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Anti-GBM glomerulonephritis: a T cell-mediated autoimmune disease?

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Summary

Anti-glomerular basement membrane (GBM) glomerulonephritis, which was among the earliest recognized human autoimmune diseases, is characterized by the presence of anti-GBM antibody. It has been a prototypical example of autoantibody-mediated autoimmune disease. However, decades of research on this disease, based either on clinical observations or experimental models, have revealed that T cell-mediated cellular immunity may potentially be a more important mediator of glomerulonephritis. We have made several breakthroughs in understanding the T cell-mediated mechanism causing this disease in a rat model based on Goodpasture's antigen, non-collagen domain 1 of $\alpha 3$ chain of type IV collagen (Col4 $\alpha 3$ NC1). We demonstrated that anti-GBM glomerulonephritis was induced by either passive transfer of Col4 $\alpha 3$ NC1-specific T cells or active immunization with the nephritogenic T cell epitope of Col4 $\alpha 3$ NC1. Immunization with the T cell epitope also triggered production of anti-GBM antibodies to diversified GBM antigens. Thus, a single nephritogenic T cell epitope alone is sufficient to induce the clinical spectrum of anti-GBM glomerulonephritis, including proteinuria, glomerular injury, and anti-GBM antibody. A possible T cell-mediated mechanism for causing human anti-GBM disease is proposed.

Key words: autoimmunity • T cells • autoantibody • glomerulonephritis

Abbreviations: Col4 α NC – non-collagen domain of $\alpha 3$ chain of type IV collagen, GBM – glomerular basement membrane.

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INTRODUCTION

Anti-glomerular basement membrane (GBM) glomerulonephritis (Goodpasture's syndrome), a cause of end-stage renal failure worldwide, is characterized by the presence of anti-GBM antibody¹⁴. Historically, anti-GBM glomerulonephritis was among the earliest recognized human autoimmune diseases²⁴, and the discovery of the anti-GBM antibody was considered a milestone in autoimmune disease research^{20, 65}. Anti-GBM glomerulonephritis has also been regarded as a prototypical example for autoantibody-mediated autoimmune diseases^{20, 65}. However, decades of research on this disease have raised several questions about the role of anti-GBM antibody in anti-GBM disease. Our recent study provided further direct evidence to show that a CD4⁺ T cell-mediated mechanism alone is sufficient not only to induce glomerular injury, but also to trigger an anti-GBM antibody response to diversified GBM antigens. Our findings argue a need to re-examine the role and production of anti-GBM antibody in human anti-GBM disease. A new hypothetical mechanism for causing human anti-GBM glomerulonephritis is proposed based on our findings.

IS ANTI-GBM ANTIBODY ALONE SUFFICIENT TO CAUSE ANTI-GBM GLOMERULONEPHRITIS?

The involvement of the antibody in glomerulonephritis was suspected at the time of the discovery of immunoglobulin. In the 1960s, anti-GBM antibody was first identified by the demonstration of deposits of antibody within glomeruli^{20, 65}. Since then, the role of anti-GBM antibody in anti-GBM glomerulonephritis has been examined in various animal models, mainly based on transfer of heterogenic anti-GBM antibodies. Those studies showed that anti-GBM antibody transferred proteinuria and glomerular damage in the experimental animals^{36, 37}. However, the transferred anti-GBM antibody only induced acute proteinuria and mild neutrophil-mediated glomerular damage, which recovered within days despite continuous linear binding of IgG to GBM in the recipients^{36, 37}. Nevertheless, the influence of those early studies led to the traditional belief that all glomerulonephritis could be explained by antibody-mediated mechanisms. It was thought that the binding of antibody to GBM triggered glomerular inflammation through a complement cascade or, more recently, Fcγ receptor pathway.

After the 1970s, it was demonstrated that severe glomerulonephritis and anti-GBM antibody were induced by immunization with various preparations of GBM antigens^{1, 11, 31, 44, 51, 55, 64, 66}. Interestingly,

despite the availability of anti-GBM antibodies or monoclonal antibodies produced in those models, the successful induction of severe anti-GBM disease by passive transfer of anti-GBM antibody was rare²¹. A few recent studies showed that anti-GBM antibodies or monoclonal antibodies of the same species were able to transfer glomerulonephritis^{56, 57, 66}. However, the transferred disease was always mild. These findings suggest a limited role of anti-GBM antibody in anti-GBM disease.

At the same time, several groups developed animal models to "augment" antibody-mediated glomerulonephritis. In these models (nephrotoxin or embedded antigen models), severe or lethal nephritis was indeed induced by anti-GBM antibody from a foreign species. However, the foreign anti-GBM antibody transferred severe glomerulonephritis only after the recipients had been previously primed by the IgG of the donor species^{19, 61}. Thus, it is difficult to interpret whether the glomerular damage is caused by transferred foreign anti-GBM antibody or the recipient's T cell/antibody response to the immunoglobulin of donor species in these models. The results from these models strongly suggest that the glomerular injury was caused by the recipient's immune response, probably T cell response, to the transferred heterogeneous immunoglobulin. First, the glomerular-infiltrating leukocytes are mononuclear cells, but not neutrophils⁶¹. Second, transfer of heterogenic anti-GBM antibody failed to induce severe glomerulonephritis in T cell-deficient animals^{29, 67}. Therefore, these models may be useful for studying the pathogenesis of glomerulonephritis, but not suitable for determining whether the anti-GBM antibody causes the disease.

Many clinical observations have also implied a limited role of anti-GBM antibody in human anti-GBM disease. The participation of antibody and associated pathways alone cannot explain many cases and aspects of human glomerulonephritis^{2, 13, 15, 45, 62}. For example, in one sub-type of human glomerulonephritis (the pauci immune form), the glomerular damage was not related to any deposit or binding of antibodies in the glomerulus^{2, 13, 15}. Although anti-GBM antibody is the hallmark of human anti-GBM glomerulonephritis, it is well known that GBM antibody titer in anti-GBM glomerulonephritis patients has little value in predicting the prognosis or the disease severity^{13, 62}. Elimination of anti-GBM antibody by plasma exchange has very limited effect on the pathogenesis of the disease⁴⁵. On the other hand, renal pathology revealed inflammation in the affected glomeruli with the feature of cell-mediated hypersensitivity. These observations provided strong evidence for involvement of cellular immunity in this disease^{4, 5, 48}.

HOW ARE ANTIBODIES TO DIVERSIFIED GBM ANTIGENS PRODUCED?

Presence of anti-GBM antibody is the hallmark of human anti-GBM disease. Numerous studies have been devoted to identification of the antigens/B cell epitopes recognized by the anti-GBM antibody. Several notable studies identified the non-collagen domain of $\alpha 3$ chain of type IV collagen (Col4 α 3NC) as the Goodpasture antigen using human anti-GBM antibodies as probes^{46, 70}. Animal models further demonstrated that immunization with Col4 α 3NC induced anti-GBM glomerulonephritis^{1, 31, 55, 66}. In addition to Col4 α 3NC1, diversified GBM proteins, including different chains of type IV collagen, collagen domains and the S7 domain of type IV collagens, and other non-collagen components of GBM, have been identified as antigens recognized by autoantibodies from anti-GBM patients^{17, 27, 34, 59, 60}. Discovery of anti-neutrophil cytoplasmic autoantibody in anti-GBM disease is another example of the complicity of the antibody response in this disease^{9, 63}.

A number of investigators attempted to further map the B cell epitopes, especially of linear types, of identified Goodpasture antigens. The mapping of B cell epitopes by recombinant polypeptides or synthetic peptides has been tried repeatedly^{25, 32, 33, 38, 42, 46, 47, 66}. One report described a linear B cell epitope encoded in C-terminal 36-residues of Col4 α 3NC³². However, synthetic peptide encoding the B cell epitope failed to induce glomerulonephritis or anti-GBM antibody^{1, 16}. Other efforts to map linear epitopes or to generate monoclonal antibodies to native GBM with peptides were not very successful, as most of the antibodies only reacted with denatured GBM proteins or did not react with GBM at all^{25, 38, 42, 47, 66}. Using more sophisticated point-mutation and other techniques, several studies demonstrated that the anti-GBM antibodies are bound to 3-D conformation of native antigens^{8, 16, 26, 39}. In summary, autoantibodies in anti-GBM disease react with highly diversified GBM antigens. The B cell epitopes recognized by the anti-GBM antibodies are mainly 3-D conformational, probably due to the complicated quaternary organization of GBM⁸.

Despite the past several decades of study on anti-GBM disease, investigation of the actual mechanism of the antibody response to diversified B cell epitope/antigens in this disease has been largely ignored. This is a fundamental question for anti-GBM disease as well as for many other autoimmune diseases. Studies based on various animal models have shown that immunization with purified GBM or GBM collagen resulted in the production of anti-GBM anti-

body^{1, 11, 21, 31, 44, 51, 55, 64}. However, the anti-GBM antibody responses in these models were induced against the immunizing antigens only, and thus cannot explain the mechanism of the anti-GBM antibody response to diversified GBM antigens as seen in human disease.

In mercuric-chloride-induced autoimmunity, production of anti-GBM antibody is one of the unique features of this model⁴³. However, the autoantibody response in this model is the result of polyclonal activation of B cells due to an abnormality in T cells, and the specificities of autoantibody are not limited to the GBM antigens⁵⁰. In addition, the antibodies to GBM and thyroglobuline are short lived, while antibodies to ssDNA and dsDNA are prolonged⁵⁰. These findings suggest that this model is more relevant to human systemic autoimmune disease than to anti-GBM diseases.

The association of various infections with anti-GBM disease or anti-GBM antibodies is well known^{10, 12, 23, 58, 68}. Several researchers have postulated “antibody mimicry” during infection as a mechanism for the autoantibody production, in which an antibody against a certain infectious agent cross-reacts with a GBM antigen. The best example is the antibody to a streptococcal cell-wall antigen, which also reacts with GBM²². However, there is no convincing evidence to demonstrate the nephritogenicity of the antibody³⁵. More importantly, it obviously failed to explain the diversified anti-GBM antibodies in human disease.

INVOLVEMENT OF A T CELL-MEDIATED MECHANISM IN ANTI-GBM GLOMERULONEPHRITIS

Based on accumulating clinical and experimental observations, many researchers have questioned the traditional belief that anti-GBM antibody is pivotal in causing glomerulonephritis. The results from many studies suggested that T cell-mediated cellular immunity may be a more important mediator of anti-GBM glomerulonephritis than anti-GBM antibody. T cells may participate in the pathogenesis of glomerulonephritis at two levels. First, T cells may act as helpers in the T-dependent antibody response to renal antigens. Second, T cells may directly participate in immunologic damage to glomeruli.

It is clear that T cells act as helpers in antibody response in anti-GBM disease, because autoantibody responses to GBM from either human patients or animal models were demonstrated to be T cell-dependent. However, this could only be regarded as a correlation to an antibody-mediated mechanism. It

has also been quite clear that T cells are involved in glomerular inflammation. Early evidence was provided by histological observations that T cells or associated delayed hypersensitivity are actually present in the glomerulus in a variety of human glomerulonephritis and experimental glomerulonephritis in animals^{4, 5, 28, 40, 41, 48}. Those glomerular-infiltrating T cells may have been activated, as revealed by their expression of interleukin 2 receptor and various T cell cytokines⁴⁰. Requirement of CD4⁺ or CD8⁺ T cells in glomerulonephritis pathogenesis has been demonstrated in animal models^{29, 67}. Recent studies have revealed the pivotal roles of T cell cytokines, such as Th1 vs. Th2, in the pathogenesis of the disease⁵³. Glomerular infiltrating mononuclear cells, which may be recruited through T cell mechanisms, have been shown to play crucial roles in crescentic glomerulonephritis^{4, 19}. The contribution of T cells to glomerulonephritis, especially of the proliferative/crescentic type, has been investigated in animal models with an interrupted B7/CD28 co-stimulation pathway⁵². In summary, these data provide strong evidence that T cells and associated cellular immunity participate in glomerular damage and may be the most important mediators of glomerulonephritis. The key question to be addressed, however, is how T cells participate in glomerular injury.

T cells may participate in glomerular injury as non-specific leukocytes recruited during glomerular inflammation. However, a more significant way is that antigen-specific CD4⁺ T cells may initiate glomerular injury *per se*. This question is difficult to be addressed directly in humans at the present time. Although several groups detected a weak T cell proliferative response specific to a GBM antigen in the peripheral blood in anti-GBM disease patients^{18, 54}, whether such a weak response is a true reflection of a T cell-mediated attack in the glomerulus remains unclear. Several animal models have been developed to address the question. Some studies have focused solely on the role of antigen-specific T cells in anti-GBM glomerulonephritis. Disease susceptibility has been strongly linked to MHC class II, suggesting the potential roles of antigen-specific T cells in glomerular damage³⁰. However, it is unclear whether T cells are merely helper cells in antibody responses, or if they directly initiate the glomerular injury. In the same study, transfer of unstimulated splenocytes from the immunized animals into naive recipients-induced mild glomerulonephritis, as well as anti-GBM antibody. An early study also showed that transfer of mononuclear cells from chicks immunized with isolated GBM-induced glomerulonephritis in naive animals⁷. Although these models used cell transfer to induce glomerulonephritis, it was difficult

to interpret data due for the following reasons. First, transferred cells might have included antigen-specific B cells, which may produce significant autoantibody *in vivo*. Actually, our study showed that antigen-specific B cells probably could survive and secrete antibody until up to 2 cycles of stimulation⁷³. Second, the specificity of the T cells was never determined. Thus, the cell transfer experiments in those studies might suggest, but do not prove, that antigen-specific T cells *per se* could induce glomerular damages.

A T CELL-MEDIATED MECHANISM ALONE MAY BE SUFFICIENT TO CAUSE ANTI-GBM GLOMERULONEPHRITIS

We have developed a rat model for anti-GBM glomerulonephritis based on Col4 α 3NC1 to test the role of T cell-mediated mechanisms in anti-GBM disease⁷⁴. We were able to establish Th1 type CD4⁺ T cell lines which were specific to Col4 α 3NC1⁷³. Passive transfer of *in vitro* activated antigen-specific CD4⁺ T cells induced proteinuria and glomerulonephritis in the naive recipients. In the T cell recipients, anti-GBM antibody was not detectable. Thus, we provide the first strong evidence that antigen-specific CD4⁺ T cells *per se* are sufficient to cause glomerular injury.

Based on these results, we believed that a nephritogenic T cell epitope(s) in Col4 α 3NC1 was responsible for the disease. We decided to map the responsible T cell epitope in Col4 α 3NC1. Although mapping the nephritogenic region in Col4 α 3NC1 has actually been attempted by several groups, all tested peptides were either non-nephritogenic or only induced mild disease^{1, 6, 42, 66}. Based on our previous research experience with other autoimmune disease models, we successfully identified one potent nephritogenic T cell epitope, pCol(28-40) (SQTTAIPSCPEGT), from Col4 α 3NC1 (Fig. 1)⁷². A single immunization with pCol(28-40) induced extremely severe glomerulonephritis in all experimental rats. Renal pathology revealed nearly 100% of glomeruli with crescentic lesions or glomerular tuft necrosis in >95% animals. We have mapped an 11 amino-acid residue pCol(28-38) (SQTTAIPSCPE) to be the core of the T cell epitope in pCol(28-40) (Fig. 1). Immunization with pCol(28-38) induced severe glomerulonephritis in all animals. Thus, this study not only demonstrated that a single T cell epitope of Col4 α 3NC1 is sufficient to induce severe glomerulonephritis, but also provides a unique model for studying T cell-mediated mechanisms in the pathogenesis of anti-GBM glomerulonephritis.

We have demonstrated that a T cell-mediated mechanism alone is sufficient to cause glomerular injury in

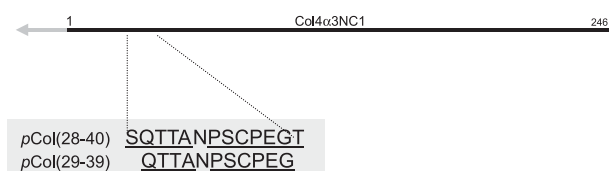


Figure 1. Amino-acid sequence of the nephritogenic T cell epitope of Col4 α 3NC1 in Wistar Kyoto rat model. The location of the T cell epitope in Col4 α 3NC1 is shown. Underlines indicate identical residues between human and rats.

the animal model. However, whether T cell-mediated mechanisms play a similar role in causing human anti-GBM disease needs to be elucidated. Several groups have detected the reactivity of peripheral lymphocytes from anti-GBM patients to GBM antigens *in vitro*^{18, 54}. We have been developing several techniques to identify potential nephritogenic T cell epitopes in humans by *in vitro* MHC-binding assay or by using human MHC transgenic animals. These techniques may allow us to investigate the role of T cell-mediated mechanisms in human anti-GBM disease in a more direct way.

A T CELL MECHANISM INITIATES ANTIBODY RESPONSE TO DIVERSIFIED GBM ANTIGENS: B CELL EPIOTOPE SPREADING

Although anti-GBM antibody has been regarded as the hallmark of human anti-GBM disease, the mechanism for the elicitation of this antibody remains unknown. Based on the T nephritogenic T cell epitope we identified, we discovered that the antibody to diversified GBM antigens was induced following immunization with the T cell epitope⁷⁵. However, the anti-GBM antibody was detectable in 76% of the immunized rats only after prominent glomerular injury. Thus, glomerular injury caused by T cells may be a prerequisite for the antibody production. Furthermore we demonstrated that the specificity of induced anti-GBM antibody was not related to the immunizing T cell epitope pCol(28-40). The anti-GBM antibody eluted from the diseased kidneys reacted only with native GBM, but not with pCol(28-40). Confocal microscopy and immunoprecipitation further demonstrated that the eluted anti-GBM antibody recognized conformational B cell epitope(s) of multiple native GBM proteins. We conclude that the autoantibody response to diversified native GBM antigens was induced by a single nephritogenic T cell epitope. Interestingly, such a phenomenon has been described in several other autoimmune disease models⁶⁹. There are several items of significance in our finding. First, anti-GBM antibody may actually be a consequence of T cell-mediated glomerulonephritis. It is necessary to re-examine classical views of the

cause-effect relationship between anti-GBM antibody and glomerulonephritis. Second, a single nephritogenic T cell epitope alone is sufficient to induce the clinical spectrum of anti-GBM glomerulonephritis, including proteinuria, glomerular injury, and anti-GBM antibody. This leads us to our new hypothesis for the occurrence of human anti-GBM glomerulonephritis.

A NEW HYPOTHESIS FOR THE CAUSE OF ANTI-GBM GLOMERULONEPHRITIS

The etiology of human anti-GBM disease remains unknown. However, the disease is highly linked to HLA DRB*1501, suggesting a critical role of CD4⁺ T cell-mediated mechanisms in this disease⁴⁹. In addition to HLA linkage, several environmental factors, including infections, have been suspected to trigger this disease. Molecular mimicry during infection has been suspected as a possible mechanism for breaking immune tolerance and causing human autoimmune diseases during infection⁶⁹. Several recent studies provided direct evidence to support this hypothesis. For example, *Chlamydia* infection may lead to myocarditis through molecular mimicry³. T cell epitope mimicry is more attractive because a T cell epitope depends primarily on the linear sequence of less than 15 amino-acid residues. Several prior studies have proposed molecular mimicry as a link between infections and anti-GBM glomerulonephritis. However, there was no experimental evidence to support this hypothesis⁷¹. Our finding that a single T cell epitope is sufficient to induce severe glomerulonephritis as well as anti-GBM antibody raises the possibility that T cell epitope mimicry may link anti-GBM etiology with infections. It will be interesting to determine whether T cell epitope mimicry during infections, especially minor infections, is a possible cause of glomerulonephritis. Figure 2 illustrates our new hypothesis for causing anti-GBM glomerulonephritis. We believe that a cer-

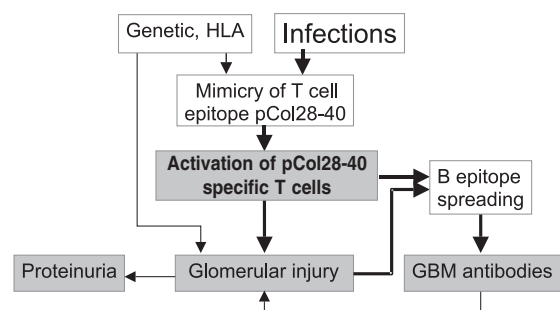


Figure 2. Schematic diagram illustrating the hypothetical mechanism by which a single T cell epitope mimicry during infection induces a full spectrum of clinical manifestations (grey boxes) of anti-GBM glomerulonephritis in a certain population with specific genetic backgrounds.

tain T cell epitope of an infectious agent may activate the CD4⁺ T cells, which cross-react with self collagen 4 α 3 chain; the activated T cells may further cause glomerular injury, and also trigger an antibody response to GBM antigens.

Recently we obtained exciting results to support our hypothesis. For example, we were able to map the

critical residues of the T cell epitope pCol(28-40). Based on the distribution pattern of the critical residues, we identified several microbial peptides. More importantly, a few of the microbial peptides induced glomerular injury as well as anti-GBM antibody. Our model may be used for the exploration of T cell molecular mimicry in glomerulonephritis.

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