Multifaceted Activities of Seven Nanobodies Against Complement C4b

Cleavage of the mammalian plasma protein C4 into C4b initiates opsonization, lysis, and clearance of microbes and damaged host cells by the classical and lectin pathways of the complement system. Dysregulated activation of C4 and other initial components of the classical pathway may cause or aggravate pathologies, such as systemic lupus erythematosus, Alzheimer disease, and schizophrenia. Modulating the activity of C4b by small-molecule or protein-based inhibitors may represent a promising therapeutic approach for preventing excessive inflammation and damage to host cells and tissue. Here, we present seven nanobodies, derived from llama (Lama glama) immunization, that bind to human C4b (Homo sapiens) with high affinities ranging from 3.2 nM to 14 pM. The activity of the nanobodies varies from no to complete inhibition of the classical pathway. The inhibiting nanobodies affect different steps in complement activation, in line with blocking sites for proconvertase formation, C3 substrate binding to the convertase, and regulator-mediated inactivation of C4b. For four nanobodies, we determined single particle cryo-electron microscopy structures in complex with C4b at 3.4 - 4 Å resolution. The structures rationalize the observed functional effects of the nanobodies and define their mode of action during complement activation. Thus, we characterized seven anti-C4b nanobodies with diverse effects on the classical pathway of complement activation that may be explored for imaging, diagnostic, or therapeutic applications.

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