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

Arije Ghannam,* Martine Pernollet, † Jean-Luc Fauquert,‡ Nicole Monnier,§ Denise Ponard,¶ Marie-Bernadette Villiers,‖ Josette Pégue-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.Ser256Pro 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 allogeneic 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 activation of C3 and subsequent production of various proteolytic fragments are crucial and a strategic function for both innate and adaptive immune defense. The liver is the main site of C3 synthesis (1), which is increased during acute inflammation. Diffusible amounts are also produced by activated monocytes, macrophages (2), and dendritic cells (DCs)3 (3, 4).

Human complete C3 deficiency with two affected alleles (OMIM 120700) was recognized as being associated with a striking susceptibility to pyogenic infections (1, 5). The rare cases of complete C3 deficiency are associated with large deletions, microdeletions, or mutations in splice sequences (for review, see Ref. 4). Heterozygous individuals with missense mutations are at risk for noninfectious diseases, such as membranoproliferative glomerulonephritis (5), age-related macular degeneration (6), and atypical hemolytic and uremic syndrome (7). Evidence that C3 is involved in adaptive immunity was demonstrated in C3−/− animals, which exhibited a substantial reduction in germinal center (GC) numbers, low IgG Ab titers after virus infection (8), and impaired T-independent and T-dependent responses (9–12). The activation and the covalent attachment of C3 to an Ag enhance the specific B cell response by at least two mechanisms. On one hand, coligation of the CD21/CD19/CD81 coreceptors lowers the threshold for B cell activation both in vivo and ex vivo (13, 14), and the ligation of CD21 is required for the survival of activated B cells within the follicles and GCs (10). In contrast, the complement receptor-dependent retention of Ag on follicular DCs provides an Ag source for the clonal selection within the GC (10, 15, 16). The direct coupling of C3d or C3b to a protein Ag lowers the amount of protein required for an optimal Ab response by as much as 10,000-fold (17, 18) and leads to more stable IgG production and better memory stimulation (19), resulting in an improved, long-lasting response (20). Similarly, the coupling of C3d to the influenza hemagglutinin in a DNA vaccine enhances the humoral response (21). C3 also appears to be critical for optimal Ag presentation in alloreactive responses (3, 22, 23). Considering the importance of C3 in complement-induced regulatory T cell, GC, germinal center; PPD, tuberculin purified derivative; SNP, single nucleotide polymorphisms; Treg, regulatory T cell.

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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 pyothrombids. 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 IgG 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 Salihi 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 Fluormount-G (Southern Biotechnology Associates). 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 CD46 FITC (BD Biosciences). The analyses were performed on a FACSCalibur 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 IU/ml tuberculin purified derivative (PPD; Aventis Pasteur), or 25 µg/ml Ag preparation from Candida albicans (ACM; BioRad) was added to 1 × 106 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 allogeneic irradiated (30 Gy) stimulator PBMCs were cocultured at 1:1 ratio in 96-well plates (200 µl; BD Falcon); 5 × 10⁴ irradiated stimulator cells were cocultured with 5 × 10⁵ responder HLA-nomatched 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 × 10⁶ 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 × 10⁵ U/mg) and 33 ng/ml human rIL-4 (20 × 10⁵ 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 CD14hi, MHC class II+, CD80hi, CD140lo 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 × 10⁴ irradiated (80 Gy) CD40L-L-transfected cells (a gift from C. Caux, Lyon, France) were seeded in the presence of 5 × 10⁴ purified B lymphocytes, with 5 × 10⁴ 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 by 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 (Hit3a; 10 µg/ml; BD Pharmingen), anti-CD28 (CD28.2; 5 µg/ml; BD Pharmingen), or anti-CD46 (20.6; 5 µg/ml) 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 from the wells of the patient (II.3), his parents (I.1 and I.2), and a healthy control (Ctrl) at day 12 (n = 2, mean ± SD). N, reference interval.

C3 (mg/L, final concentration)

<table>
<thead>
<tr>
<th>C3 (mg/L, final concentration)</th>
<th>Ctrl</th>
<th>&lt;43</th>
<th>86</th>
<th>216</th>
<th>432</th>
<th>1,080</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemolytic Activity (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3 (mg/L) (N=825-1140)</td>
<td>517</td>
<td>559</td>
<td>542</td>
<td>436</td>
<td>&lt;43</td>
<td></td>
</tr>
<tr>
<td>CH50 (%) (N=82-102)</td>
<td>84</td>
<td>111</td>
<td>100</td>
<td>106</td>
<td>&lt;1</td>
<td></td>
</tr>
</tbody>
</table>

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</th>
<th>Unit</th>
<th>Serum Titre 4 mo after Vaccination</th>
<th>Serum Titre 4 Years after Vaccination</th>
<th>Threshold</th>
</tr>
</thead>
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<tr>
<td>Mumps</td>
<td>IgG, UI/L</td>
<td>500</td>
<td>230</td>
<td>230</td>
</tr>
<tr>
<td></td>
<td>IgM, UI/L</td>
<td>500</td>
<td>&lt;230</td>
<td>230</td>
</tr>
<tr>
<td>Measles</td>
<td>IgG, UI/L</td>
<td>&gt;300</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td>IgM, UI/L</td>
<td>&gt;300</td>
<td>150</td>
<td>300</td>
</tr>
<tr>
<td>Poliovirus</td>
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<td>&lt;10</td>
<td>&lt;10</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Type 2, dilution</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Type 3, dilution</td>
<td>160</td>
<td>80</td>
<td>40</td>
</tr>
<tr>
<td>Orthomyxovirus influenzae</td>
<td>Dilution</td>
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<td>&lt;20</td>
<td>20</td>
</tr>
<tr>
<td>Orthomyxovirus parainfluenza</td>
<td>Types 1–3, dilution</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>20</td>
</tr>
<tr>
<td>Tetanus toxoid</td>
<td>kU/L</td>
<td>0.10</td>
<td>&lt;0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Diptheria toxoid</td>
<td>kU/L</td>
<td>0.18</td>
<td>&lt;0.10</td>
<td>0.10</td>
</tr>
<tr>
<td><em>S. pneumoniae</em></td>
<td>µg/mL</td>
<td>1.9</td>
<td>1.6</td>
<td>0.3</td>
</tr>
<tr>
<td><em>H. influenzae</em> B</td>
<td>IgG, µg/mL</td>
<td>5.8</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td><em>Bordetella pertussis</em> (immunoblot)</td>
<td>Pertussis toxin</td>
<td>Absence</td>
<td>Absence</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adenylate-cyclase</td>
<td>Absence</td>
<td>Absence</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fimbrial hemagglutinin</td>
<td>Absence</td>
<td>Absence</td>
<td></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, the CD1a expression on the DCs was drastically reduced to 550 of mean fluorescence intensity, compared with the high 1079 and 4980 expression levels on DCs from the parents and control, respectively. In addition, proband DCs expressed slightly lower HLA-DR (Fig. 4B). We next estimated the IL-12p70 secretion during the maturation step of the DCs upon

**FIGURE 3.** Allogeneic reactivity and Ag proliferation assay. Irradiated PBMCs were cocultured with allogeneic PBMCs for up to 6 days, using the patient’s stimulation capacity (A) and responder capacity (B) (C3–/–) and control HLA-nonmatched cells (T1, T2, and T3). The proliferation was measured by [3H]thymidine incorporation (n = 3, mean ± SD). Values of p (Student’s t test) for comparisons between data are given. When not indicated, the differences were not significant. C, PBMCs were incubated with Ag, and their proliferation was measured using [3H]thymidine incorporation (n = 3, mean ± SD). Values of p (Student’s t test) for comparisons between data are given. Ctrl, cells without Ag.

**FIGURE 4.** Differentiation of myeloid DCs. A, DC culture of the proband and a healthy control at day 6 in the presence of GM-CSF and IL-4. B, Histogram panels from cytometric analysis of immature DCs at day 6. C, IL-12p70 in mature DC supernatants harvested after 24-h LPS activation (n = 2, mean ± SD). C3–/–, Proband; Ctrl, healthy control; Fa, father; Mo, mother.
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. 5Ai–ii). 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

FIGURE 5. B cell analysis. A–C, Phenotypes of peripheral blood B cells. Peripheral blood cells were stained with anti-CD19 PC7, anti-IgD PE, IgM APC, and anti-CD27 FITC. The percentage of positive cells is indicated relative to the CD19-gated cells. Panels are identified as proband (A, i–iv), father (B), and mother (C). D–F, B cell proliferation and IgM and IgG secretion assays. Highly purified B lymphocytes were 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 detrimental 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−/− mice (3, 22, 23). Mouse C3−/− 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 depletion 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 C3nullC3Pro550 proband lacks much of the expected switched memory B cells (IgD+/CD27+) in the periphery, with as little as 2.3% of switched IgM 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 C4D 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 monococytes 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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