

HOW IMMUNE COMPLEXES FROM CERTAIN IgG NAbS AND ANY F(ab')₂ CAN MEDIATE EXCESSIVE COMPLEMENT ACTIVATION

Hans U. Lutz

*Institute of Biochemistry, Swiss Federal Institute of Technology, ETH Hönggerberg, Zurich, Switzerland
Email: hans.lutz@bc.biol.ethz.ch*

Abstract: In sepsis death follows an excessive inflammatory response involving cytokines and complement that is activated primarily via the amplifying C3/C5 convertase. Excessive stimulation of complement amplification requires IgG-containing or F(ab')₂-containing immune complexes (IC) that capture dimeric C3b on one of their heavy chains or heavy chain fragments. The ability of IgG-IC to capture dimeric C3b by the Fab portion is dependent on an affinity for C3 within the Fab portion, but outside the antigen-binding region. This property is rare among IgG NAbS. In contrast to this, the lack of the Fc portion renders the Fab regions of any F(ab')₂-IC accessible to nascent C3b, but dimeric C3b deposits only if F(ab')₂-IC form secondary IC with anti-hinge NAbS that rigidify the complex and thereby promote deposition of dimeric C3b. Both types of complexes, C3b₂-IgG-IC and C3b₂-F(ab')₂-IC/anti-hinge NAbS, are potent precursors of alternative C3 convertases and stimulate complement amplification along with properdin up to 750 times more effectively than C3b and properdin. F(ab')₂ fragments are not normally generated, but are formed from NAbS by enzymes from pathogens and neutrophils in sepsis. Unlike IgG-IC F(ab')₂-IC are not cleared by Fc-receptor dependent processes and circulate long enough to form secondary IC with anti-hinge NAbS that rigidify the complexes such that they capture dimeric C3b and gain the potency to stimulate complement amplification.

INTRODUCTION

NAbS are a priori beneficial, but some of them can, under very unique conditions, mediate excessive systemic complement activation and thereby promote disease without

having mutated to auto-aggressive antibodies. This phenomenon occurs in diseases that do not exert an evolutionary pressure like infarction or SIRS in sepsis and trauma. For example, the acute inflammatory response as in infarction and ischemia/reperfusion involves NABs as was first observed by Carroll's group in ischemic skeletal muscle.¹ Ischemia is accompanied by hypoxia and pH changes, which result in sudden exposure of normally hidden autoantigens. The liberated autoantigens get in contact with IgM and IgG NABs that are present in the locally available plasma. NABs against many of the exposed proteins exist in plasma to clear such components, when liberated in a homeostatic process. During ischemia the release of such components is, however, massive, such that the large number of liberated autoantigens get more and more in contact with NABs upon being locally replenished during reperfusion. Immune complexes formed by these NABs and liberated autoantigens activate complement not only by initiating the classical complement pathway, but to a significant extent by stimulating complement amplification via the alternative complement pathway, since damage to intestinal cells was only 1/3 in factor D knockouts subjected to ischemia/reperfusion.² The phenomenon is reviewed in Chapter 13 by S.D. Fleming in this volume.

In contrast to the situation in ischemia, excessive complement activation during SIRS appears to be stimulated primarily by immune complexes generated from certain IgG NABs and any F(ab')₂ fragments.³ SIRS can develop from severe sepsis, but also in patients with trauma having no infections. Excessive complement activation in SIRS can induce paralysis of neutrophils, the cytokine storm of macrophages, the release of tissue factor,⁴ and death by destructive processes. As long as nascent C3b deposits to immune complexes that initiate complement activation via the classical complement pathway, the number of excessively produced nascent C3b is limited. If, however, the immune complexes stimulate complement amplification, millions of nascent C3b molecules are generated, many of which bind covalently not only to opsonized pathogens, but also to nearby self components without being opsonized. This destructive process accompanied by proinflammatory cytokines will eventually result in binding, entrapment and phagocytosis of self structures and thereby exerts a devastating role in an organism. Until recently it has remained unclear how immune complexes stimulate excessive complement activation via the alternative complement pathway, but the phenomenon as such is known for 30 to 40 years.⁵ The mechanism will be reviewed in this chapter.

STIMULATION OF COMPLEMENT AMPLIFICATION BY C3b₂-IgG CONTAINING IMMUNE COMPLEXES

Complement amplification is normally stimulated by C3b newly generated by one of the three complement pathways. A newly generated C3b molecule nucleates an alternative C3 convertase (C3bBb) that is stabilized by properdin (C3bBbP) and catalyzes the cleavage of additional C3 molecules to C3a and C3b. This process is eventually limited by factor H and I, which together will inactivate C3b to iC3b. This control is overrun if the number of generated C3b molecules is ten to hundred times higher, implying that the precursor of such superactive C3 convertases must be far more stable than monovalently bound C3b and that the generated precursor or enzyme has to be far more effective than a C3bBb complex. One such effective C3 convertase precursor is formed on IgG immune complexes (IgG-IC) that captured dimeric C3b by one of the heavy chains. Dimeric C3b deposits to immune complexed IgG antibodies that have an affinity for C3

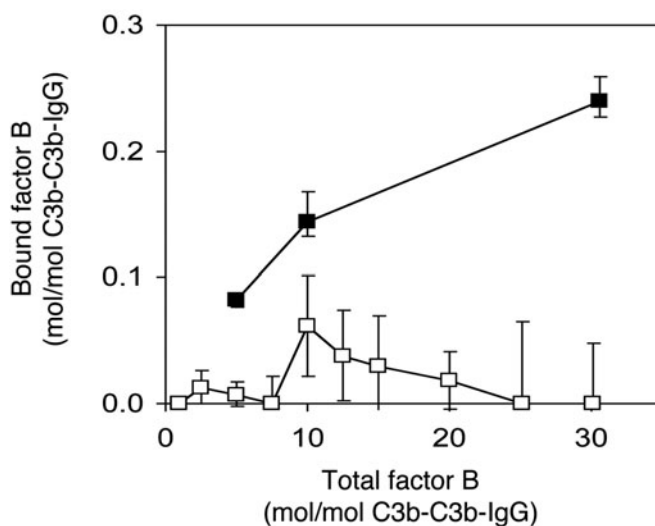


Figure 1. Binding of factor B to C3b₂-IgG complexes before or after preincubation with properdin. Labeled factor B (10–290 nM) was incubated with 1.93 nM C3b₂-IgG complexes without properdin (open squares) or with 147 nM properdin (closed squares) in veronal buffered saline at pH 7.4 with 5 mM MgCl₂. Results are from triplicates and are given as means \pm SD. Reproduced with permission, from Jelezarova et al. *Biochem J* 2000; 349:217–223;⁸ ©2000 the Biochemical Society.

outside the antigen-binding sites.⁶ An immune complexed IgG NAb having this affinity for C3 preferentially forms C3b₂-IgG complexes⁷ and these complexes are far more effective C3 convertase precursors than C3b. The reason for their high efficiency is that the dimeric C3b within this complex has a longer half life than C3b⁷ and the dimeric C3b provides an increased affinity for properdin that exists in oligomeric form in plasma. As a consequence a C3b₂-IgG containing immune complex first binds oligomeric properdin which in its bound state greatly increases the affinity for factor B (Fig. 1).⁸ The prolonged half life of C3b₂-IgG complexes and the stimulation by an increased affinity for factor B, render such complexes about 750 times more effective in generating C3 convertases than monovalently bound C3b. Such IgG molecules that stimulate complement amplification appear to be rare, but have not been searched for systematically. They appear to recruit primarily from IgG NABs^{9,10} and remain active in Mg²⁺ EGTA- treated human serum.¹¹ While Valim and Lachmann suggested that the IgG2 subclass predominantly activated the alternative complement pathway,¹² Banda et al. claim that IgG molecules capable of initiating the murine alternative complement pathway require N-glycans bound to Asp227 within the Fc portion of the heavy chains.¹³ In none of the two cases it has been investigated how complement amplification is stimulated and whether this stimulation was in any way related to the formation of C3b-containing IgG-IC. In contrast to this, generation of C3b₂-IgG complexes in the course of an alternative complement pathway stimulation is known for 30 years. In fact, Gadd and Reid were the first to notice that IgG-IC that stimulate complement amplification, capture C3b molecules by one of their heavy chains.¹¹ While their finding suggested the presence of either monomeric or dimeric C3b on one heavy chain, we could demonstrate by 2-dimensional SDS PAGE with a hydroxylamine treatment between the dimensions that C3b-carrying IgG-IC contain

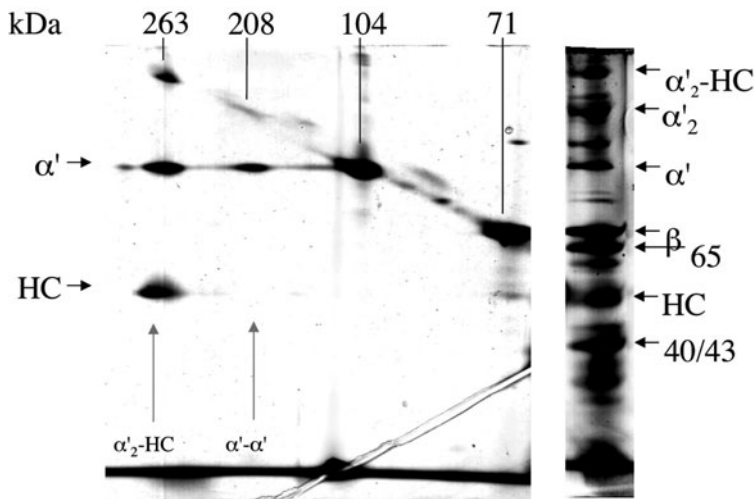


Figure 2. Two-dimensional SDS PAGE of C3b₂-IgG complexes. Purified C3b₂-IgG complexes were reduced, alkylated and run on a 6% polyacrylamide gel. Gel strips were cut from top of the separating gel to the β band of C3b, treated with hydroxylamine as described and loaded on an 8% gel for the second dimension. A silver stained gel is shown. The calculated MW of complexes are given in kDa: 263 for α C3₂-HC, 208 for α C3₂. This figure was originally published as Figure 3B, by Jelezarova E, et al. *J Biol Chem* 2003; 278:51806–51812;¹⁴ ©2003 the American Society for Biochemistry and Molecular Biology.

exclusively ester-bonded dimeric C3b on one heavy chain, but are contaminated by free C3b dimers (Fig. 2).¹⁴ These findings are in favor of the mechanism that we have suggested for the stimulation of complement amplification by IgG-IC. Long known additional findings support the mechanism further, namely that not only IgG-IC, but also F(ab')₂-IC stimulate complement amplification at least to the same extent^{9-11,15} with up to 56% of C3 being activated in 100% plasma,¹⁶ by generating C3b₂-F(ab')₂-IC.^{11,15} These findings demonstrate that the ability of IgG-IC to stimulate complement amplification differed from their ability to activate the classical pathway, because stimulation of complement amplification did not require the Fc portion. The ability of almost any type of F(ab')₂-IC to stimulate complement amplification appeared obvious, because the lack of the Fc portion may have increased the accessibility for nascent C3b to deposit to the CH1 domain of F(ab')₂-IC complexes.

ANTI-HINGE NABs RIGIDIFY F(ab')₂-IC TO CAPTURE DIMERIC C3b AND TO STIMULATE COMPLEMENT AMPLIFICATION

Several groups have demonstrated that not only IgG-IC, but also F(ab')₂-IC stimulate complement amplification to similar extents.^{11,16-19} There is, however, an important difference already discovered in 1971 by Kevin Reid,¹⁶ namely that F(ab')₂-IC require aside of the complement proteins an unknown, non-complement serum factor to stimulate complement amplification. In our attempt to search for this serum factor we hypothesized that "IgG anti-hinge NABs" that had been studied more recently in quite some details

by the group of Terness (for reviews see refs. 20, 21), may represent this serum factor. Anti-hinge NAbs have even earlier been studied and named “pepsin agglutinators,” because they agglutinated IgG upon its treatment with pepsin that generates $F(ab')_2$ and Fc.^{22,23} IgG anti-hinge antibodies are germline-encoded NAbs that bind to a conformational hinge region epitope that becomes accessible exclusively upon cleavage of IgG1 by pepsin.²⁴ Anti-hinge NAbs have an important role in the regulation of the B-cell antibody production. Their plasma concentration increases slightly when B cells start producing antibodies and eventually anti-hinge NAbs stop B-cell antibody production by binding to the hinge region of antigen-carrying B-cell receptor and Fc receptor of the antibody-producing B cell.²¹ Thus, anti-hinge NAbs have an important regulatory function within the innate and adaptive immune system.

Despite having this immune regulatory role, the very same IgG NAbs can exert damaging effects. In studying the damaging role of anti-hinge NAbs, C3b-F(ab')₂-IC formation and C3 activation induced by these complexes was studied with whole serum, with serum absorbed on F(ab')₂, and with serum absorbed on F(ab')₂ to which purified anti-hinge NAbs were added. Anti-hinge NAbs had the expected effect and restored complex formation and C3 activation in serum absorbed on F(ab')₂, although at a 10 time higher concentration than expected, presumably because the purity of the material was not yet 100%.²⁵ Interestingly, F(ab')₂-IC added to serum generated complexes that carried two and even three C3b molecules on one of the shortened heavy chains. Hence, our data strongly suggest that anti-hinge NAbs represent Reid's serum factor which rigidifies F(ab')₂-IC and thereby facilitates covalent binding of dimeric C3b to the Fd region of one arm of an immune-complexed F(ab')₂ in generating a potent C3 convertase precursor (Fig. 3).

We have also verified this hypothesis on F(ab')₂-IC from affinity-purified anti-spectrin NAbs. Since these NAbs lack a CH1-mediated affinity for C3, they stimulate complement amplification as F(ab')₂-IC, but not as IgG-IC in normal human serum. Absorption of

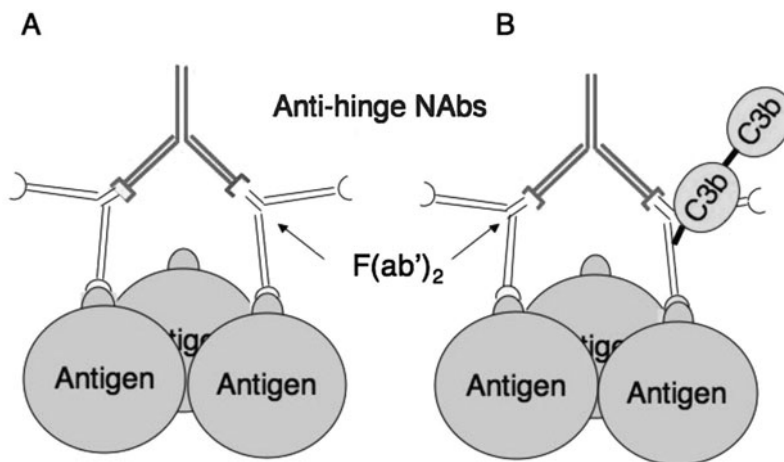


Figure 3. Generation of potent C3 convertase precursors. A. Anti-hinge NAbs form secondary IC with F(ab')₂-IC. (B). F(ab')₂-IC rigidified by anti-hinge NAbs allow nascent C3b to deposit as ester-linked C3b dimer onto the shortened and rigidified heavy chain of a F(ab')₂. Reproduced with permission from Fumia et al. *Mol Immunol* 2008; 45:2951–2961.²⁵ ©2008 Elsevier.

normal serum on immobilized $F(ab')_2$ removed anti-hinge NAbs and abrogated the ability of $F(ab')_2$ -IC to stimulate complement amplification, while supplementation with purified anti-hinge NAbs restored stimulation of complement amplification.²⁵ Thus, anti-hinge NAbs represent the serum factor that is required to rigidify $F(ab')_2$ -IC such that nascent C3b can deposit as a dimer to one Fab portion of $F(ab')_2$ -IC. This finding may further explain why the efficacy by which $F(ab')_2$ -IC stimulate complement amplification is high with human serum, but decreases with serum from more primitive species that are known to have lower concentrations of anti-hinge NAbs.²⁶

$F(ab')_2$ -CONTAINING IMMUNE COMPLEXES FORM SECONDARY IC WITH ANTI-HINGE NABS IN PLASMA OF SEPTIC PATIENTS

$F(ab')_2$ fragments from IgG molecules may be dangerous, because they can form immune complexes with autoantigens and eventually potent alternative complement pathway C3 convertases. The reason is that $F(ab')_2$ -IC persist long enough in plasma to form secondary immune complexes with IgG anti-hinge NAbs, because $F(ab')_2$ -IC, unlike IgG-IC are not cleared via Fc-receptor carrying phagocytes. Eventually, anti-hinge NAbs bind to $F(ab')_2$ -IC and stabilize antigen-bound $F(ab')_2$ to the point that the rigidified $F(ab')_2$ captures dimeric C3b on one Fab and thereby forms an effective C3 convertase precursor.

The most specific enzyme that generates $F(ab')_2$ fragments from IgG molecules, pepsin, is restricted to the gastrointestinal tract in higher organisms. However, a number of other proteases that can also cleave IgG into $F(ab')_2$ and $F(ab')_2$ -like fragments (in which only one heavy chain is cleaved) can occur in blood during inflammation and bacterial infections. These $F(ab')_2$ -generating proteases originate from endogenous sources, like neutrophils (elastase),²⁷⁻²⁹ from pathogens like *Staphylococci*³⁰ (glutamyl endopeptidase), from *Streptococci* (streptococcal immunoglobulin-degrading enzyme, IdeS),³¹ and many others.

It was our goal to investigate whether $F(ab')_2$ fragments are generated during severe sepsis and whether these fragments upon complexing autoantigens form secondary $F(ab')_2$ -IC with anti-hinge NAbs and give rise to excessive complement activation. Nine patients in intensive care with several types of bacterial infection, elevated CRP values and neutrophil numbers have been studied for elastase, generation of $F(ab')_2$ fragments, factor Bb concentration, and formation of secondary immune complexes comprised of $F(ab')_2$ -IC and anti-hinge NAbs. Both the concentration of $F(ab')_2$ and that of the activated complement factor B (Bb) increased linearly with the total concentration of elastase in plasma (Fig. 4).²⁵ These results provide suggestive evidence for the role of elastase in generating $F(ab')_2$ from IgG and the ability of secondary IC to act as potent precursors of alternative C3 convertases. Gelfiltrations on plasma proteins from these patients revealed that the total concentration of $F(ab')_2$ was about three $\mu\text{g/ml}$ plasma, but less than 10% of this material migrated with its MW of about 100 kDa.³ The majority of $F(ab')_2$ ($1.7 \pm 0.4 \mu\text{g/ml}$, $n = 9$) was recovered in pool A with MWs in the range from 200 to 800 kDa and $0.4 \pm 0.1 \mu\text{g/ml}$ migrated in pool B with MWs of about 150 kDa. In contrast to this, none of the pools from controls contained measurable concentrations of $F(ab')_2$. Hence, these results do not only confirm the formation of $F(ab')_2$ during severe sepsis, but illustrate the formation of secondary immune complexes, of which the largest one (pool A) contained aside of $1.7 \mu\text{g/ml}$ $F(ab')_2$, unknown antigens, and $0.9 \mu\text{g/ml}$ anti-hinge NAbs.²⁵ At first it appears that a total concentration of $F(ab')_2$ of $3 \mu\text{g/ml}$ is minute, but

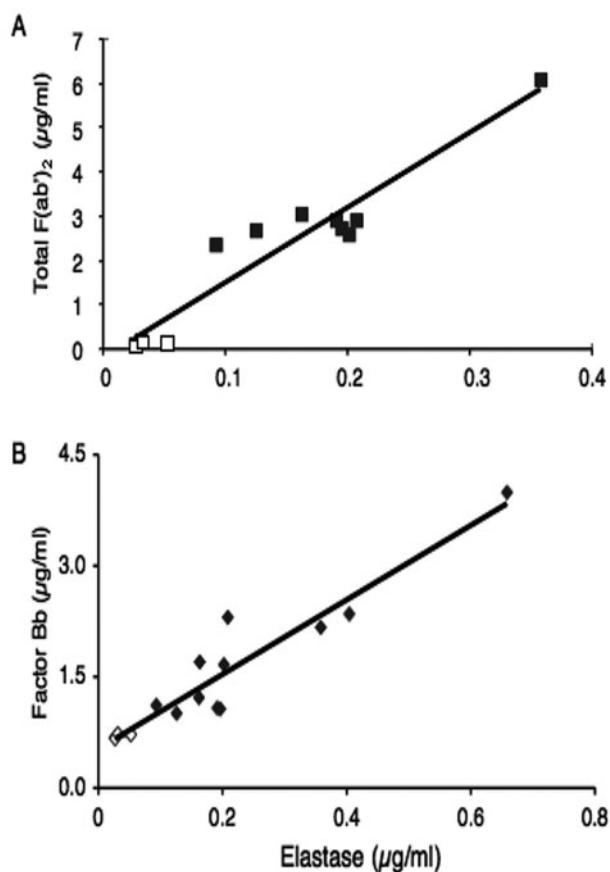


Figure 4. The plasma concentration of F(ab')₂ and that of activated factor B (Bb) correlated with that of elastase in plasma from septic patients. The total concentration of F(ab')₂ in plasma (A), data from patients number 4–12) and that of activated factor B (B), data from patients number 1–12). Values from control plasma are shown by open symbols. Reproduced with permission from Fumia et al. *Mol Immunol* 2008; 45:2951–2961.²⁵ ©2008 Elsevier.

if all the F(ab')₂-IC in pool A formed pairwise secondary immune complexes, of which all captured dimeric C3b and each of them allowed assembly of C3 convertases, these convertases would have generated theoretically 600 µg/ml C3b. This estimate would mean activation of 50% of C3, a huge portion, but not unrealistic for immune complexes that stimulate complement amplification.

HOW TO PREVENT F(ab')₂-IC FROM STIMULATING COMPLEMENT AMPLIFICATION?

Theoretically there are mainly two routes by which the massive complement amplification induced by F(ab')₂-IC could be stopped. One calls for inhibitors of elastase and similar types of proteases, another route prevents F(ab')₂-IC from being rigidified by

anti-hinge NAb. The latter goal is reached by injecting a sufficient amount of irrelevant $F(ab')_2$ fragments that bind to anti-hinge NAb and thereby prevent them from rigidifying $F(ab')_2$ -IC. This approach has been taken by Dr. Dietrich 45 years ago in form of Gamma Venin, a $F(ab')_2$ preparation from pooled whole human IgG.³² It turned out to be quite effective in nine patients with severe sepsis. His explanation of why the treatment was effective, was, however, inappropriate on the basis of what was known. He argued that the many types of $F(ab')_2$ helped clearing pathogens, although efficient clearance normally requires immune complex formation by IgG antibodies and their recognition by phagocytic receptors via the Fc portion of these antibody molecules. Therefore the immunologists may have concluded that IgG should have been used instead of $F(ab')_2$ and they convinced the companies to isolate and pool human IgG for intravenous application (IVIG).

In the meantime the argument of Dietrich has turned out to be partially correct, because work from Yano et al.²⁶ and Brezski et al.³³ indeed suggested that anti-hinge NAb can aid in clearance of targets that have bound antigen-specific $F(ab')_2$. This type of cooperation of anti-hinge NAb with $F(ab')_2$ is, however, highly dependent on the relative concentrations of total $F(ab')_2$, antigen-specific $F(ab')_2$ and anti-hinge NAb. For example, anti-hinge NAb that existed in varying concentrations in nonhuman primates induced a rapid clearance of about 75% of platelets purposely tagged with a monoclonal anti-platelet $F(ab')_2$ in 5 of 30 animals.²⁶ On the other hand, an injection of $F(ab')_2$ from pooled human IgG in amounts that can complex all available anti-hinge NAb, as performed by Dietrich, must have prevented anti-hinge NAb from binding to newly generated $F(ab')_2$ -IC and thereby exerted a beneficial effect in patients with a severe sepsis.

Intact IgG (IVIG) as a replacement of $F(ab')_2$ from pooled human IgG cannot exert an analogous effect, because the binding site for anti-hinge NAb is not accessible in intact IgG. Expectedly, IVIG failed to cure patients with severe sepsis irrespective of the brand.³⁴ Nevertheless, high concentrations of IVIG stimulate factor I and H dependent inactivation of C3b₂-containing complexes by a factor of two,³⁵ a value evidently too small to cure from severe sepsis. IVIG has since been used successfully in an increasing number of autoimmune diseases, where it suppresses autoaggressive antibody formation by several means.³⁶ Hence, it may be appropriate to repeat isolation of $F(ab')_2$ from pooled whole human IgG (Gamma Venin) and to study its efficacy in treating patients with sepsis and SIRS.

The other route in treating patients with massive complement overreaction calls for inhibitors of elastase and similar proteases capable of generating $F(ab')_2$ from IgG. This approach has been used successfully in disease models using animals.³⁷⁻³⁹ Recently elastase inhibitors have also been applied to humans. In patients with acute respiratory distress syndrome (ARDS/SIRS) sivelestat had beneficial effects on pulmonary functions.⁴⁰ Among patients with multi organ failure sivelestat reduced hospital mortality from 33 to 6 percent.⁴¹ Togo et al. studied the effect of sivelestat on survival of patients who developed gastrointestinal septic ARDS and found that sivelestat was most effective if patients were well oxygenated.⁴² In all cases treatment with the elastase inhibitor reduced the inflammatory process, but none of the authors has so far related the benefit of such a treatment to the inhibition of $F(ab')_2$ generation. In general they referred to the reduced degradation of elastin and matrix proteins resulting in reduced lung injury³⁸ and its inhibiting effect on NF-kappaB.⁴³ Thus, there is ample room for clinical trials in which effective drugs like sivelestat are applied and the patients studied for IgG cleavage and the parameters of complement amplification.

CONCLUSION

The results summarized have revealed that in sepsis and SIRS proteases from neutrophils and pathogens can generate F(ab')₂ fragments from IgG NAbs and preformed Abs. In case these F(ab')₂ fragments form immune complexes (IC) with autoantigens the F(ab')₂-IC have a high probability to form secondary IC with anti-hinge NAbs. Binding of anti-hinge NAbs to F(ab')₂-IC rigidifies these complexes and thereby enables them to capture C3b dimers. Bound C3b dimers are precursors of alternative C3 convertases, being about 750 times more effective than bound C3b. The extent to which the generated C3 convertases stimulate excessive complement activation via the amplification loop is difficult to predict. Excessive complement activation is dependent on the concentration of anti-hinge NAb-stabilized F(ab')₂-IC and thus is a function of antigen-specific F(ab')₂, the concentration of immune complexes, and that of anti-hinge NAbs. The process is inhibitable by irrelevant F(ab')₂ fragments that bind to anti-hinge NAbs and prevent it from stabilizing F(ab')₂-IC. The findings can explain why F(ab')₂ from whole human IgG, but not IgG itself (IVIG), was effective in stopping severe sepsis.

REFERENCES

1. Weiser MR, Williams JP, Moore FD et al. Reperfusion injury of ischemic skeletal muscle is mediated by natural antibody and complement. *J Exp Med* 1996; 183:2343-8. PMID:8642343 doi:10.1084/jem.183.5.2343
2. Stahl GL, Xu Y, Hao L et al. Role for the alternative complement pathway in ischemia/reperfusion injury. *Am J Pathol* 2003; 162:449-55. PMID:12547703 doi:10.1016/S0002-9440(10)63839-4
3. Lutz HU, Fumia S. Stimulation of complement amplification by F(ab')₂-containing immune complexes and naturally occurring anti-hinge antibodies, possible role in systemic inflammation. *Autoimmun Rev* 2008; 7:508-13. PMID:18558371 doi:10.1016/j.autrev.2008.04.017
4. Ward PA. The dark side of C5a in sepsis. *Nat Rev Immunol* 2004; 4:133-42. PMID:15040586 doi:10.1038/nri1269
5. Ratnoff WD, Fearon DT, Austen KF. The role of antibody in the activation of the alternative complement pathway. *Springer Semin Immunopathol* 1983; 6:361-71. PMID:6364431 doi:10.1007/BF02116280
6. Lutz HU, Nater M, Stammler P. Naturally occurring anti-band 3 antibodies have a unique affinity for C3. *Immunology* 1993; 80:191-6. PMID:8262548
7. Lutz HU, Stammler P, Fasler S. Preferential formation of C3b-IgG complexes in vitro and in vivo from nascent C3b and naturally occurring anti-band 3 antibodies. *J Biol Chem* 1993; 268:17418-26. PMID:8349625
8. Jelezarova E, Vogt A, Lutz HU. Interaction of C3b₂-IgG complexes with complement proteins properdin, factor B and factor H: implications for amplification. *Biochem J* 2000; 349:217-23. PMID:10861231 doi:10.1042/0264-6021:3490217
9. Nelson B, Ruddy S. Enhancing role of IgG in lysis of rabbit erythrocytes by the alternative pathway of human complement. *J Immunol* 1979; 122:1994-9. PMID:376729
10. Schenkein HA, Ruddy S. The role of immunoglobulins in alternative complement pathway activation by zymosan. I. Human IgG with specificity for zymosan enhances alternative pathway activation by zymosan. *J Immunol* 1981; 126:7-10. PMID:6778918
11. Gadd KJ, Reid KBM. The binding of complement component C3 to antibody-antigen aggregates after activation of the alternative pathway in human serum. *Biochem J* 1981; 195:471-80. PMID:7316962
12. Lucisano Valim YML, Lachmann PJ. The effect of antibody isotype and antigenic epitope density on the complement-fixing activity of immune complexes - a systematic study using chimeric anti-NIP antibodies with human Fc regions. *Clin Exp Immunol* 1991; 84:1-8. PMID:1707767 doi:10.1111/j.1365-2249.1991.tb08115.x
13. Banda NK, Wood AK, Takahashi K et al. Initiation of the alternative pathway of murine complement by immune complexes is dependent on N-glycans in IgG antibodies. *Arthritis Rheum* 2008; 58:3081-9. PMID:18821684 doi:10.1002/art.23865
14. Jelezarova E, Luginbuehl A, Lutz HU. C3b₂-IgG complexes retain dimeric C3 fragments at all levels of inactivation. *J Biol Chem* 2003; 278:51806-12. PMID:14527961 doi:10.1074/jbc.M304613200

15. Gadd KJ, Reid KB. Importance of the integrity of the inter-heavy-chain disulphide bond of rabbit IgG in the activation of the alternative pathway of human complement by the F(ab')₂ region of rabbit IgG antibody in immune aggregates. *Immunology* 1981; 42:75-82. PMID:6780451
16. Reid KB. Complement fixation by the F(ab')₂-fragment of pepsin-treated rabbit antibody. *Immunology J* 1971; 20(5):649-58. PMID:5006125
17. Sissons JG, Cooper NR, Oldstone MB. Alternative complement pathway-mediated lysis of measles virus infected cells: induction by IgG antibody bound to individual viral glycoproteins and comparative efficacy of F(ab')₂ and Fab' fragments. *J Immunol* 1979; 123:2144-9. PMID:489977
18. Akagaki Y, Inai S. Activation of the alternative complement pathway by the immune precipitate formed with F(ab')₂ fragment of human IgG antibody. *Mol Immunol* 1983; 20:1221-6. PMID:6419059 doi:10.1016/0161-5890(83)90146-3
19. Joiner KA, Goldman RC, Hammer CH et al. Studies of the mechanism of bacterial resistance to complement-mediated killing. V. IgG and F(ab')₂ mediate killing of E. coli 0111B4 by the alternative complement pathway without increasing C5b-9 deposition. *J Immunol* 1983; 131:2563-9. PMID:6355296
20. Terness P, Opelz G. Natural anti-immunoglobulin autoantibodies: Irrelevant by-products or immunoregulatory molecules? *Int Arch Allergy Immunol* 1998; 115:270-7. PMID:9566349 doi:10.1159/000069457
21. Terness PI, Navolan D, Dufter C et al. Immunosuppressive anti-immunoglobulin autoantibodies: Specificity, gene structure and function in health and disease. *Cell Mol Biol* 2002; 48:271-8. PMID:12030431
22. Kormeier LC, Ing JT, Mandy WJ. Specificity of antiglobulin factors in normal human serum reacting with enzyme digested gamma-G-globulin. *J Immunol* 1968; 100:612-21. PMID:4966843
23. Heimer R, Wolfe LD, Abruzzo JL. The specificity of antibodies to the F(ab')₂ fragment of human IgG. *Arthritis Rheum* 1985; 28:562-8. PMID:3873943 doi:10.1002/art.1780280516
24. Terness P, Kohl I, Hübener G et al. The natural human IgG anti-F(ab')₂ antibody recognizes a conformational IgG1 hinge epitope. *J Immunol* 1995; 154:6446-52. PMID:7539020
25. Fumia S, Goede JS, Fischler M et al. Human F(ab')₂-containing immune complexes together with anti-hinge natural antibodies stimulate complement amplification in vitro and in vivo. *Mol Immunol* 2008; 45:2951-61. PMID:18339427 doi:10.1016/j.molimm.2008.01.029
26. Yano S, Kaku S, Suzuki K et al. Natural antibodies against the immunoglobulin F(ab')₂ fragment cause elimination of antigens recognized by the F(ab')₂ from the circulation. *Eur J Immunol* 1995; 25:3128-33. PMID:7489753 doi:10.1002/eji.1830251121
27. Baici A, Knöpfel M, Fehr K et al. Kinetics of the different susceptibilities of the four human immunoglobulin G subclasses to proteolysis by human lysosomal elastase. *Scand J Immunol* 1980; 12:41-50. PMID:6902981 doi:10.1111/j.1365-3083.1980.tb00039.x
28. Donnelly SC, MacGregor I, Zamani A et al. Plasma elastase levels and the development of the adult respiratory distress syndrome. *Am J Respir Crit Care Med* 1995; 151:1428-33. PMID:7735596
29. Gardinali M, Padalino P, Vesconi S et al. Complement activation and polymorphonuclear neutrophil leukocyte elastase in sepsis. Correlation with severity of disease. *Arch Surg* 1992; 127:1219-24. PMID:1417490
30. Konstan MW, Hilliard KA, Norvell TM et al. Bronchoalveolar lavage findings in cystic fibrosis patients with stable, clinically mild lung disease suggest ongoing infection and inflammation. *Am J Respir Crit Care Med* 1994; 150:448-54. PMID:8049828
31. Ryan MH, Petrone D, Nemeth JF et al. Proteolysis of purified IgGs by human and bacterial enzymes in vitro and the detection of specific proteolytic fragments of endogenous IgG in rheumatoid synovial fluid. *Mol Immunol* 2008; 45:1837-46. PMID:18157932 doi:10.1016/j.molimm.2007.10.043
32. Dietrich H. Report on the experience in the treatment of septic diseases with Gamma-Venin. *Dtsch Med J* 1966; 17:709-10. PMID:4167085
33. Brezski RJ, Luongo JL, Petrone D et al. Human anti-IgG1 hinge autoantibodies reconstitute the effector functions of proteolytically inactivated IgGs. *J Immunol* 2008; 181:3183-92. PMID:18713989
34. Werdan K, Pilz G, Bujdoso O et al. Score-based immunoglobulin G therapy of patients with sepsis: The SBITS study*. *Crit Care Med* 2007; 35:2693-701. PMID:18074471 doi:10.1097/01.CCM.0000295426.37471.79
35. Lutz HU, Stammler P, Jelezarova E et al. High doses of immunoglobulin G attenuate immune aggregate-mediated complement activation by enhancing physiologic cleavage of C3b in C3b_n-IgG complexes. *Blood* 1996; 88:184-93. PMID:8704173
36. Vani J, Elluru S, Negi VS et al. Role of natural antibodies in immune homeostasis: IVIg perspective. *Autoimmun Rev* 2008; 7:440-4. PMID:18558359 doi:10.1016/j.autrev.2008.04.011
37. Kambe M, Bessho R, Fujii M et al. Sivelestat reduces myocardial ischemia and reperfusion injury in rat hearts even when administered after onset of myocardial ischemia. *Interact Cardiovasc Thorac Surg* 2009; 8:629-34. PMID:19279053 doi:10.1510/ievts.2008.195933
38. Toda Y, Takahashi T, Maeshima K et al. A neutrophil elastase inhibitor, sivelestat, ameliorates lung injury after hemorrhagic shock in rats. *Int J Mol Med* 2007; 19:237-43. PMID:17203197
39. Suda K, Takeuchi H, Hagiwara T et al. Neutrophil elastase inhibitor improves survival of rats with clinically relevant sepsis. *Shock* 2010; 33:526-31. PMID:19953005

40. Okayama N, Kakihana Y, Setoguchi D et al. Clinical effects of a neutrophil elastase inhibitor, sivelestat, in patients with acute respiratory distress syndrome. *J Anesth* 2006; 20:6-10. PMID:16421669 doi:10.1007/s00540-005-0362-9
41. Hoshi K, Kurosawa S, Kato M et al. Sivelestat, a neutrophil elastase inhibitor, reduces mortality rate of critically ill patients. *Tohoku J Exp Med* 2005; 207:143-8. PMID:16141683 doi:10.1620/tjem.207.143
42. Togo S, Matsuo K, Ishibe A et al. Usefulness of a selective neutrophil elastase inhibitor (sivelestat) in septic ARDS patients after gastrointestinal surgery. *Hepatogastroenterology* 2008; 55:967-73. PMID:18705309
43. Hagiwara S, Iwasaka H, Togo K et al. A neutrophil elastase inhibitor, sivelestat, reduces lung injury following endotoxin-induced shock in rats by inhibiting HMGB1. *Inflammation* 2008; 31:227-34. PMID:18536984 doi:10.1007/s10753-008-9069-z