell response will be

determined not only by the types of receptor engaged but also upon the degree to which the various receptors are represented in the final signalling complex.

Promotion of B-cell interactions with FDC

Follicular dendritic cells of the spleen and lymph nodes express three receptors for C3 fragments (CR1, CR2 and CR3, CD11b/CD18[67]) as well as a receptor for the Fc portion of IgG, FcγRIIb.[68] These receptors enable the FDC to take up and retain on their surfaces, in a process primarily involving CR1 and CR2,[15,68,69] opsonized IC for presentation to activated, antigen-specific B cells. The consequences of the interaction appear to be twofold. First, rescue of the antigen-activated B cells from apoptosis;[70,71] and second, the promotion of somatic hypermutation[72,73] and class switch,[26,74,75] concomitant with the development of a memory B-cell population. With regard to rescue from apoptosis, at least, it would appear that CR2 plays a central role in the process.[76,77] This occurs via a pathway independent of that involving CD40,[78] and the required activation of CR2 on B cells takes place either by its association with C3 fragments deposited on the FDC or through association with FcγRII (CD23) borne on the FDC.[79]

Recently, it has been demonstrated that antigen presentation may be enhanced by direct C3 fragment deposition on murine B lymphoblasts or macrophages.[80] It had previously been established that B cells[81] and FDC[82,83] (which constitutively express CR2), as well as non-CR2-expressing murine and human macrophages,[80,84,85] are the targets for the covalent deposition of C3 fragments. In the case of human B cells, it has been shown that this deposition is mediated by CR2[86,87] which, by virtue of its capacity to bind the hydrolysed form of C3 (iC3), assembles an AP convertase at its ligand-binding site.[88] Nascent C3b fragments, generated by the convertase, then attach themselves to secondary acceptor sites on the B-cell surface.[89] Although the full significance of this deposition for the development of an immune response remains to be clarified, there is some evidence to suggest that it may play a subsidiary role by promoting intercellular interactions involving the binding of C3 fragments deposited on one cell to CR2 expressed on another.[80] Thus, covalent C3 deposition on B cells may enhance their interaction with CR2 on FDC (and vice versa) or on the subset of T cells, which expresses this receptor.[90]

Finally it should be noted that, in addition to its role in regulating B-cell responses, complement also plays a role in modulating the cytokine profile of helper T cells. Thus, it has been reported that interferon-γ (IFN-γ) synthesis is reduced in C1q-deficient mice[91] while interleukn-4 (IL-4) production remains normal. This deficiency has a dual effect. It blocks the switch to IgG2a and IgG3 production, which is under IFN-γ control,[92,93] and it ablates the induction of localized C3 synthesis in lymph nodes activated by antigen challenge, thereby diminishing the potency of the amplification mechanisms described above. In other words, it would appear that complement exerts a comprehensive and integrated influence on all aspects of the acquired immune response.

THE ROLE OF COMPLEMENT IN MAINTENANCE OF IMMUNE TOLERANCE

Although the prevalence of homozygous complement deficiency is low, association of complement deficiency in the early components of the classical pathway with systemic lupus erythematosus (SLE) is important. This relationship is paradoxical, as complement deficiency is associated with an impaired antibody response to foreign antigens, whereas SLE is characterized by high levels of antibodies against intracellular and cell-surface antigens. The susceptibility to SLE and the severity of the disease are related to the position of the missing complement component in the classical activation pathway (reviewed in reference 94). Thus, individuals with complete C1q deficiency have the highest prevalence of SLE and the most severe disease manifestations. By contrast, just a minority of the patients with C3 deficiency develop clinical features associated with autoimmune disease, and mild, lupus-like illness has been reported in only three families.

Animal deficiencies in C2, C4 and C3 do not result in spontaneous disease, although C2- and C4-deficient guinea-pigs develop elevated levels of IgM and rheumatoid factors, which are not seen in C3-deficient dogs (reviewed in reference 95). In contrast, dogs with C3 deficiency develop a glomerulonephritis that is very similar to that reported in humans.[96] The genes for C3, C4, CR1/2, Factor B and C1q have been successfully targeted in mice. No phenotype associated with spontaneous autoimmune disease has been observed, with the exception of C1q-deficient mice. A significant percentage of C1qâ−/− animals develop high titres of antinuclear antibodies and glomerulonephritis, and exhibit high numbers of apoptotic bodies in the glomeruli. Recent findings indicate that C1q is involved in the clearance and processing of self-antigens contained within surface blebs generated by apoptotic cells.[97,98] Thus, SLE associated with C1q deficiency may be related to an impairment of the clearance of self-antigens, which then chronically stimulate an autoimmune response.[99]

The development of SLE in humans is associated with abnormalities of B-cell functions, and an altered expression pattern of both CR1 and CR2 on B lymphocytes has been reported.[100] A similar decrease in C3 receptor expression was found in MRL/*lpr* mice – considered as a spontaneous model of human SLE – and was shown to precede the development of clinical and autoimmune manifestations.[101] A number of lines of evidence (discussed above) indicate that the CD19/CD21 complex regulates transduction thresholds governing humoral immunity to T-dependent antigens. In addition, CD19 plays a key role in the development of the $CD5^+$ B-1 subset of B cells in mice. B-1 cells constitute a small population of the B lineage, which is the major producer of serum IgM but generates only a restricted antibody repertoire dominated by a specific set of V genes, many of which encode autoreactive antibodies.[102] B-1 cells produce natural antibodies (IgM and IgG3) of low affinity that have a broad specificity for bacterial polysaccharides, lipids and proteins, as well as autoantigens such as single-strand DNA (ssDNA) and IgG. A dominant feature of CD19-deficient mice is a dramatic decrease of $CD5^+$ B-1 cells and of anti-DNA autoantibodies, while mice that overexpress CD19 have significantly increased numbers of $CD5^+$ B cells and high titres of anti-DNA antibodies of IgG isotype.[103,104] Deficient $Cr2^{-/-}$ mice were also found to have a reduction in the

$CD5^+$ population of peritoneal B-1 cells, although their serum levels of IgM were within the range of normal mice.[21] Interestingly, $Cr2^{-/-}$ mice lack Ab with specificity for certain antigens commonly found in wild-type mice, such as those towards lipopolysaccharide (LPS), *Escherichia coli* and hypoxia antigen, demonstrating an altered antibody repertoire.[105] Thus, the binding of C3 fragment-coated self-antigens to CD19/CD21, together with low-affinity BCRs of B-1 cells, may promote clonal expansion of this subset and be involved in the selection of the repertoire of natural antibodies.

Although it remains unclear whether pathogenic IgG autoantibodies are derived from switched B-1 cells or from conventional B-2 cells, the inappropriate or prolonged generation of C3 fragments during inflammatory or infectious episodes may alter signalling thresholds of B cells, leading to breakdown of tolerance and positive selection of autoreactive B-cell clones. Indeed, recent studies have established a role for the CD19/CD21 complex in the control of peripheral tolerance. The role of CD19 in tolerance regulation was examined by crossing mice that overexpress a human CD19 transgene with transgenic mice expressing a model autoantigen (sHEL) and high-affinity HEL-specific antigen-receptor (IgHEL).[106] Whereas B cells of double-transgenic mice sHEL/IgHEL were functionally anergic and did not produce autoantibodies, overexpression of CD19 resulted in the breakdown of peripheral tolerance and the production of anti-HEL antibodies. Thus, by augmenting antigen receptor signalling, CD19 overexpression shifts the balance to autoimmunity, suggesting that inappropriate CD19 expression or function contributes to autoimmunity by disrupting tolerance.

Involvement of complement in the negative selection of self-reactive B cells has also been reported.[107] B cells from double-transgenic mice (HEL/IgHEL) crossbred with Cr2 mice accumulated in secondary lymphoid organs and responded to stimulation with autoantigen by calcium mobilization, suggesting a defect in anergy development of the B cells. However, no autoantibodies against HEL were detected in the serum of the animals. Similar results were obtained in double-transgenic mice crossed with $C4^{-/-}$ mice but not with $C3^{-/-}$ mice, suggesting a predominant role of C4 in the negative selection of self-reactive B cells. The authors propose that uptake of C4b-coated antigens by CD35(CR1-)-positive cells within the bone marrow and secondary lymphoid organs would provide a mechanism for concentration of self-antigens and ensure contact with immature self-reactive B cells. This new role for complement in negative selection of self-reactive B cells would provide an explanation for the apparently paradoxical association between complement deficiency and autoimmunity.

CONCLUSIONS

In this review, we have described in outline the considerable body of evidence that links the complement system not only to the development of humoral immunity but also to the induction of self-tolerance in developing B cells. While the former serves to underline the high degree of interdependency that exists between the innate and acquired elements of the immune system, the latter provides a rationale for the, at first sight, paradoxical association between deficiencies of the early complement components and autoimmune disease. While the past decade has witnessed major advances in our understanding of the role(s) of complement in the induction of acquired immunity and B-cell tolerance, there remain, nevertheless, unanswered questions of both a conceptual and practical nature. For the first, the contention that complement provides the organism with a means of distinguishing between harmless antigens and dangerous (microbial) antigens, on the basis of their capacity to activate complement directly, requires re-examination in light of the findings that many primary antigens can form complement-activating IC with natural antibodies. In this case, it is the natural Ab repertoire that is decisive, and this includes not only specificity for carbohydrate antigens of microbial origin but also for a wide range of autoantigens.

The role of complement in tolerance induction illustrates once again the familiar paradigm concerning the initiation of cellular activation or death at different stages of development of the cell. While the mechanism for induction presumably involves tagging circulating autoantigens with C4b, it remains to be clarified how this labelling takes place: is it through a spontaneous activation process or is it consequent upon complex formation with natural autoantibodies?

The new insights that have been acquired regarding the role of complement in the development and regulation of humoral immune responses are likely to result in the development of novel strategies for immunization against pathogenic micro-organisms, on the one hand, and open the way to new forms of treatment for immune complex-associated autoimmune diseases, on the other.

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