

# Sulphilimine cross-links in Goodpasture's disease

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## Introduction

Goodpasture's (GP) disease is an autoimmune disease mediated by autoantibodies that attack the glomerular and alveolar basement membranes. GP autoantibodies target the non-collagenous domain (NC1) of the  $\alpha 3$  and  $\alpha 5$  chain of collagen IV, both of which are part of the  $\alpha 345$  NC1 hexamer, the GP autoantigen [1]. A three-dimensional model of the GP autoantigen has been built by homology modelling using the crystal structure of the  $\alpha 121$  NC1 hexamer (Fig. 1) [2,3]. The hexamer is formed by the interaction of the trimeric NC1 domains of two adjoining triple-helical collagen IV molecules, and is stabilized by novel sulphilimine bonds that cross-link the interface of the trimeric NC1 domains [3,4]. Understanding the structure of the autoantigen and its epitopes is of pivotal importance in deciphering clues about aetiology of GP disease.

## Sulphilimine cross-links and Goodpasture's (GP) disease epitopes

The  $\alpha 345$  NC1 hexamer requires dissociation into subunits for epitope formation [1,5,6]. Cross-linked hexamer is inert to binding of GP antibody, but upon dissociation into dimer and monomer subunits by low pH or denaturants, the subunits bind antibodies. In contrast, un-cross-linked hexamer can also be dissociated by GP antibody into monomer subunits that bind the antibody, revealing that sulphilimine cross-links provide a constraint against dissociation (Fig. 2). Hexamer dissociation involves conformational changes that

## Summary

The sulphilimine cross-link of the Goodpasture (GP) autoantigen is a novel molecular mechanism (structural constraint) for conferring immune privilege to a site which otherwise is susceptible to structural changes that induce an immunogenic and pathogenic conformation. Perturbation of the assembly or cleavage of the sulphilimine cross-links could be a key factor in the aetiology of Goodpasture's disease in susceptible individuals.

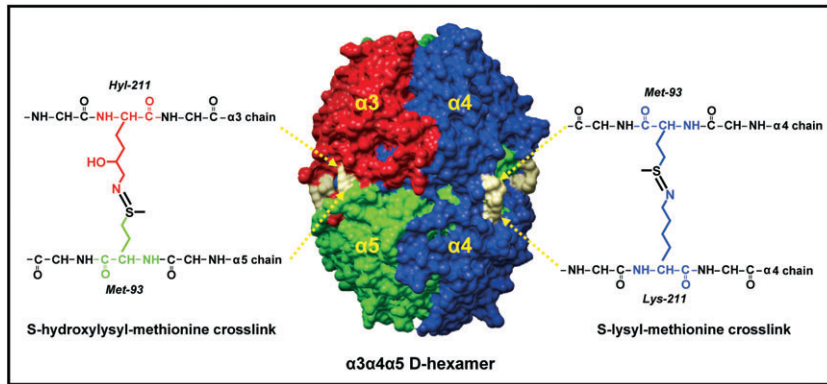
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unlock domain-swapping interactions between subunits and exposes residues sequestered by neighbouring subunits [1,7,8]. Concomitantly, hexamer dissociation transforms the non-pathogenic Ea and Eb regions of the  $\alpha 3$  and  $\alpha 5$  NC1 subunits into pathogenic conformations (epitopes) that confer antibody binding within the dissociated subunits. The sulphilimine cross-links, together with other quaternary interactions, represent a novel molecular mechanism (structural constraints) for conferring immune privilege to sites which otherwise form an immunogenic and pathogenic conformation.

## Sulphilimine cross-links and B and T cell epitopes

GP disease is presumed to result from activation of 'ignorant' lymphocytes by an unknown stimulant coupled with epitope presentation and kidney and/or lung susceptibility. B cells are activated upon binding of the B cell receptors (BCR) to the soluble antigen, with assistance from T cells. BCRs presumably do not bind to the native form of the GP autoantigen, the cross-linked hexamer, but bind subunits upon hexamer dissociation into subunits. The un-cross-linked hexamer is more susceptible to dissociation; therefore, the loss of sulphilimine cross-links may be a predisposing factor for binding of BCR to hexamer subunits.

The sulphilimine cross-links may also influence the generation of T cell epitopes. Unlike B cells, T helper cells (CD4<sup>+</sup>) recognize GP antigens in the form of linear peptides bound to human leucocyte antigen (HLA) class II molecules on the surface of antigen-presenting cells (APC). GP disease



**Fig. 1.** Sulphilimine bonds reinforce the structure of the  $\alpha 345$  non-collagenous domain (NC1) hexamer, the Goodpasture (GP) autoantigen. The illustration shows a space-filling representation of the three-dimensional structure of the  $\alpha 345$  NC1 hexamer, the GP autoantigen. It is composed of two trimeric caps, each composed of  $\alpha 3$  (red),  $\alpha 4$  (blue) and  $\alpha 5$  (green) NC1 domains. Residues Met<sup>93</sup> and Lys<sup>211</sup> (or its hydroxylated form Hyl<sup>211</sup>) are connected through a sulphilimine bond, shown in grey, forming two different types of cross-links: s-hydroxyl-methionine (sHM, left) and s-lysyl-methionine (sLM, right).

has strong positive and negative major histocompatibility complex (MHC)-II associations [9]. Previous studies on the processing of GP antigen by feeding human B cells homozygous for HLA-DR15 with recombinant  $\alpha 3$ NC1 monomer have determined that enzymatic processing, rather than affinity of peptides for HLA molecules, drive GP antigen presentation [10]. The  $\alpha 3$ NC1 peptides were presented as two core sequences with medium affinity for HLA molecules and their presentation was enhanced by inhibition of the lysosomal protease asparagine endopeptidase.

As a natural mechanism to prevent the activation of T cells, sulphilimine bonds may interfere with the generation of  $\alpha 3$ NC1 sequences at different stages of proteolytic processing, peptide binding to MHC class II molecules and/or

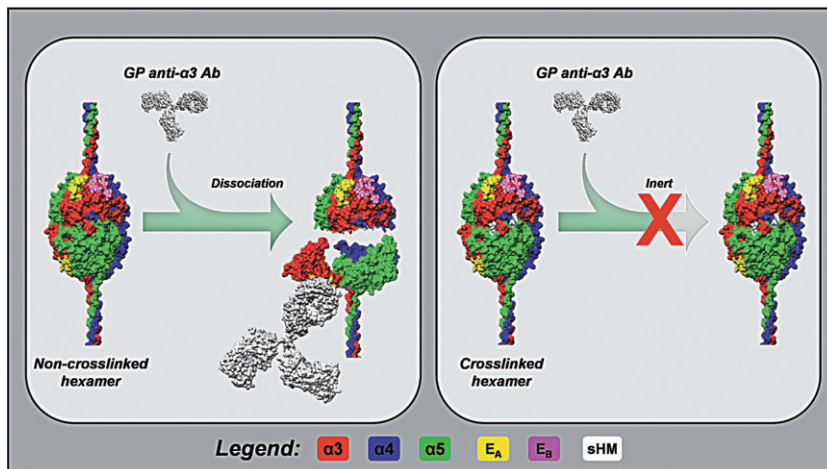
the presentation of peptides–MHC complexes for recognition by CD4<sup>+</sup> T cells. However, it is plausible that under pathophysiological conditions that favour the absence of sulphilimine cross-links, antigen processing and presentation may be enhanced, and therefore contribute to the aetiology of GP disease.

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#### Disclosure

None.



**Fig. 2.** Sulphilimine cross-links provide a structural constraint against Goodpasture (GP) antibody binding. The  $\alpha 345$  non-collagenous domain (NC1) hexamer is shown in the un-cross-linked (left panel) and cross-linked (right panel) forms. Ea and Eb regions are shown in yellow and magenta, respectively, whereas the sulphilimine cross-links are shown in white. The left panel shows an immunoglobulin G (IgG) molecule (white) binding the  $\alpha 3$ NC1 domain inducing hexamer dissociation and transitioning the Ea region into an epitope. Experimental evidence suggests that hexamer dissociation involves a conformational change within the NC1 domains, which unlocks domain-swapping interactions between subunits. The sulphilimine cross-link provides a constraint against dissociation, rendering the hexamer inert to antibody dissociation and binding.

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