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Molecular Immunology 42 (2005) 1393–1403
Molecular Immunology
www.elsevier.com/locate/molimm

IgG naturally occurring antibodies stabilize and promote the generation of the alternative complement pathway C3 convertase

Emiliana Jelezarova, Hans U. Lutz*

Institute of Biochemistry, Swiss Federal Institute of Technology, ETH Hoenggerberg, HPM D14, Schafmattstrasse 18, CH-8093 Zurich, Switzerland

Received 15 December 2004; accepted 22 December 2004
Available online 16 February 2005

Abstract

Normal human IgG contains naturally occurring anti-C3 antibodies (anti-C3 NAbs) that have been proposed to regulate complement amplification. Here, we report a novel procedure for anti-C3 NAb purification. Pooled human IgG was fractionated on a DEAE column prior to affinity chromatography on IgG and then on C3. Anti-C3 NAbs co-purified with anti-F(ab′)$_2$ NAbs. In a refined protocol, IgG fractions were absorbed on Fc, F(ab′)$_2$, and C3, which allowed to isolate the directly accessible NAbs and to remove IgG hinge-region-specific NAbs. Since a substantial fraction of total anti-C3 NAbs in whole IgG pre-existed as complexes, IgG that did not bind to the three affinity columns was treated with urea and the affinity chromatography repeated to collect the dissociated NAbs. The urea-accessible anti-F(ab′)$_2$ NAbs were rather pure but anti-C3 NAbs yet contained substantial amounts of anti-F(ab′)$_2$ NAbs. Anti-C3 NAbs showed up to 400-fold and anti-F(ab′)$_2$ NAbs, up to 30-fold enrichment as compared to pooled normal human IgG. Anti-C3 NAb preparations exhibited nephritic factor activity that was up to 60 times stronger than that of total IgG from a patient with membranoproliferative glomerulonephritis type 2. In addition, anti-C3 NAbs promoted C3 convertase generation, when added to the convertase precursor or during convertase assembly, suggesting a non-nephritic-factor mechanism. Factors H and I reduced the overall level of activity but had no influence on the NAb dose–response curve meaning that NAbs did not interfere with factor H binding. Convertase promoting activity during assembly correlated with the content of anti-C3 NAbs in NAb complexes. In conclusion, anti-C3 NAbs associated with framework-specific anti-idiotypic NAbs stabilize C3 convertase and promote its generation but their activity is compensated for in whole IgG.

Keywords: Antibodies; Complement; Inflammation; Human; Autoimmunity

1. Introduction

The amplification loop of complement is maintained by the alternative pathway of complement (AP)[3] C3 convertase, C3bBb, which cleaves C3 to C3b that in its turn can recruit factor B and nucleate a new enzyme. The AP C3 convertase is subject of strict regulation. Its inherent instability and the proteolytic inactivation of C3b by factor I prevent excessive or inappropriate activation (Weiler et al., 1976). Factor H provides co-factor activity for factor I, but has decay accelerating function as well, which makes it the major soluble protein that regulates the half-life of the C3 convertase (reviewed in Pangburn, 2000). In addition, factor H discriminates between self and non-self by recognizing surface polyanions (Meri and Pangburn, 1990). Properdin is the single physiological positive regulator of the alternative pathway known and it operates by stabilizing the C3 convertase against the intrinsic decay (Fearon and Austen, 1975). C3 nephritic factor (C3NeF) is an autoantibody found in patients with membranoproliferative glomerulonephritis (MPGN), which binds to the alternative pathway C3 convertase and prolongs its half-life (Daha et al.,

Abbreviations: AP, alternative pathway of complement; C3NeF, C3 nephritic factor; GPBS, PBS, containing 0.1% gelatin; GVBS, VBS, containing 0.1% gelatin; IK, immunoconglutinins; IVIG, pooled human IgG for intravenous application; MPGN, membranoproliferative glomerulonephritis; NAbs, naturally occurring antibodies; VBS, veronal-buffered saline

* Corresponding author. Tel.: +41 1 632 30 09; fax: +41 1 632 12 69.
E-mail address: hlutz@bc.biol.ethz.ch (H.U. Lutz).

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doi:10.1016/j.molimm.2004.12.014

1976). However, despite the similar effect, two important differences exist between properdin and C3NeF. While C3NeF recognizes only the assembled active enzyme, C3bBb (Daha and van Es, 1979), properdin can bind also to the precursors of the C3 convertase, C3b (DiScipio, 1981; Farries et al., 1988) and $C3b_2$–IgG complexes (Jelezarova et al., 2000). In addition, C3NeF stabilizes the C3 convertase against the decay-accelerating function of factor H (Weiler et al., 1976), which properdin cannot do.

Nephritic factors and C3NeF in particular have been shown to be part of the normal immune repertoire of a healthy adult (Spitzer and Stitzel, 1988). Pokeweed-stimulated mononuclear cells from healthy adults and neonates produced IgG and IgM C3NeF to the same extent as the corresponding cells of MPGN patients (Spitzer et al., 1990). Not only did C3NeF IgG represent a significant fraction of the total IgG synthesized (12–17 %), but it also appeared to express a very limited idiotype, suggesting clonal selection. Interestingly, C3NeF was not found in the plasma of healthy subjects or normal newborn infants, implying that the ability to produce C3NeF is present from birth but is subject of regulation by anti-idiotypic antibodies (Spitzer et al., 1992). Indeed, the nucleotide sequence of an IgG C3NeF produced by an EBV transformation exhibited extensive rate of mutation, characteristic of antigenic selection and affinity maturation (Victor et al., 1993). The high affinity of C3NeF is another feature reminiscent of an induced Ab. The affinity constants determined for IgG C3NeF from MPGN patients were $0.8–4.8 \times 10^8$ l/mol. The high specificity, limited clonal usage, and the high affinity probably reflect the singularity of the antigen.

Naturally occurring IgM and IgG antibodies (NAbs) are on the other end of the "antibody scale": they are low titer, low affinity germ line-derived antibodies directed to conserved autoantigens or to non-self antigens (Avrameas, 1991). Generation of autoreactive NAbs requires the availability of respective B cell clones and is dependent on the expression of the autoantigen (Hayakawa et al., 1999). NAbs have a role in tissue homeostasis. They induce complement deposition to senescent (Lutz et al., 1987; Rettig et al., 1999) and apoptotic cells (Kim et al., 2002) and mediate their clearance by phagocytosis. NAbs are also involved in mounting an adaptive immune response (Ochsenbein and Zinkernagel, 2000) and may in conjunction with the powerful complement system direct an immune response to non-self epitopes (Dempsey et al., 1996; Lutz, 1999; Thornton et al., 1994).

Pooled human IgG for intravenous application (IVIG) contains anti-C3 NAbs that have been proposed to regulate complement amplification in vitro (Lutz et al., 1993, 1996). We recently could demonstrate for the first time that high dose IVIG attenuates complement amplification also in vivo by stimulating the inactivation of $C3b_2$-containing complexes in dermatomyositis (Lutz et al., 2004). High dose IVIG does not inhibit classical complement pathway, but attenuates complement amplification to the extent that even extra C3 activation via the classical pathway is compensated for. Thus, IVIG indeed contains antibodies that effectively regulate complement amplification. The actual mechanism of convertase modulation has not yet been clarified.

Another group of antibodies in plasma is closely related to anti-C3 NAbs: immunoconglutinins (IK). IKs are present in many species including humans and are defined as antibodies binding to fragments of C3. Their exact specificity is poorly defined, but most investigators determined them by studying serum immunoglobulin binding to intact C3 in ELISA. Interestingly, IgM and IgA IKs did not vary greatly with disease states in systemic lupus erythematosus, while IgG IKs were generally higher in several types of autoimmune disease than in controls. Attempts to correlate IK titers with disease states gave an unclear picture. For example, in systemic lupus erythematosus patients IgG IK levels were generally higher than in controls, but the highest titers were observed before or after an acute flare rather than at exacerbation (Nilsson et al., 1992). Hence, IgG molecules that bound to C3 (defined as IKs) were indicative of recovery or remission rather than disease activity. IKs, but not conglutinins, correlated with recovery of calves from respiratory tract disease (Purdy et al., 2000). The exact role of IKs is not understood. IKs were purified from plasma containing no IgM and IgA on immobilized C3 in the presence of a detergent. The purified material had properties both of an enhancer of complement deposition and of complement inhibitor, since it inhibited factor I dependent C3b cleavage, but also inhibited C5 convertase activity (Nilsson et al., 1990). The uncertainty about the properties of IKs may have several reasons. One reason is that none of the IKs purified on immobilized C3 is either homogeneous or pure. The different specificity of individual IK preparations suggests different epitopes and/or a polyclonal response (Nilsson et al., 1992). In addition, such preparations are contaminated to different extent by other IgG molecules, mainly due to IgG–IgG interactions.

Our goal is to understand how NAbs modulate C3 convertase activity in healthy individuals and how pathological autoantibodies to C3b and C3 convertase can be counteracted or modulated by NAbs. Studying the role of anti-C3 NAbs requires reasonably pure material, but their isolation from pooled human IgG is hampered by their low concentration and low affinity. In addition, autoreactive NAbs appear to be masked in human serum, presumably by anti-immunoglobulin (anti-Ig) NAbs (Avrameas, 1991; Avrameas and Ternynck, 1993). A specific problem with the affinity isolation of anti-C3 NAbs is the fact that most of the IgG molecules have a low intrinsic affinity for C3 (Kulics et al., 1983). In order to remove some of the unwanted IgG molecules, we fractionated total human IgG on a DEAE column and treated it with urea prior to affinity chromatography on IgG fragments and C3. Here, we report on NAb binding and functional properties tested in a variety of functional assays specifically developed to study C3 convertase modulation.