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REVIEW

Proteinase-nicked IgGs: an unanticipated target for tumor immunotherapy

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ABSTRACT

The host immune system adopts multiple mechanisms involving antibodies to confront cancer cells. Accordingly, anti-tumor mAbs have become mainstays in cancer treatment. However, neither host immunity nor mAb therapies appear capable of controlling tumor growth in all cases. Structural instability of IgG was overlooked as a factor contributing to immunosuppression in the tumor microenvironment. Recently, physiological proteinases were identified that disable IgG immune effector functions. Evidence shows that these proteinases cause localized IgG impairment by selective cleavage of a single IgG peptide bond in the hinge-region. The recognition of IgG cleavage in the tumor microenvironment provides alternatives for tumor immunotherapy.

Introduction

IgG proteinolysis and the generation of therapeutic IgG fragments

Historically, a limited number of enzymes were known to be capable of IgG attack. This led to the long-held perception that IgGs were resistant to proteinase attack. The few enzymes known to attack IgGs included papain, an enzyme from the papaya plant and pepsin from the mammalian stomach (pH optimum <2).[1,2] These enzymes were fundamental tools for the resolution of IgG structure but seemed of little relevance for human systemic physiology. In seeming contradiction, there have been frequent reports of human antibodies circulating to sites of papain and pepsin IgG hinge cleavage.[2-9]

Circulating blood platelets proved to be an instructive target system for antibody-mediated clearance of papain and pepsin-mediated antibody fragments. The platelet receptor $\alpha_{IIb}\beta_3$ is essential for normal hemostasis but also responsible for platelet thrombosis in diseased arteries. This receptor was identified as a target for immunotherapeutic blockade.[10] To inhibit platelet function without platelet clearance, therapeutic monoclonal antibodies were modified by removal of the Fc domain of the IgG by proteinolytic cleavage. This modification was expected to avoid the Fc-mediated cell killing or clearance functions while leaving its antigen targeting actions in place. Two anti-platelet mAbs, each containing the human IgG1 hinge region, can be highlighted as examples. One, mAb YM337, was fragmented to bivalent F(ab')$_2$ with pepsin (Figure 1(a)).[11] However, in preclinical testing, the YM337 F(ab')$_2$ unexpectedly promoted platelet clearance – a finding that was traceable to circulating anti-hinge antibodies (AHAs). This unwelcome outcome indicated that AHAs to pepsin-generated F(ab')$_2$ could present barriers for human therapeutic applications.[5] In contrast, platelet clearance was minimal when the papain-generated Fab fragment of mAb c7E3 was tested in humans (Figure 1(a)).[12] The immunological differentiation between Fab- and F(ab')$_2$-mediated platelet clearance revealed an unexpected type of cell eradication with potential relevance for cancer therapy.

Molecular specificity of anti-hinge antibodies (AHAs)

The C-termini of the IgG proteinolytic fragments were found to be important components of antigenicity.[13] We adopted a peptide analog approach to more fully define AHA specificity. An ELISA system was configured using individual, solid-phase peptides with free C-termini corresponding to each sequential position in the upper through the lower IgG1 hinge. These peptides were presented outwardly as if in a cell-bound IgG fragment (Figure 1(b)).[14] Test sample sources were typically small pools of donor sera or purified intravenous gamma globulin preparations isolated from thousands of donors (Figure 1(b)). When individual donor sera were assessed, AHA ELISA reactivity levels were highly variable. However, when tested over time, these reactivity levels were relatively constant at least a year (REJ, unpublished observations).[14] In any case, the results consistently revealed a large disparity between average low levels of AHA binding to the papain cleavage site (threonine 224, EU numbering) and higher reactivity to the pepsin site (leucine 234). These results suggest a connection to the previously described disparity of platelet clearance. A similar papain vs. pepsin cleavage site disparity was confirmed in a recent, independent study.[5] However, all of these findings still left open the question: why is AHA reactivity generated to IgG hinge cleavage sites arising from non-physiological proteinases? Attention turned to human and disease-related proteinases that might also cleave IgG *in vivo*.
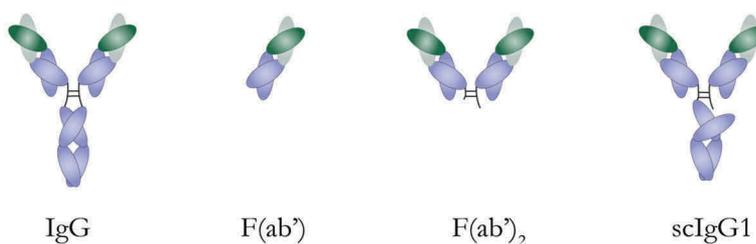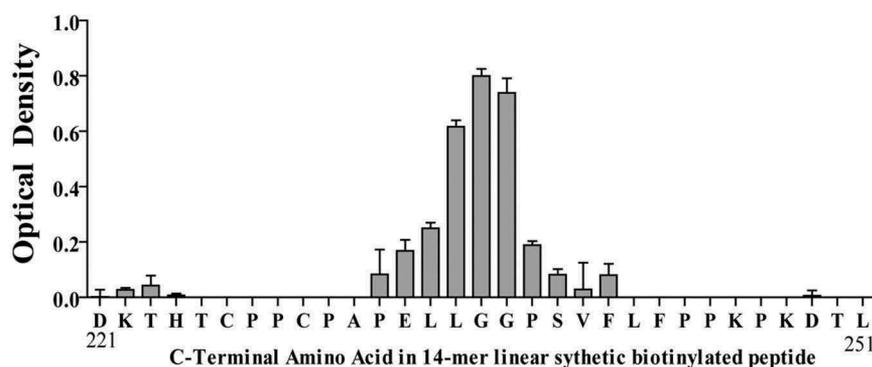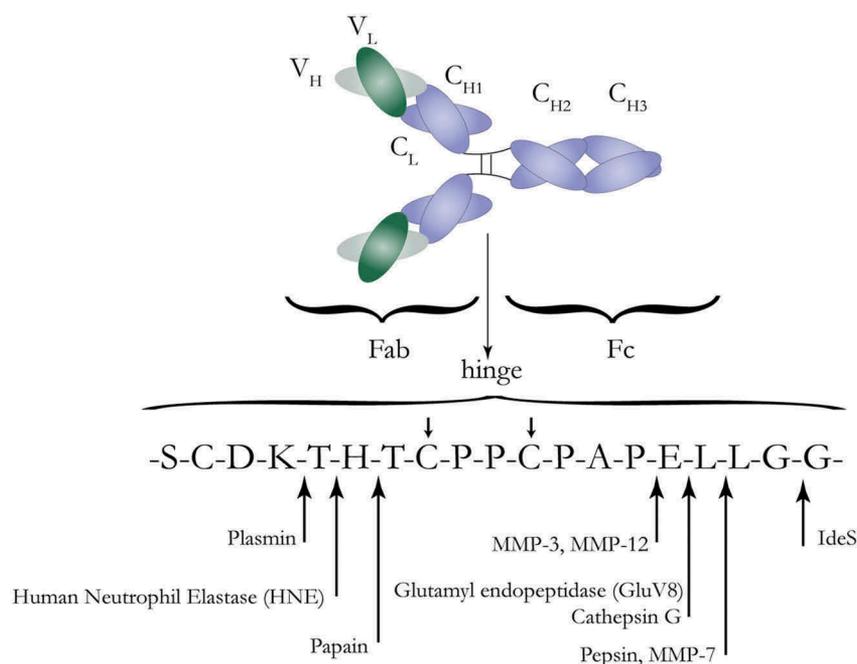

Figure 1. Structures and antigenic features of proteinolytic fragments of human IgG1. (a) A schematic depiction of IgG1 and antigen-binding fragments (Fab, F(ab')$_2$, and scIgG1) resulting from proteinolytic action on the hinge region. (b) ELISA binding of human serum autoantibodies to sequential peptide analogs corresponding to all potential sites of cleavage in the IgG1 hinge. The peptides each possess a respective free C-terminus that largely defines the neoepitopes exposed in the IgG1 hinge.[14] (c) The sequence of the IgG1 hinge and the sites of proteinolytic cleavage determined for selected proteinolytic enzymes.[31]

Additional context to AHA biology is provided by the proposal that anti-hinge antibodies may be members of a broader group of anti-post-translational protein modifications. This expanded group includes not only IgG hinge cleavage but also modifications such as glycation, citrullination, acetylation, and deamidation.[15,16] This would be consistent with an explanation of AHAs as an adaptive immune response rather than as natural, cross-reactive, germline IgG

sequences. Additional evidence supporting this hypothesis is cited below.[17] It can be noted here that no similar AHA anti-peptide reactivity was detected to N-terminal sequences of the cleaved Fc domain. This difference may be of fundamental antibody-antigen interest but is outside of the scope of this review.[18]

Proteinases and igg

Most IgG-cleaving proteinases are not uniquely selective for IgG and ordinarily target other protein substrates (IdeS from *Streptococcus pyogenes* being a notable exception).[19,20] In this regard, they are dissimilar from a group of bacterial proteinases that evolved a strict specificity for human IgA.[21] Also, IgG fragmentation with human and most bacterial proteinases has generally been found to be slower than with papain or pepsin. This difference largely explains the historical emphasis placed on the latter proteinases. However, the hydrolysis of the flexible IgG hinge by physiological enzymes in solution may not reflect that of cell-bound IgGs *in vivo* (arguably the most likely targets for host immune recognition). Notwithstanding, several enzymes were identified that split the IgG lower hinge at tightly defined peptide bond locations. For example, a number of cancer-associated cysteine proteinases including MMP-3/7/9/12/13 were found to cleave IgG in proximity to proline residues. This cleavage pattern is consistent with their bond specificity in less flexible connective tissue components.[3,22,23] The list of proteinases that catalyze specific hinge cleavages of IgG has increased over time and includes tumor-associated enzymes as well as enzymes secreted by pathogenic bacteria.[24,25]

Among bacterial enzymes catalyzing specific cleavage of IgG is glutamyl endopeptidase (gluV8) from the bacterium *Staphylococcus aureus*. GluV8 cleaves the lower IgG1 hinge after glutamic acid–236 .[22,26] A particularly important proteinase was identified from the *S. pyogenes* bacterium, which is associated with often lethal necrotic fasciitis. This proteinase, IdeS (for Immunoglobulin degrading enzyme of *S. pyogenes*), was found to rapidly cleave the human IgG hinge with precise peptide bond specificity. IdeS is unique in its selectivity for human IgGs and, in addition to its reagent uses, has potential therapeutic applications for counteracting autoimmunity.[19,21,27,28] IdeS hydrolyzes bonds in the lower hinge of all four human IgG subclasses – but not those of other mammalian groups. The absence of extraneous proteolysis largely avoids complicating side effects in the tumor microenvironment, making IdeS ideal for engineered tumor cell modeling studies.

The proteinolytic cleavage sites in the IgG1 hinge determined for different enzymes are shown in Figure 1(c). These sites are in obvious alignment with the AHA autoantibody immunity targets determined by ELISA (Figure 1(b)). This correspondence lent further support to the concept that IgG breakdown *in vivo* generates neo-hinge epitopes that are targeted by AHAs.

Reagents for detecting specific igg hinge cleavage in tissue

The essential link between concept and actuality was the demonstration of IgG hinge cleavage *in situ*. However, the detection of hinge cleavage in tissue samples posed a major challenge since the amounts of damaged Abs were predicted to be low in comparison to the high concentrations of intact IgG in blood (10–20 mg/mL). A specific methodology for detecting cleavage site neo-epitopes was needed. The solution proved to be unexpectedly straightforward. Specifically, rabbits mounted vigorous responses to cleaved human IgG1 hinge analog peptides (conjugated to KLH). The resulting antibodies were immunogen-specific with minimal cross-reactivity to intact IgG.[22] This finding also indicated that the cleaved hinges are presented and recognized differently than the intact IgG structure. This conclusion has now been confirmed in independent structural studies.[17,29] For tissue immunohistology (especially solid tumors), it proved useful to employ a "cocktail" of rabbit polyclonal Abs to three cleavage sites associated with cancer and infectious diseases: CPPCPAP [MMPs], CPPCPAPE [gluV8], and CPPCPAPELLG [IdeS].[30]

A single peptide bond scission in the hinge disables igg effector functions

IgG cleaving proteinases such as MMP-3 were observed to proceed via an intermediate single-chain scission rather than direct dual chain breakage.[3] Since no loss of Fc or other structural elements was associated with the scission of one peptide bond, it was at first presumed that this type of breakage would leave IgG functions intact. Surprisingly, scIgGs were found to be functionally incapacitated to the same degree as $F(ab')_2$s in both antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) assays (Figures 2(a,b), respectively). The loss of these functions was subsequently correlated with impaired interaction of scIgG with Fc receptors on immune cells (e.g. NK cells) and with complement.[31] Not surprisingly, the hinge region where proteinases acted contained a sequence previously shown to be involved in the interactions of IgG with these immune components.[32-35] Possible explanations considered for the functional scIgG impairments were structural or flexibility disruptions in the cleaved hinge. Another possible explanation was a requirement for the duplex hinge for Fc receptor docking and complement binding. The previously unsuspected scIgG dysfunctional phenomenon is depicted in Figure 3.

In vivo parallels to the *in vitro* phenomena were readily found. Among these was an inability of the single-cleaved version of mAb GK1.5 (anti-CD4) to clear splenic CD4⁺ T cells in mice. Intact GK1.5 was effective in this regard.[31] An analogous study in dogs showed that single-cleaved c7E3 IgG, in contrast to the intact version, was dysfunctional in clearing circulating platelets.[31] The anti-HER2 mAb, trastuzumab, was found to be substantially impaired compared to the intact mAb in blocking the growth of a human xenograft tumor in mice.[36,37] The proteinolytic impairment of Fc-mediated cell clearance was thus replicated using multiple human mAbs in the immune effector systems in animal models.[36,37]

A related and complex corollary to the loss of effector function by scIgGs is an impaired recruitment of immune cells to the tumor environment. Macrophages, in particular, are active in clearing invasive cells.[38] However, mice treated with single-cleaved trastuzumab had markedly inhibited macrophage recruitment to HER2 overexpressing xenografts as compared
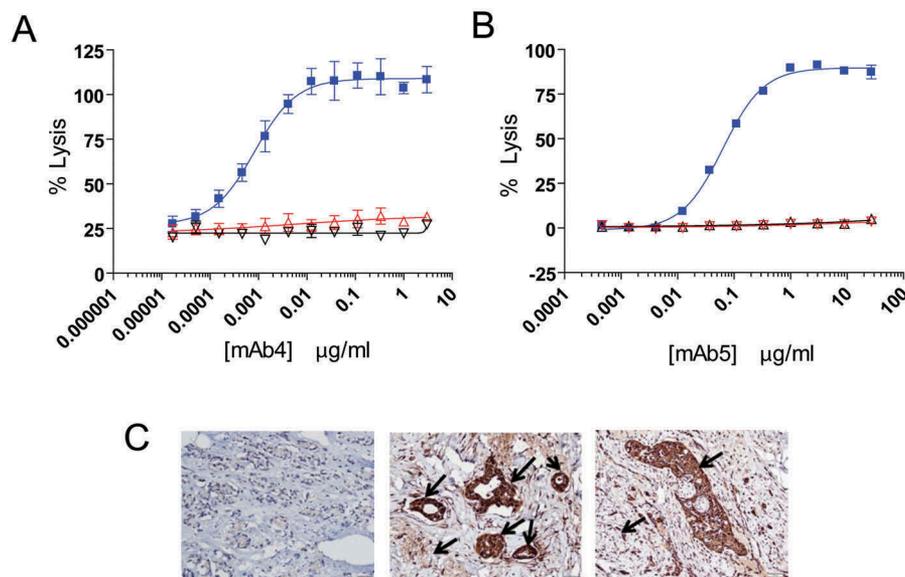
Figure 2. Impacts of proteolytic cleavage on cell killing functions of IgG1. (a) Concentration-dependent effects of an intact mAb IgG (blue), its single-cleaved version (scIgG) (red) and the F(ab')$_2$ fragment (black) on antibody-dependent cellular cytotoxicity (ADCC). MDA-MB-231 cancer cells were the targets and human PBMCs were the effector cells.[31] (b) Complement dependent cytotoxicity using an intact mAb IgG1 and the corresponding derivatives described in panel A. In this case, WIl2-S cancer cells were the targets.[31] (c) Detection of cleaved IgG in breast tumor tissues detected by immunohistochemistry.[38] The middle and right images demonstrate the staining (dark) of cleaved IgG in tumors from two patients with high HER2-expressing breast cancer after treatment with trastuzumab. The image on the left is a negative staining control without added primary antibodies. The methods were described previously.[36]

to mice treated with intact trastuzumab.[37] Similarly, decreased recruitment of macrophages was observed in human tumors when host antibodies exhibited evidence of proteolytic cleavage.[38] Thus, not only does proteolytic hinge cleavage of an anti-tumor mAb prevent effector functions such as ADCC and CDC while simultaneously blocking competent mAb binding (below), it can also further inhibit the arrival of effective immune clearance cells. This was an unanticipated convergence of lost functionality due to proteinase action.

Direct detection of cleaved IgGs *IN SITU*

Regarding tumor detection, it should be reemphasized that scIgGs do not lose antigen-binding activity, and the Fc domain remains fully attached if only by a single hinge heavy chain (Figure 2(c)). It can be speculated that by their retained affinity/avidity attachments to surface antigens, scIgGs may provide a barrier to the binding of subsequent and functional IgGs to the same antigen. This could be important not only for antibody functions on an individual cell but could also have impacts on IgG diffusion and access to sites within the tumor compartment.[39] Demonstrations that intact IgGs and scIgGs exhibit similar antigen binding are at least consistent with this conjecture. This blockade function could be envisioned to contribute to tumor escape from humoral immunity (Figures 3(a, b)). Analogous shielding speculations had been made for cleaved IgAs (in those cases catalyzed by bacterial proteinases) in tissues and in the digestive tract.[21,40] Moreover, it has proven difficult to demonstrate hinge-cleaved IgGs in circulation – an observation consistent with retained, localized binding of these IgG derivatives.

A further insight came when it was found that the cleavage of trastuzumab in a xenograft system only occurred on cells expressing high levels of the HER2 receptor. Low expressing cells did not yield trastuzumab cleavage and none was detected in the media.[37] Confirmation of a preferential accumulation of cell surface scIgG (rather than F(ab')$_2$) was made when cell-bound mAbs were exposed to endogenous and exogenous proteinases or when the cells were engineered to express the IgG cleaving enzyme, IdeS.[31,36–38,41] These findings indicated that an initially more rapid first and then slower second chain cleavage of the IgG hinge, previously noted in solution at low enzyme to IgG ratios, was even more exaggerated with cell surface-bound IgGs. Intact IgGs, compared to scIgGs, evidently present the hinge heavy chain differently for proteinase attack, despite their identical sequence. The reason that the above inter-connected phenomena had gone undetected may be that cell surface scIgGs get masked amidst the overwhelming excess of intact vascular IgGs (10–20 mg/mL). Also, the customary method of detection of tissue IgGs by anti-IgG immune reagents, anti-Fc, is unable to differentiate between intact and scIgGs that differ by one split peptide bond in the ~150kDa IgG structure. Thus, an appreciation of cell-bound scIgGs (e.g. within tumors) was fundamentally hindered by the lack of specific tools.

The newly developed anti-human hinge cocktail antibody approach was deployed for immuno-histochemistry (IHC) in a number of pathological settings. Successful uses included the detection of cleaved IgGs in synovial fluid samples from individuals with rheumatoid arthritis,[22,31] in freshly obtained intestinal mucosa from patients with inflammatory bowel disease,[42] and in extracts from commercially obtained tumors.[31] Earlier investigations using frozen tissue bank tumor samples provided evidence
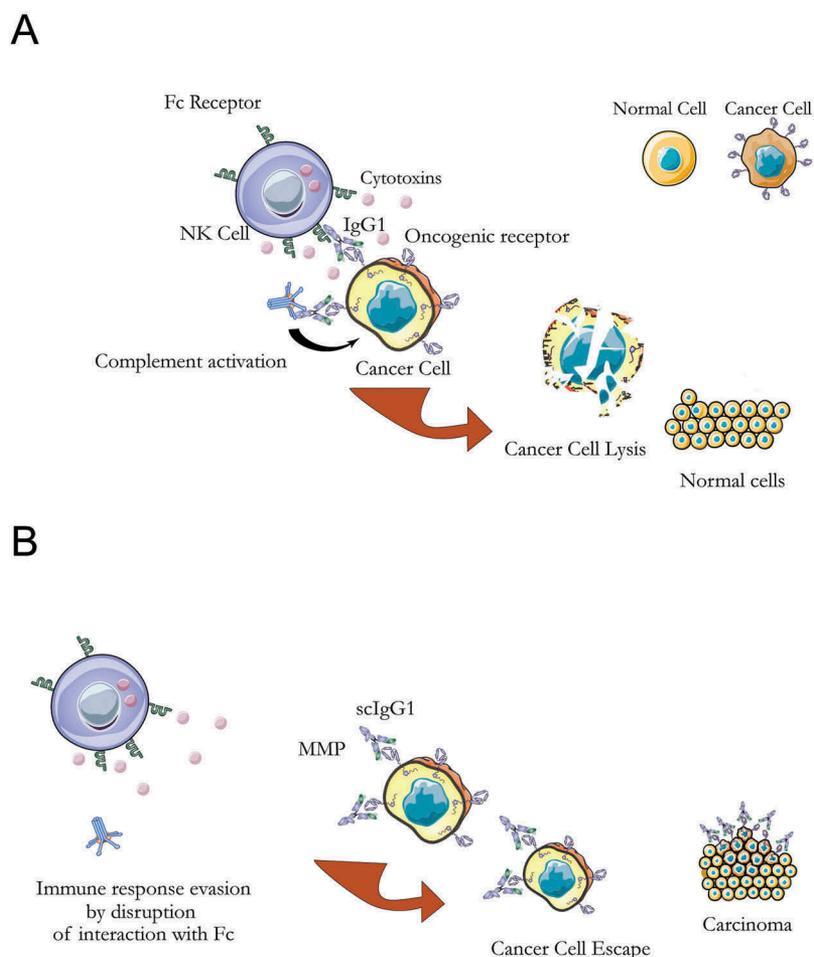
A

B

Figure 3. The roles of the IgG1 Fc domain in engaging immune cell killing mechanisms. (a) A depiction of intact IgG binding to oncogenic receptors on cancer cells to direct ADCC, ADCP and/or CDC. The intact IgG Fc domain can engage Fc receptors on natural killer cells or complement to trigger their respective lytic mechanisms against the cancer cells. (b) A proposed mechanism of evasion of host antibody immunity when the cancer environment contains proteinolytic enzymes (e.g. MMP) that can cleave IgG. As shown, single cleaved IgGs can accumulate on the cell surface but cannot interact with NK cells or complement thereby permitting the cancer cells to survive and proliferate.

for IgG cleavage in squamous cell carcinomas of the head and neck.[30] Also, in a xenograft tumor model (human HER2 positive BT474 cell line) in immune-deficient mice, evidence for dysfunctional trastuzumab confirmed the cell-bound, limited IgG cleavage.[36]

To specifically investigate IgG proteolysis in breast cancer, a clinical collaboration was established within the University of Texas Health Science Center at Houston for the acquisition of tumor tissues. Tumor samples were obtained from 60 breast cancer patients and immediately snap-frozen in liquid N_2 in the surgical suite. These precautions were adopted to minimize additional proteinase activity after tissue removal. Upon analysis, a high degree of tissue specificity was indicated by the detection of cleaved IgGs within tumors but not in adjacent or related normal tissue or in circulation (Figure 2(c)).[38]

There was no reason to suspect that proteolysis of therapeutic mAbs would be fundamentally different than for host IgGs in tumor microenvironments. Indeed, *in vitro* studies in purified solution as well as those using cancer cell lines as proteinase sources showed that mAbs such as trastuzumab, cetuximab, pertuzumab as well as infliximab, c7E3 IgG, and an anti-tissue factor mAb (all human IgG1-based mAbs) were

susceptible to proteolysis.[31,36–38,41] Important *in vivo* correlations were provided by demonstrations that clinically administered trastuzumab was detectable as scIgG-T in tumor extracts from patients with breast cancer or by IHC in biopsy samples.[36,37]

Functional restoration to cleaved IgGs by anti-hinge antibodies

The exceptional specificity had already turned attention toward whether anti-hinge antibodies could have a functional impact. To this end, human serum AHAs were isolated by affinity chromatography on cleaved IgG fragments (e.g. F(ab′)₂ generated with IdeS or gluV8).[22] Low serum concentrations necessitated using IgG pools from large numbers of healthy donors as a source and presented a challenge for purification. However, in the end adequate amounts of purified AHAs were obtained. When supplemented to cell-bound F(ab′)₂, purified AHAs readily restored the ADCC and CDC cell killing functions to the otherwise inactive IgG fragments. Unexpectedly, the isolated AHA preparations consistently contained elevated levels of the IgG3 subclass compared to

normal circulating IgG ratios. The prevalence of IgG3s signaled that AHAs had undergone class switch recombination and were unlikely to be germ-line sequences. A potential physiological role this autoantibody population is suggested by the substantial cell-killing potential of IgG3s in ADCC.[43,44]

Recently, human AHAs with sequences that are substantially altered from germline were obtained from cloned human B cells.[17] In certain cases, the cloned AHAs demonstrated picomolar affinity vs. antibody fragments – a finding that is most consistent with an adaptive immune response. With regard to target selectivity, several of the cloned AHAs were specific to linear amino acid hinge sequences with a free carboxyl terminus, but not to intact IgG.[29] This epitope selectivity had previously been noted for an anti-cleavage site mAb. Predictable questions arise as to why the widely prevalent AHAs (active in vitro when concentrated) are inadequate to protect against host tumors and infections in vivo. Again, AHA serum concentrations are generally quite low, notwithstanding their recently established high affinities. Perhaps AHA functions, as determined in early immunological development, are sufficient to remove normally occurring damaged IgGs (the so-called housekeeping function), but are less capable of overriding IgG damage in acute and virulent settings.

Host AHAS Were Effective in a *Staphylococcus aureus* infection setting in rabbits

Glutamyl endopeptidase, gluV8, is expressed by the *S. aureus* bacterium and selectively cleaves human IgG1 at glutamic acid 236 (EU numbering) in the lower hinge in physiological conditions. Mammalian exposure to *S. aureus* is nearly universal from birth and immunity to it can readily be demonstrated.[45] However, such natural humoral immunity is apparently inadequate to control acute colony growth such as that generated in an experimental tissue cage model.[46] To test whether IgG cleavage might contribute to the lack of protective immunity in this highly stringent model (employed frequently for antibiotics drug development), we stimulated host immunity. Host anti-hinge antibody responses were elicited by immunization with a peptide analog of the predicted gluV8 cleavage site in rabbit IgG. After anti-peptide antibodies had been shown to be present in the tissue cage, *S. aureus* was introduced and colony growth then monitored. In unimmunized controls as well as animals immunized with a scrambled peptide, colony growth was vigorous for up to 3 weeks. However, in rabbits immunized with the cleaved hinge analog peptide, a greatly inhibited colony growth occurred that in some cases extended to over three log reductions to the limit of detection (a bactericidal threshold).[47] The results strongly suggested that this prophylactic immunization approach had induced endogenous Abs that protected against microbial proliferation. If so, the protective immunity had been gained without the need for targeting the bacterium directly. This finding had little precedent and prompted questions about whether the anti-microbial example could be further applied in vivo.

An anti-hinge mAb was a critically needed tool

A more incisive immunological tool for cellular and in vivo studies was required, and a human/rabbit chimeric mAb to the IdeS cleavage site in human IgG1 was developed. Rabbits provided an exquisitely specific mAb to the IdeS cleavage site peptide analog. Joining the rabbit mAb variable regions to human constant domains yielded the desired chimeric antibody, mAb, 2095–2, which bound equally well to several IgG1 mAbs that had been clipped by IdeS to either scIgGs or F(ab′)₂s.[48] The characteristics of mAb 2095–2 included both high affinity and specificity for the IdeS cleavage site (terminating in glycine-236) with negligible reactivity for intact IgGs. In fact, mAb 2095–2 bound to the homologous IdeS cleavage site sequence in human IgG4 to confer ADCC and CDC functions to a subclass that, when intact, has no such activity. As a tool for studying the restoration of functional activity to IdeS-cleaved IgGs, mAb 2095–2 proved to be highly versatile.[48]

Platelets proved again to be an important immunological cell clearance model. The c7E3 mAb (parent of abciximab) binds to the $\alpha_{IIb}\beta_3$ receptor on human platelets and the homologous receptors on canine and rat platelets (although with lower affinity). As predicted, the c7E3 IgG mAb caused a dose-dependent and progressive removal of platelets in dogs but not the IdeS-derived F(ab′)₂ fragment. However, a co-administration of 2095–2 to platelet-bound F(ab′)₂ induced rapid and profound platelet clearance.[48] The extent of clearance was greater than that achievable with c7E3 IgG itself and occurred more rapidly and at substantially lower doses (2095–2 compared to c7E3 IgG). Thus, not only was restoration of function realized but also, the results pointed to an unexpected amplification of antibody-mediated cell clearance.

A rat model of platelet clearance further exemplified the enhancement of cell clearance by mAb 2095–2.[48] In this example, a 1 mg/kg dose of c7E3 F(ab′)₂ (ineffective alone) was followed in other groups by subsequent escalating dose administrations of mAb 2095–2. Even at the lowest tested dose (0.1 mg/kg: 1/10 ratio of 2095–2 to F(ab′)₂), the extent of platelet clearance exceeded that achieved with intact c7E3 IgG at a 10-fold higher level. These findings confirmed the earlier amplification phenomenon and further highlighted an unexpected interplay between surface bound immune complexes and cell killing mechanisms.

Cancer investigations

Solid tumors have extended persistence. Certainly, if only for this reason, they are not analogous to discrete or transient cellular targets such as circulating platelets, CD4⁺ T cells, or the *S. aureus* bacterium. Moreover, tumor environments are well known to be immunosuppressive in nature. The suppression and evasion of host immunity have recently been recognized as important cancer hallmarks.[49,50] The cellular targets above have been explored repeatedly in the context of proteinase impacts on IgG functions and modeling approaches to restore function with anti-hinge antibodies. Tumor environments naturally express abundant proteinases including MMPs that are also capable of cleaving IgGs. These

observations raised the possibility of localized antibody dysfunction and immune evasion in tumors.[3,51,52] Hypothetically, the restoration of proteinase-damaged IgG functions could provide a valuable supplement to existing anti-tumor therapies in these immunosuppressive environments. Complicating factors will surely include the actual identities of proteinases with hinge cleaving properties for different IgG isotypes, and the timing and amounts of enzyme expression over time throughout the heterogeneous tumor environment.

The first attempts of the anti-hinge therapy concept in cancer were similar to those used in the previous cell clearance models. Namely, anti-tumor scIgG or F(ab')$_2$ was first administered, followed by the anti-hinge mAb 2095–2. Although early anti-tumor benefits were seen in a pilot murine xenograft model (REJ, unpublished observations), convincing longer-term statistical significance was not achieved. The approach required multiple administrations of the mAb fragments over several weeks coupled with mAb 2095–2. Thus, transient local benefits may have been diverted by the formation of immune complexes of the two agents in circulation that prevented tumor-directed effects. Basic elements of the tumor environment including local expression of proteinases and disablement of IgG on cell surfaces were clearly not present.

It should be highlighted that an alternative therapeutic approach consists of immunizing the host against the point of proteinase attack in the IgG hinge. This concept was previously investigated in an infectious disease setting.[47] Specifically, Staphylococcus aureus colony growth occurs rapidly in a tissue cage model in rabbits. The gluV8 proteinase of S. aureus cleaves IgG in the hinge and rabbit immunity is normally incapable of controlling bacterial proliferation. However, in a setting in which rabbits were pre-immunized against the gluV8 cleavage site in rabbit IgG, substantial reductions of colony growth were observed. This strongly suggested that endogenous anti-hinge immunity could be supplemented to bactericidal effect. Those encouraging findings prompted attempts to identify parallel model systems in mouse models. A methodological modification would be needed to evolve short-term cancer models.

Ides-expressing cancer cells with accelerated cleavage of IgGs on their surface

Cancer cells were engineered to express a proteinase that would induce localized IgG inactivation. The choice of IdeS as the foundation of the model was influenced at several levels including that 1) IdeS afforded rapid IgG cleavage and had been shown to catalyze hinge cleavage in cell-bound mAbs, 2) few side effects were likely due to its high IgG specificity, and 3) IdeS can be readily cloned into cancer cell lines. Although IdeS is not a cancer proteinase, its impacts on IgG functions parallel those of the MMPs and it provided a highly workable platform for animal model development. Indeed, IdeS expression in BT474 cells greatly accelerated trastuzumab and pertuzumab cleavage on cell surfaces possessing high levels of the HER2 receptor.[36,37,41] The lack of comparable hinge cleavage on low HER2-expressing cells confirmed the preferential cleavage of surface IgGs compared to those in the surrounding soluble phase.

The extraordinary potency of cell-expressed IdeS readily disabled targeting mAbs such as trastuzumab and pertuzumab, but also impaired the ability of mAb 2095–2 to restore function.[36,41] The latter impairment was due to cleavage in the homologous IgG1 hinge of 2095–2. Thus, the expression of IdeS proteinase that was advantageous for model situations illustrating local IgG disablement was at the same time disadvantageous for the purposes of a corrective mAb. These observations prompted the exploration of IgG structures with an ability to resist proteinase attack.

Engineering proteinase-resistant mAbs

It was previously mentioned that hinge structures that confer IgG effector functions are often also susceptible to proteinase cleavage. Therefore, the goal of engineering proteinase resistance while retaining critical IgG interactions with Fc receptors and complement appeared problematic. The challenge was met because effector functions could be successfully maintained when specific mutations of the C_H2 domain were coupled with the substitution of a short stretch of the human IgG2 hinge (naturally proteinase-resistant) for the IgG1 Gly-236 residue.[30] This is schematically shown in Figure 4(a). The versatility of this proteinase-resistant backbone was that IgG variants could be chosen to restore either ADCC or CDC as dictated by clinical needs (Figure 4(b)). Presuming that attributes such as antibody stability, pharmacodynamics, immunogenicity, and biodistribution are positive, this mAb engineering approach holds promise for enhanced functionality. Certainly, the first in vivo results in IdeS-expressing cancer murine models were encouraging.[36,37,41]

Concluding remarks

This review has highlighted at least two divergent approaches to cope with the disablement of IgGs in proteinase-rich environments. One is the conversion of current therapeutic anti-cancer mAbs (trastuzumab, pertuzumab, etc.) to proteinase-resistant versions. The available results suggest that this approach can succeed when the proteinases in the target environment are known (e.g. MMPs in cancer; IdeS in S. pyogenes infection). The limitation of this approach is that it is most applicable for mAbs to already defined targets and thereby represents an incremental improvement to their actions.

The second approach involves the anti-hinge antibody. In this strategy, the target of therapy is the limited neo-epitopes revealed by proteinase action on any host antibodies in the tumor microenvironment. In this scheme, there would be no need to know the actual antigens on the cancer cell for host antibodies (there could be multiple) since the anti-hinge mAb would theoretically target all anti-TAA (tumor-associated antigen) antibodies that became damaged. In the case of above normal proteinase expression (as in IdeS-expressing tumor models), the anti-hinge approach could be bolstered by its own proteinase resistance. A consideration here is that several anti-hinge mAbs might be required if multiple proteinases were active in the tumor site. Nevertheless, this concept would represent a significant departure from current cancer immunotherapies.
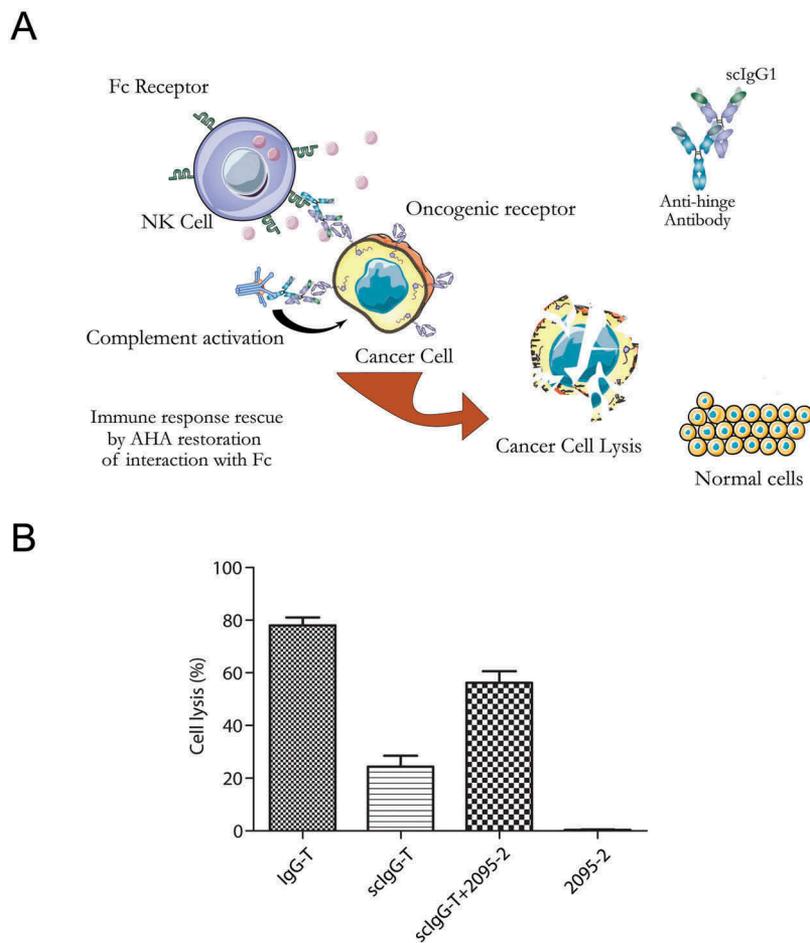
Figure 4. Restoration of function to cleaved IgGs. (a) Several alternatives have been shown to restore function to disabled scIgGs by secondary binding of intact anti-hinge antibodies. The sources of the anti-hinge Abs have been AHAs isolated from human serum,[14] a monoclonal anti-hinge mAb,[48] immunization-derived immune responses to peptide analogs of the cleaved hinge,[47] and proteinase-resistant versions of a monoclonal anti-hinge mAb.[53] The anti-hinge antibodies bind to the cleaved primary antibodies on the cell surface to provide an intact hinge and Fc that are capable of restoring ADCC and complement against the cancer cells. (b) Quantitative assessments of ADCC by intact and single-cleaved trastuzumab and the anti-hinge mAb 2095–2 alone and in combination with the scIgG. The combination, in this case, restored substantial function to the cleaved mAb.[36]

In summary, evidence has now accumulated for proteinolytic inactivation of host IgGs within tumors and infectious environments. Historically, this highly localized event had gone unnoticed. In the course of developing antibody reagents to detect the cleavage, it was found that these novel antibodies could themselves be used to overcome the functional incapacity of scIgG and F(ab′)₂. The collective findings provided a new insight into the functioning of antibody-based immunity in hostile environments. The ongoing challenge will be to translate these findings into effective anti-cancer therapies.

Acknowledgments

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

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