Human C3 Deficiency Associated with Impairments in Dendritic Cell Differentiation, Memory B Cells, and Regulatory T Cells

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Human C3 Deficiency Associated with Impairments in Dendritic Cell Differentiation, Memory B Cells, and Regulatory T Cells

Arije Ghannam,* Martine Pernellet, † Jean-Luc Fauquert, ‡ Nicole Monnier, § Denise Ponard, ¶ Marie-Berandette Villiers, †† Josette Péguet-Navarro, ‡‡ Arlette Tridon, ‡ Joel Lunardi, § Denis Gerlier,* and Christian Drouet‡‡

Primary C3 deficiency, a rare autosomal inherited disease (OMIM 120700), was identified in a 2-year-old male suffering from recurrent pyogenic infections from early infancy with undetectable total complement hemolytic activity (CH50) and C3 values. The nonconsanguineous parents and the two patients’ two siblings had 50% normal serum C3 concentration. The molecular abnormality associated a paternal allele coding C3 with the missense mutation p.Ser550Pro and an apparently null maternal allele, with production of a defective protein that could no longer be secreted. Vaccination of the child did not induce a long-term Ab response. Accordingly, switched memory IgG+CD27+ B cells were barely detected, amounting to only 2.3% of peripheral blood CD19+ cells. Cells were significantly defective in stimulating alloreactive responses. The in vitro development of immature dendritic cells and their maturation capacity were greatly impaired, with decreased CD1a expression and IL-12p70 secretion ability. These cells were unable to induce autologous B cell proliferation and Ig secretion in the presence of CD40L and C3. Finally, the regulatory T cell development ability of CD4+ T cells after CD3 and CD46 activation in the presence of IL-2 was significantly impaired. Thus, the association of important functional defects of dendritic cells, acquisition of B cell memory, and regulatory T cells with human C3 deficiency strongly supports a major role for C3 in bridging innate and adaptive immunity in humans. The Journal of Immunology, 2008, 181: 5158–5166.
the recovery from primary infection, the costimulation of B cells via the CD21/CD19/CD81 coreceptors to recruit leukocytes at the infection site has been suggested as a minor pathway (24). In contrast, the ability of C3 to enhance specific CD4+ and CD8+ T cell responses can be critical in mediating antiviral protection (24, 25). All of these findings may have implications in vaccine development.

In the present study, we characterized a complete C3 deficiency in a nonconsanguineous French family. In addition to a new missense C3 mutant, our results identify several new immune dysfunctions (4). An important defect of memory B cells was found, associated with the impairment of vaccine Ab production. The in vitro differentiation of myeloid DCs was greatly impaired. The complement-induced regulatory T cells (Tregs) were lacking in the young patient and his heterozygous parents. Altogether, our findings further emphasize the critical and multiple roles of C3 in the development of adaptive immunity in humans.

Materials and Methods

Case report and blood collections

Since early infancy, a 2-year-old boy had suffered from recurrent pyogenic infections, including severe meningitis, pneumonopathy, otitis, and pyothroiditis. The hepatic functions were normal. His two siblings (5 and 7 years old) and parents (mother and father, 38 and 40 years old, respectively) were apparently healthy, but his twin brother had died in the first week of life from fulminant meningitis (Fig. 1A). Blood was collected from all family members for complement assays, cell phenotype, and function analyses. The study was conducted in agreement with the European Union and French ethical policies and was approved by the local ethics committee at the Centre Hospitalier Universitaire of Clermont-Ferrand.

Complement assays and molecular analyses

Complement hemolytic activity (CH50) was determined (26), and serum concentrations of complement proteins and IgGs were measured by laser nephelometry (detection limit for C3 = 43 mg/L; Dade Behring). Complementation analyses were conducted using the human C3 protein, after purification to homogeneity, as detailed by Al Salhi et al. (27). The C3 concentration in the supernatants of monocyte monolayers was measured by ELISA, using purified C3 as standard, as described previously (20). At day 12 of the culture and after a 24-h activation by 0.1 mg/ml Escherichia coli LPS, total RNA was prepared from monocyte lysates (TRizol; Invitrogen) and reverse transcribed using the Transcriptor system (Roche Biochemicals). Seven overlapping fragments were amplified with primers chosen using the cDNA reference sequence (GenBank accession NM_000064). Amplicons were directly sequenced using the ABI PRISM Big Dye Terminator Cycle Sequencing v3.0 reaction kit, and were analyzed on an ABI 3100 DNA Analyzer (Applera).

Immunofluorescence analysis of intracellular C3

Monocytes were prepared from PBMCs by negative selection (Dynabeads MyPure Monocyte Kit2), with a purity of the CD14+ population greater than 95%, as assessed by flow cytometry. The CD14+ monocytes/macrophages were grown for 12 days on LabTEK coverslips (Nunc-Fisher) in culture medium and then stimulated for 24 h in the presence of 0.1 μg/ml E. coli LPS. The cells were washed with PBS and fixed with 4% paraformaldehyde in PBS for 10 min; the cells were permeabilized with 1% Tween 20 in PBS for 10 min. After three washes in PBS, the cells were incubated for 45 min at room temperature with the mouse anti-human C3 β-chain Ab WMI (dilution 1/20 in PBS-1% BSA) (28). Following washes, the cells were incubated with goat anti-mouse Ab conjugated with Alexafluor 488 (Invitrogen; dilution 1/100) for 45 min. Cells were washed twice in PBS and once with water, air dried, and mounted with Flourmount-G (Southern Biotechnology Association). Fluorescence was observed using a laser confocal microscope (Zeiss LSM410).

Cell phenotyping

Cells were typed using the following Abs: CD19 PC7, CD27 FITC, IgD PE, IgM APC, CD1a FITC, CD14 PE, HLA-DR PerCP, CD80 APC, and CD45 FITC (BD Biosciences). The analyses were performed on a FACScanlibur instrument using the CellQuest software (BD Biosciences).

Ag proliferation assays

PBMCs were prepared by density-gradient centrifugation over lymphocyte separation medium (Eurobio). A total of 50 μg/ml tetanus toxoid (AT; Aventis Pasteur), 2500 UI/ml tuberculin purified derivative (PPD; Aventis Pasteur), or 25 μg/ml Ag preparation from Candida albicans (ACM; Bio-Rad) was added to 1 × 10^6 PBMCs in triplicate in 96-well plates (200 μl; BD Falcon). Mitogen (0.5 μg/ml)-activated cells and nonstimulated cells were used as positive mitogenic and negative control, respectively. The proliferation was monitored after an 18-h [3H]thymidine (1.0 μCi/ml) incorporation at day 6 for each Ag. Tests were conducted in triplicate, and the results were expressed as mean net cpm ± SD. Each experiment was conducted twice, and the experiments shown are representative of all the data.

Alloreactive mixed culture

Responder and alloengeneic irradiated (30 Gy) stimulator PBMCs were cocultured at 1:1 ratio in 96-well plates (200 μl; BD Falcon); 5 × 10^4 irradiated stimulator cells were cocultured with 5 × 10^4 responder HLA-nonmatched cells/well for 6 days. The proliferation was monitored after an 18-h [3H]thymidine (1.0 μCi/ml) incorporation at day 6. Tests were conducted in triplicate, and the results were expressed as mean net cpm ± SD.

Generation of myeloid DCs

CD14+ cells were prepared, as described above, and cultured (1 × 10^6 cells/ml) in six-well culture plates (BD Falcon) in RPMI 1640 medium supplemented with 10% FCS (Invitrogen), 2 mM glutamine, and 25 mM HEPES, in the presence of 200 ng/ml human rGM-CSF (2 × 10^5 U/mg) and 33 ng/ml human rIL-4 (20 × 10^5 U/mg), which were gifts from Schering-Plough Research Institute, Kenilworth, NJ. GM-CSF and IL-4 were added again at days 3 and 5. At day 7, DCs were checked for CD14^high MHC class II^+, CD80^high, CD14^low phenotype (CD14 PE, HLA-DR PerCP, CD1a FITC), and IL-12p70 expression in supernatants (CBA; BD Biosciences) after maturation by 0.1 mg/ml LPS for 24 h.

Coculture of B cells and DCs

B lymphocytes were purified from PBMCs by negative selection using Dynal Biotech B Cell Negative Isolation Kit, with a purity of the CD19+ population greater than 90%. B cell activation was evaluated in the presence of DCs, according to Dubois et al. (29). Briefly, 2.5 × 10^5 irradiated (80 Gy) CD40L-L-transfected cells (a gift from C. Caux, Lyon, France) were seeded in the presence of 5 × 10^5 purified B lymphocytes, with 5 × 10^5 immature DCs harvested at day 7 in a 96-well culture plate (200 μl; BD Falcon). When indicated, purified C3 was added (100 μg/ml, final concentration, for 4 h). B cell proliferation was monitored after a 16-h [3H]thymidine (1 μCi/ml) incorporation at day 6. Tests were conducted in triplicate, and the results were expressed as mean cpm ± SD. To determine IgM and IgG production, supernatants were recovered after 13 days and assayed by nephelometry (Dade Behring).

Tregs

CD4+ T cells were positively isolated from PBMCs using magnetic beads (CD4 isolation kit II, Dynabeads; Dynal Biotech), with greater than 90% purity. CD4+ T cells were then cultured in 96-well plates precoated with the mAbs anti-CD3 (HI3; 10 μg/ml; BD Pharmingen), anti-CD28 (CD28.2; 5 μg/ml; BD Pharmingen), or anti-CD46 (20.6; 5 μg/ml) (30) in the presence of human rIL-2 (40 U/ml; Genzyme) for 3 days. IL-10 secretion was analyzed in the supernatants using CBA (BD Biosciences). Cell surface expression of CD46 on CD4+ T cells was assessed by cytometry using anti-CD46 FITC.

Statistical analysis

Two-tailed Student’s t test was used to compare patient and control data. All data are represented as mean ± SD; n = 2 or 3. Value of p < 0.05 was considered significant.

Results

Complement and vaccine Ab profiles

Fig. 1A illustrates the pedigree of the family and the symptomatic proband. The patient’s CH50 and C3 levels were below the detection limit (CH50 < 10%, C3 < 43 mg/L; Fig. 1B), whereas other complement proteins, including C4, were within the normal range. His parents and siblings displayed nearly half the normal C3 concentration in serum (Fig. 1B). Complementation of the patient’s plasma...
by increasing C3 concentrations with purified human C3 restored the hemolytic activity in a dose-dependent manner (Fig. 1C).

To confirm the diagnosis of C3 deficiency, we performed a biosynthesis experiment using monocyte monolayers prepared from the patient, his parents, and a healthy control. As shown in Fig. 1D, C3 was found below the detection limit in the patient's cell supernatants, suggesting a C3 deficiency, in agreement with the absence of plasma C3 nef activity (data not shown).

The ability of the proband’s macrophages to synthesize, but not to secrete C3 was then investigated. After intracellular immunostaining, the macrophages from the proband exhibited a very bright fluorescent intensity, with strong accumulation in a vesicular system resembling the distribution of the Golgi apparatus (Fig. 2A, i and ii). In the macrophages from a healthy individual (control), a faint C3 labeling was found distributed throughout the cytoplasm, from the nucleus to the plasma membrane (Fig. 2A, iii and iv). Quantification of the fluorescence signal (mean fluorescence intensity) from the individual cells ranked from fainter to brightest ones illuminates the overall much brighter C3 labeling of the proband’s cells (Fig. 2Aiv). Remarkably, in the C3 labeling of the proband’s cells (Fig. 2Aiv), the frequent infections in the patient raised the question of his Ab protection toward vaccine Ags. When the proband was 5 years old, the adaptive immunity was evaluated. Total IgG level in serum was found lower than normal with 3.7 g/L (IgG1 3.5 g/L; IgG2 0.5 g/L; IgG3 0.15 g/L; IgG4 < 0.07 g/L). Most of the anti-vaccine Ag Ab titers were found to be lower than normally needed for protection. When positively detected after vaccination and tested 4 years after vaccination, the Ab titers lowered to near or under the threshold value, except for Streptococcus pneumoniae and Haemophilus influenzae, two pathogens that have reiteratively infected the proband (Table I).

Decreased ability of peripheral cells to stimulate alloreactive cells in vitro

The potency of the patient’s cells to stimulate alloreactive cells was tested. For this purpose, PBMCs prepared from the patient, his parents, and a healthy control were irradiated and cocultured in the presence of allogeneic PBMCs. The patient’s cells exhibited lower stimulation capacity toward the control cells T1, T2, and T3, in comparison with the matching situations involving control cells (p < 0.05; Fig. 3A). In the reverse situation, the patient’s cells developed...
normal responder properties (Fig. 3B). This indicates a possible impairment of the Ag presentation ability of proband’s cells.

Decreased proliferation response to specific recalled Ags

The cellular responses to soluble Ags such as PPD, AT, and ACM were investigated using Ag proliferation assays. Compared with the control, significantly lower responses were found in the patient’s cell culture stimulated by PPD and AT (p < 0.02 and p < 0.01, respectively). No significant difference was found in the culture stimulated by ACM (p > 0.05) (Fig. 3C). The response to mitogen was similar for the patient and the controls. These data further support an impairment of the patient’s PBMCs in Ag processing and/or presentation.

**Table I. Ab titres of the patient**

<table>
<thead>
<tr>
<th>Type, Unit</th>
<th>Serum Titre 4 mo after Vaccination</th>
<th>Serum Titre 4 Years after Vaccination</th>
<th>Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mumps IgG, UI/L</td>
<td>500</td>
<td>230</td>
<td>230</td>
</tr>
<tr>
<td>IgM, UI/L</td>
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<td>&lt;230</td>
<td>230</td>
</tr>
<tr>
<td>Measles IgG, UI/L</td>
<td>&gt;300</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>IgM, UI/L</td>
<td>&gt;300</td>
<td>150</td>
<td>300</td>
</tr>
<tr>
<td>Poliovirus Type 1, dilution</td>
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<td>&lt;10</td>
<td>32</td>
</tr>
<tr>
<td>Type 2, dilution</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>32</td>
</tr>
<tr>
<td>Type 3, dilution</td>
<td>160</td>
<td>80</td>
<td>40</td>
</tr>
<tr>
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<td>&lt;20</td>
<td>20</td>
</tr>
<tr>
<td>Orthomyxovirus parainfluenzae</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>20</td>
</tr>
<tr>
<td>Tetanus toxoid kUI/L</td>
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<td>&lt;0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Diptheria toxoid kUI/L</td>
<td>0.18</td>
<td>&lt;0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>S. pneumoniae µg/mL</td>
<td>1.9</td>
<td>1.6</td>
<td>0.3</td>
</tr>
<tr>
<td>H. influenzae B IgG, µg/mL</td>
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<td></td>
<td>5.8</td>
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<td>Bordetella pertussis (immunoblot)</td>
<td>Pertussis toxin Absence</td>
<td>Absence</td>
<td>Absence</td>
</tr>
<tr>
<td>Adenylate-cyclase</td>
<td>Absence</td>
<td>Absence</td>
<td>Absence</td>
</tr>
<tr>
<td>Fimbrial hemagglutinin</td>
<td>Absence</td>
<td>Absence</td>
<td>Absence</td>
</tr>
</tbody>
</table>
Decreased ability to differentiate myeloid DCs

DC maturation was recently demonstrated to be C3 dependent (3, 31), and we investigated the ability of patient cells to mature into DCs in vitro. Purified human monocytes were allowed to differentiate into immature DCs by culture in the presence of GM-CSF and IL-4 in FCS-supplemented medium (Fig. 4A). Compared with the parents’ monocytes, the total number of DCs that could be collected at the end of the differentiation process was much lower when derived from the patient monocyte culture, compared with the parents and control (0.27 vs 0.90% of the monocyte population). Furthermore, proband DCs expressed slightly lower HLA-DR (Fig. 4B). We next estimated the IL-12p70 secretion during the maturation step of the DCs upon
LPS activation. In the proband’s supernatants, IL-12p70 was nearly absent and parents’ DCs exhibited half the production found in the control (Fig. 4C). Thus, the ability of the patient’s monocytes to develop into immature DCs and their capacity to acquire the maturation phenotype were greatly altered.

Absence of memory B cells

Because of the defective Ab responses to vaccine Ags, peripheral B cells were next analyzed. Memory B cells are distinguished in two subtypes according to the expression of CD27 and IgD with IgD+/CD27+ (as switched cells) and IgD+/CD27− (as nonswitched cells) (32). The number of memory IgD−CD27+ B cells was greatly decreased in the patient (2.3% of the CD19+ cells), and was found in reduced amounts in his mother and father (13.1 and 14.2%, respectively; Fig. 5, A–C), as compared with the usual 30–40% in controls (33). The percentage of CD19+ B cells in the total lymphocyte population was within the normal value. Consequently, cells with naive IgD+CD27− phenotype represented more than 95% of the patient’s circulating B cells, with rare memory CD27+CD19+ B cells belonging to the IgM+IgD− phenotype (Fig. 5Aiiii). Thus, the C3 deficiency was associated with a very low level of memory B cells.

Impaired B cell activation and differentiation in response to C3

In mixed cultures of DCs and B cells, the presence of CD40L has been demonstrated to enhance both the B cell proliferation and Ig production (29), and the influence of exogenously added C3 on B cell functions was investigated in these conditions. In the absence of C3, the proliferative responses and Ig secretion of B cells from the proband, his parents, and the control were comparable (Fig. 5, D–F). In contrast, exogenous C3 increased the proliferation of B cells from the control and, to a lesser extent, the parents’ cells, but not those from the patient’s B cells (Fig. 5D). Furthermore, C3 induced IgM secretion in the control and parents’ and patient’s B cells, with isotypic commutation in control B cells, but not in the parents’ and patient’s B cells (Fig. 5, E and F). Thus, B cell activation and differentiation in response to C3 were impaired in both the patient and his parents.

Alteration of CD46-activated Tregs

CD46, a receptor for C3b ligand, is expressed on all nucleated cells. It is a potent costimulator for human CD4+ T lymphocytes that leads them to acquire a distinct Treg-like phenotype called complement-induced Tregs (cTregs; phenotype Tr1) (34, 35). This prompted us to evaluate the CD46-costimulatory function in cells with C3 deficiency. Purified human CD4+ lymphocytes from the patient, his parents, and a healthy donor were stimulated with immobilized mAbs (anti-CD3, anti-CD46, anti-CD28), and their cytokine profile was analyzed. The CD3- and CD46-mediated stimulation of CD4+ T from the patient and his parents in the presence of IL-2 resulted in low IL-10 secretion (Fig. 6A), whereas no difference was found upon anti-CD28 and anti-CD3 costimulation. FoxP3+CD4+ T cells were within the normal range (8.15% of the CD4+ cells; reference 3–12%). Because CD46 was expressed at a normal level on the patient’s CD4+ T cells (Fig. 6B), the lack of cultured with irradiated CD40L-L cells (left histogram) or together with immature DCs (middle histogram). When indicated, purified C3 was added to the culture (B + CD40L + immature DCs) for 4 h, and then the medium was changed. Cells were incubated with [3H]thymidine uptake at day 6 (D), and the supernatants were harvested at day 13 and subsequently assayed for their IgM (E) and IgG (F) contents (n = 3, mean ± SD). C3+/–, Proband; Ctrl, healthy control; Fa, father; Mo, mother.
Tr1 activation could result from an impaired signaling function of CD46 in C3 deficiency.

**Discussion**

Human primary C3 deficiency has been described to date in 28 patients from 20 families in the world. The deficiency combining the p.Ser550Pro mutation and an apparently null allele further emphasizes the heterogeneous characteristics of C3 deficiency. A similar situation has been reported in C3 deficiency associated with connective tissue disease (36). In agreement with the first descriptions (OMIM 120700), this novel French case has a long history of recurrent infections, mainly associated with Gram-positive bacteria and *H. influenzae*; both parents and two siblings are asymptomatic and phenotypically heterozygous, with the C3 concentration reduced to approximately half of that normally present in serum. The coding mutation is located in the middle of a β-sheet in the MG5 domain, as defined in the recently solved three-dimensional structure (37). Because the C3Pro550 is not secreted by monocytes, but accumulates intracellularly, the Ser550Pro mutation most likely prevents the correct folding into the β-barrel structure of the MG5 domain. This intracellular accumulation could be deleterious to some macrophage functions. Likewise, because liver and kidney are the main source of C3 production, these organs could be predicted to exhibit functional defects. However, there were no clinical nor routine biological signs that could have alerted the clinician about liver or renal dysfunction in both proband and father. In the absence of thorough investigation of liver and renal functions, we cannot, however, exclude any dysfunctions of these organs.

The lower ability of proband’s cells to stimulate allogeneic cells is in agreement with recent findings in C3<sup>−/−</sup> mice (3, 22, 23). Mouse C3<sup>−/−</sup> cells displayed reduced cell surface expression of MHC class II and B7.2, with subsequent impairment of the Th1-polarizing molecule IL-12 and reduction of alloreactive T cell priming. Thus, the data from mouse and human cells support the role for C3 in the development of functional APCs. Indeed, monocytes from the C3 deficiency poorly differentiate in vitro into DCs, with reduced expression of CD1a and failed aptitude for secretion of IL-12p70. In the absence of C3 from the culture medium, DCs express a lower amount of the differentiation markers, including DC-SIGN, HLA-DR, CD1a, CD80, and CD86, and produce many fewer cytokines (31). Accordingly, in the mouse, C3 triggers the differentiation of monocytes into DCs (31). This suggests a novel role played by C3 as a critical cofactor in DC differentiation and maturation.

The C3 deficiency was associated with a prominent B cell defect, in agreement with the pioneering studies in mice, in which the
GC reaction was inhibited after depletions of C3 by cobra venom factor (38), and with the more recent observations made in C3−/− mice (9) (for reviews, see Carroll (16, 39)). Surprisingly, the C3null/C3Pro550 proband lacks much of the expected switched memory B cells (IgD/CD27+) in the periphery, with as little as 2.3% of switched IgG memory B cells among the CD19+ cells, and the IgM/IgD/CD27+ B cells were nearly the only circulating memory B cell population. Because B cells from the present C3 deficiency were almost pure nonswitched memory IgM/IgD/CD27+ B cells, the response to exogenously added C3 observed in vitro, that is increased IgM secretion without stimulation of IgG secretion and B cell proliferation, can be considered as specific properties of this B cell memory subset. Whether these properties are independent of the presence or absence of C3 in vivo or resulted from a lack of proper B cell education due to a C3-free environment remains to be determined. The absence of switched IgM memory B cells in the proband correlated with the low Ab titers after vaccination. This indicates that C3 should deliver a third signal required for the accumulation of proliferating B cells, isotypic switching, and differentiation into Ab-secreting cells. Overall, this B cell phenotype strongly suggests a defect in the development of the GC, a failure in the maturation process within the GC, and/or a failure in the transfer process to the marginal zone from where originate the memory B cells that secrete IgM (33, 40, 41).

The impairment of IL-10 secretion by T cells from the proband and his heterozygous parents upon anti-CD46 activation suggests that the Tr1 phenotype could be dependent on a critical expression level of C3 for development. This lack of regulatory function in the absence of the natural CD46 ligand is in agreement with the sharply decreased IL-10 secretion and abnormal T cell response observed in the contact-hypersensitivity reaction by CD46 transgenic mice (42). The cTreg cells favor the maturation of DCs (see below) (35) and enhance both the activation and the Ab secretion ability of B cells (43, 44). Thus, the poor cTreg function in the absence of C3 could contribute, at least in part, to the B cell defect and to the DC’s hindered ability to mature in vitro. The role of triggering agonist of the innate immune receptors in the development of the adaptive immunity has been recently recognized by showing that TLR triggering is required for full B cell activation, isotypic switching, and efficient Ab production (45). The defects in three master pieces of the adaptive immune response observed in this C3 deficiency, that is the poor maturation of monocytes into DCs, the lack of B memory cells, and the inability of CD4+ T cells to give rise to cTregs, strongly suggest that C3 and/or C3-derived proteolytic products act as a triggering agonist toward complement receptors expressed by cells from the immune system. Because cells from the proband developed normal expression of complement receptors, we propose that C3 is required as a triggering agonist for priming specific intracellular signaling in monocytes and/or lymphocytes during ontogeny. This is in agreement with the recent concept of dependence receptor, in which the receptor expression leads to the cell becoming dependent on the presence of the ligand for its survival (46). Furthermore, because the C3 heterozygosity in the parents is also associated with a reduced number of memory B cells and lower ability of CD4+ T cells to differentiate into cTregs, the optimal maturation of these cells may rely on a critical local threshold of C3 level. A related observation in the mouse argues for this hypothesis, as follows: irradiated mice reconstituted with C3−/− bone marrow displayed a defect in Ab response when immunized locally, despite having normal level of circulating C3, whereas the reverse chimera responds normally (47).

Collectively, our data further emphasize the major role of complement in bridging innate and adaptive immunity. C3 directly or indirectly provides a costimulatory signal or sets a threshold needed for monocyte maturation into DCs, B cell isotypic switching and differentiation into memory cells, and cTreg activity. The importance of complement in regulating cell-mediated immunity is likely to have a more central role in adaptive immunity than has previously been suspected.

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Disclosures

The authors have no financial conflict of interest.

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