

CD21 deficiency, complement, and the development of common variable immunodeficiency

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In this issue, Thiel and colleagues¹ present the first patient identified who lacks the membrane receptor protein CD21. This receptor is mainly present on B cells and follicular dendritic cells.² In this 28-year-old man, the deficiency is associated with an immune defect, common variable immunodeficiency (CVID). As such it adds to the growing list of genetic deficiencies associated with the development of CVID. It is not surprising that CD21 deficiency contributes to CVID because CD21 is part of the molecular complex consisting of CD21, CD81, and CD19 present on the membrane of B cells.² This molecular complex markedly augments the B-cell receptor response upon triggering by its cognate antigen. Defects in CD19 and CD81 have been previously associated with defects in antibody generation and CVID.^{3,4}

Our understanding of CVID is still incomplete, and it is particularly interesting to analyze the role of CD21 deficiency in CVID, because of CD21's well-defined biological activity. CD21 is also known as CR2,² the second of the series of complement receptors to be characterized. To fully appreciate the role of CD21 in patients with CVID, one must first understand the role of CD21 in complement function. CD21 binds a degradation fragment of plasma C3, known as C3d, and is also reported to bind CD23 and INF- α .^{5,6} C3 is expressed at high levels in plasma and is present in most normal individuals at about 1.2 mg/mL.^{7,8} It is one of the most important opsonins in blood and is a central protein in the activity of all 3 of the known complement activation pathways, the lectin pathway, the classical pathway, and the alternative pathway. C3 mediates the phagocytosis of microorganisms, facilitates the production of inflammatory factors such as C5a, and allows the complement cascade to reach the steps that cause lysis of susceptible organisms. By mediating these biological activities, C3 plays a key role both in complement activation and in host defense, often working through a series of specific receptors for C3 fragments. As discussed below, it also plays an important role in the immune response. Because it can play a highly destructive role if it causes unregulated inflammation and tissue damage, C3 is under tight regulatory control.

C3 activity is regulated by a series of cell membrane and blood proteins.⁹ Native C3, unlike IgG, does not interact with any known cellular membrane receptor. It is a 195,000-kD protein composed of 2 chains, alpha (about 120 kD) and beta (about

75 kD) (Fig 1). Activation of C3 is caused by a series of specific cleavages in the alpha chain by activating enzymes. The first of these cleavages releases a small 8900-kD fragment, C3a, from the alpha chain. The remainder of the molecule undergoes a conformational change and now is termed C3b. This conformational change reveals a highly reactive group, previously buried within the alpha chain, which can form amide or ester covalent bonds with a target of attack such as the surface of a microbe. If a covalent bond is not formed in milliseconds, the reactive site is hydrolyzed and the C3b no longer can bind to the target of attack. C3b can bind C5, the next protein in the hemolytic sequence, to continue the inflammatory cascade. C3b can also interact with proteins of the alternative complement pathway to promote further complement activation. Unlike intact native C3, C3b does react with a variety of specific receptors that may lead to specific biological consequences.¹⁰ The major receptor for C3b is the membrane protein CD35, or in the complement nomenclature CR1. CD35 is present on all B cells, on all phagocytes, and on a variety of other cells such as dendritic cells. No one has described a patient missing this receptor. Targeted antigens such as viruses or bacteria coated with C3b bind to the receptor. In the presence of the appropriate second signal, the target is phagocytosed and destroyed. C3b is often deposited on circulating immune complexes following complement activation. CD35 is present on all erythrocytes and can bind these immune complexes, effectively removing them from the plasma and allowing their transfer to phagocytes in the liver and the spleen. There, the immune complexes are stripped from CD35 and destroyed.

The conformational change in C3 that leads to the formation of C3b also enables a circulatory plasma regulatory protein, factor H, to bind to C3b. Factor H acts as a cofactor for the further degradation of C3b and can be thought of as a docking site for the circulating enzymatically active protein factor I.¹¹ Following binding of factor H, factor I causes additional alpha chain cleavages of C3b that produce the fragment iC3b. As indicated by the added "i," this form of C3b is hemolytically inactive and cannot continue activating the complement cascade. However, it is capable of interacting with its own set of receptors. It interacts weakly with CD 35 but strongly with the B2 integrin receptors CD11b/CD18 (CR3) and CD11c/CD18 (CR4). These integrin receptors are prominent on the surface of phagocytes, a subset of B cells, and natural killer cells and dendritic cells where they bind antigens with iC3b. Patients who are missing CD18 have no membrane CR3 or CR4 and exhibit major defects both in the phagocytosis of iC3b-coated particles and in binding to the integrin receptors on endothelial cells that allow the phagocytes normal egress from vessels. This disease is termed leukocyte adhesion deficiency.¹² CD11b/CD18 (CR3) and CD11c/CD18 (CR4) are markers for dendritic cells that bind iC3b-coated antigens and act as antigen-presenting cell antigens to facilitate a normal immune response.

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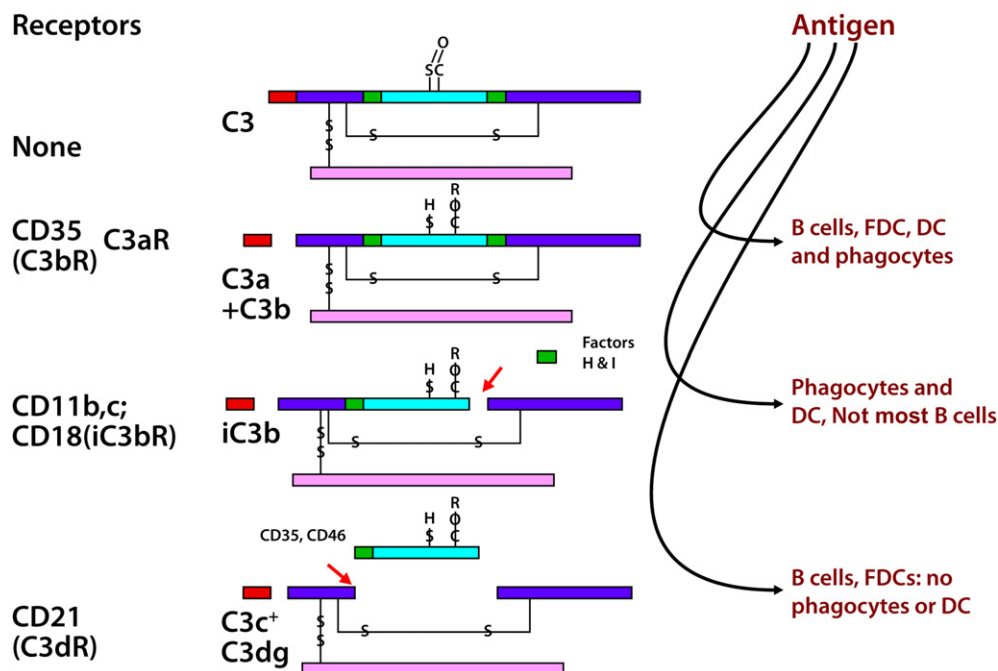


FIG 1. A schematic showing the complex degradation pattern of C3. On the *left* are the receptors to which the various fragments bind. On the *right* are the principle cells to which antigens coated with these fragments bind. The striking point is that native C3 is recognized by no receptors and the various fragments are directed to different sets of cells. The small fragment C3a is shown in *red* and in the *bottom panel* the C3dg is shown in *green* and *light blue*. It can be trimmed to C3d in *light blue*. C3c consists of all the beta chain, and the discontinuous *dark blue* fragments of the alpha chain, all still disulfide linked. DC, Dendritic cell; FDC, follicular dendritic cells.

iC3b can undergo further degradation in the presence of the enzyme factor I and a suitable cofactor. In general, the cofactors that allow for further degradation are cell membrane bound and include CD35 and CD46. iC3b is further cleaved by factor I to form a 40-kD fragment of the alpha chain, which is called C3dg and remains bound to the antigen target. C3dg can be trimmed to C3d, remaining bound to the target. C3dg and C3d bind weakly to the integrins but bind well to CD21, the object of this report. CD21 is present on a more limited number of cells. It is not present on most phagocytes, but it is present on all B cells representing part of the multimolecular complex described at the beginning of this report. It is also present on follicular dendritic cells and some other dendritic cells. CD21 is particularly well represented on B cells in the marginal zone of secondary lymphoid tissue follicles. This is a point of entry of microbes and antigens into secondary lymphoid tissue.

When foreign antigens or microbes enter the circulation, they may activate complement directly or bind antibody, which, in turn, activates the complement. This leads to the deposition of C3b and ultimately C3d. Thus, microbes or antigens that enter the circulation can be swept into the marginal zone of secondary lymphoid tissues and coated with the appropriate C3 fragments to bind to CD21. This interaction appears to be a critical control point in the initial stages of infection. Rapid generation of antibody by B cells in the marginal zone may be essential to control infection, long before an adaptive immune response develops, which may take days. Similarly, under some circumstances, the binding of antigens to CD21 via C3d is essential in promoting the immune response. While small antigens can position themselves to initiate an immune response by entering

canaliculi that pass from the marginal zone into follicles at the B-T-cell interface, larger antigens, and presumably antigens that are coated with C3d, bind to cells in the marginal zone via CD21. These cells with bound antigens are triggered to leave the marginal zone and migrate into follicles near the T-B-cell border.^{13,14} Within the follicle some of the antigen coated with C3d is transferred to CD21 on follicular dendritic cells where it is maintained intact for prolonged periods of time, facilitating a prolonged immune response. It is in the center of follicles that germinal centers are formed and B cells undergo waves of somatic hypermutation leading to the formation of antibody with increasing affinity for antigen. Long-lived memory B cells are also formed within the germinal centers that then migrate to the bone marrow as they transform into long-lived plasma cells. Thus, the formation of germinal centers is a critical step in the normal humoral immune response. Knockout mice with loss of C3 or CD21 have a major defect in their immune response. They form germinal centers poorly, have short-lived memory cells, and exhibit short-lived antibody responses. In studies of antigens synthesized with an added sequence containing multiple copies of C3d it was noted that the added C3d increased the immune response by as much as a thousandfold.² It appears that the binding of C3d-coated antigens to CD21 offers an extra site for binding and thereby stabilizes the binding to the B-cell receptor. It is likely that concurrent binding of CD19 provides the additional stimulus for B-cell activation.¹⁵

Given the importance of CD21 in antigen trapping, antigen transport, and the generation of a normal immune response, it is not surprising that an individual lacking CD21 has an abnormal immune response. In the limited studies that have been done in

mice lacking CD21, large doses of antigen and antigen given with adjuvant such as alum can compensate for the antibody-promoting effects of CD21.^{16,17} Thus, CD21 is not absolutely required for these effects, but it facilitates a normal immune response, particularly with low doses of antigen.

The patient described in this report is unique and potentially quite instructive. CD21 has been used as a marker of B cells for decades, yet no individual missing CD21 has been previously described. The analysis of this patient is detailed, and the defects in the CD21 gene are well documented. It is interesting that although this patient has immunodeficiency, the immunodeficiency is not severe, and is most notable for polysaccharide antigens. Presumably, the patient has been exposed to microbial antigens multiple times and exposed to vaccine antigens administered with adjuvant. It is striking that having had infections in childhood, this patient went 20 years without infection. The description of this patient completes the identification of individuals missing CD19, CD81, and CD21, all part of the same molecular complex on B cells and all of whom have CVID.

It is hoped that studies of this patient will continue. For example, it would be of great interest to challenge this patient with bacteriophage ϕ X 174 and measure the response of this patient to neoantigens. On the basis of our current knowledge of CD21, we would predict that the patient would have a markedly abnormal and short-lived immune response with the generation of few memory cells.¹⁸ The authors are to be congratulated for a thorough analysis of this patient's defect and for presenting the preliminary studies that allow us to further understand the important role of CD21 in the immune response.

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