

## Accepted Manuscript

Linked help from bacterial proteins drives autoantibody production in small vessel vasculitis

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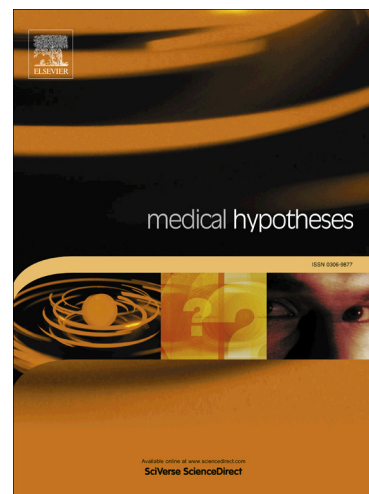
PII: S0306-9877(17)31122-2  
DOI: <https://doi.org/10.1016/j.mehy.2018.01.008>  
Reference: YMEHY 8774

To appear in: *Medical Hypotheses*

Received Date: 26 October 2017  
Accepted Date: 14 January 2018

Please cite this article as: D.B.G. Oliveira, Linked help from bacterial proteins drives autoantibody production in small vessel vasculitis, *Medical Hypotheses* (2018), doi: <https://doi.org/10.1016/j.mehy.2018.01.008>

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## Summary

The small vessel vasculitides granulomatosis with polyangiitis (GPA) and microscopic polyangiitis are associated with autoantibodies to neutrophil cytoplasm antigens (ANCA), principally proteinase-3 (PR3) and myeloperoxidase (MPO). There is an association between GPA and nasal carriage of *Staphylococcus aureus*. The recent finding that *S. aureus* produces proteins that bind tightly to and block the function of both PR3 and MPO suggests a mechanism for ANCA formation. The bacterial protein-autoantigen conjugate is recognised by B cells with ANCA specificity, internalised, and the bacterial protein processed and presented to T cells with specificity for bacterial peptides. The T cell can then provide help to the B cell, allowing class switching, affinity maturation and the production of pathogenic ANCA. This mechanism predicts that T cells with this specificity will be found in patients, and that the bacterial protein-autoantigen conjugate will be particularly efficient at eliciting ANCA production.

The small vessel vasculitides granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA) are serious systemic inflammatory conditions associated with autoantibodies to neutrophil cytoplasmic antigens (ANCA). GPA is characterised by granulomatous inflammation of the airways, and is usually associated with antibodies to proteinase-3 (PR3), whereas MPA does not feature this type of inflammation, and is usually associated with antibodies to myeloperoxidase (MPO); however, the opposite associations (anti-MPO with GPA, and anti-PR3 with MPA) are well recognised. There is considerable evidence that ANCA are involved in the pathogenesis of these diseases (1), but the mechanism leading to their formation is unclear. One factor that has been linked to GPA and anti-PR3 antibodies in particular is nasal carriage of *Staphylococcus aureus* (2,3); furthermore, treatment with co-trimoxazole has been shown to decrease the frequency of relapses in GPA (4). Why the *Staphylococcus*, why these particular autoantigens, and how self-tolerance is broken are questions with no widely accepted answers.

A possible explanation is suggested by studies showing that the *Staphylococcus* secretes proteins that bind to and block the function of precisely these autoantigens. In the case of PR3 the bacterial proteins are the extracellular adherence protein (Eap) and the Eap-homologues 1 and 2 (5), hereafter collectively referred to as Eap. For MPO, the inhibiting protein has been named the staphylococcal peroxidase inhibitor (SPIN) (6). These interactions are strong (affinity constants in the low nanomolar range), and pathophysiologically relevant: bacteria in whom the Eap genes are deleted are less virulent in a mouse model (5), and SPIN is a component of the mechanisms employed by the bacteria to resist killing by neutrophils (6).

Once the bacterial protein-autoantigen complex has been formed then linked T cell help can provide the stimulus to autoantibody formation. The autoantigen component is recognised by B cells that are part of the normal immune repertoire. These cells are responsible for the production of natural non-pathogenic autoantibodies (7); pathogenic ANCA, however, are class switched and of high affinity, attributes which require T cell help. Once the bacterial protein-autoantigen complex has been internalised by the B cell, the bacterial component can be processed, and peptides derived from it presented in the context of class II MHC molecules; they can then be recognised by bacterial peptide-specific T cells which go on to provide the requisite help for production of pathogenic ANCA (see Figure). Once tolerance has been broken in this way mechanisms such as epitope spreading may allow a more general autoimmune response to develop. This mechanism may explain the observation that the MHC associations found in these diseases are actually closer with the type of ANCA rather than with the phenotype of the disease (8): different bacterial proteins are selected by anti-PR3 and anti-MPO antibodies, and the resulting differing spectrum of bacterial peptides has preferential affinity for different MHC molecules.

Although the proposed mechanism has been illustrated with a B cell for simplicity (and other details required for B cell activation omitted) this does not preclude the involvement of other antigen presenting cells. For example, an interaction between T cells and monocytes may be important in granuloma formation, and neutrophil extracellular traps are an efficient way of transferring ANCA antigens to dendritic cells (9). The key point is the link provided by the ANCA antigen-bacterial protein conjugate between the autoreactive B cell on the one hand and the bacterial peptide-specific T cell on the other.

The hypothesis does not directly account for the link between the *Staphylococcus*, anti-PR3 ANCA and the GPA phenotype. PR3 differs from the other serine proteases found in neutrophil primary granules (neutrophil elastase, cathepsin G) in being expressed on the cell surface by resting neutrophils, and also being present in secretory granules (1). This ready availability of PR3, combined with nasal carriage of *Staph. aureus*, may serve to focus the initial autoimmune response to the

upper airway, with the development of a subsequent T cell response to Eap driving granuloma formation. These differences may also account for the fact that although Eap binds to and inhibits both neutrophil elastase and cathepsin G these protein are not major autoantigens in small vessel vasculitis. MPO is similarly not expressed on the surface of resting neutrophils (11), but its status as the most abundant neutrophil protein, making up 5% of the dry weight of the cell (12), may facilitate its role as an autoimmune target. The lack of a strong association between the *Staphylococcus*, anti-MPO antibodies, and MPA may imply, if the mechanism outlined in this hypothesis is correct, that other microorganisms produce analogous MPO-binding proteins.

This proposal does not exclude a contribution from other mechanisms that have been proposed to account for the link between vasculitis and the *Staphylococcus*. These include non-specific stimulation by staphylococcal superantigens (13), stimulation by bacterial DNA motifs via Toll-like receptors (14), a specific response to endothelial bound-staphylococcal acid phosphatase (15), or to peptides translated from the antisense RNA from the PR3 gene that have homology to *Staph. aureus* proteins (16). Staphylococcal protein A, via its ability to bind to surface immunoglobulin on B cells and deliver a stimulatory signal, could serve as an amplifier of autoantibody production (17).

The hypothesis makes the following predictions. T cells with reactivity towards peptides from the bacterial proteins should be present in patients with vasculitis. Specifically, T cells with reactivity towards Eap and SPIN will be found in patients positive for anti-PR3 and anti-MPO ANCA, respectively.

The bacterial protein-autoantigen conjugates will be particularly efficient at stimulating the production of ANCA in an *in vitro* system, as compared to either component added alone. This is a critical prediction, in the sense that if this proposed linked help cannot be demonstrated the hypothesis is effectively falsified.

Peptides from Eap and SPIN will have affinity for the MHC molecules coded for at the *HLA-DP* and *HLA-DQ* loci respectively (8). In addition, Eap peptides may have affinity for the product of the *HLA-DRB1\*1501* allele (18).

Although not a direct prediction of the linked help hypothesis, it seems plausible that microorganisms other than the *Staphylococcus* will have evolved similar countermeasures against host defences. Such putative blocking proteins may have a particular role in non-*Staphylococcal* associated anti-MPO small vessel vasculitis, and substitute for SPIN in the predictions above.

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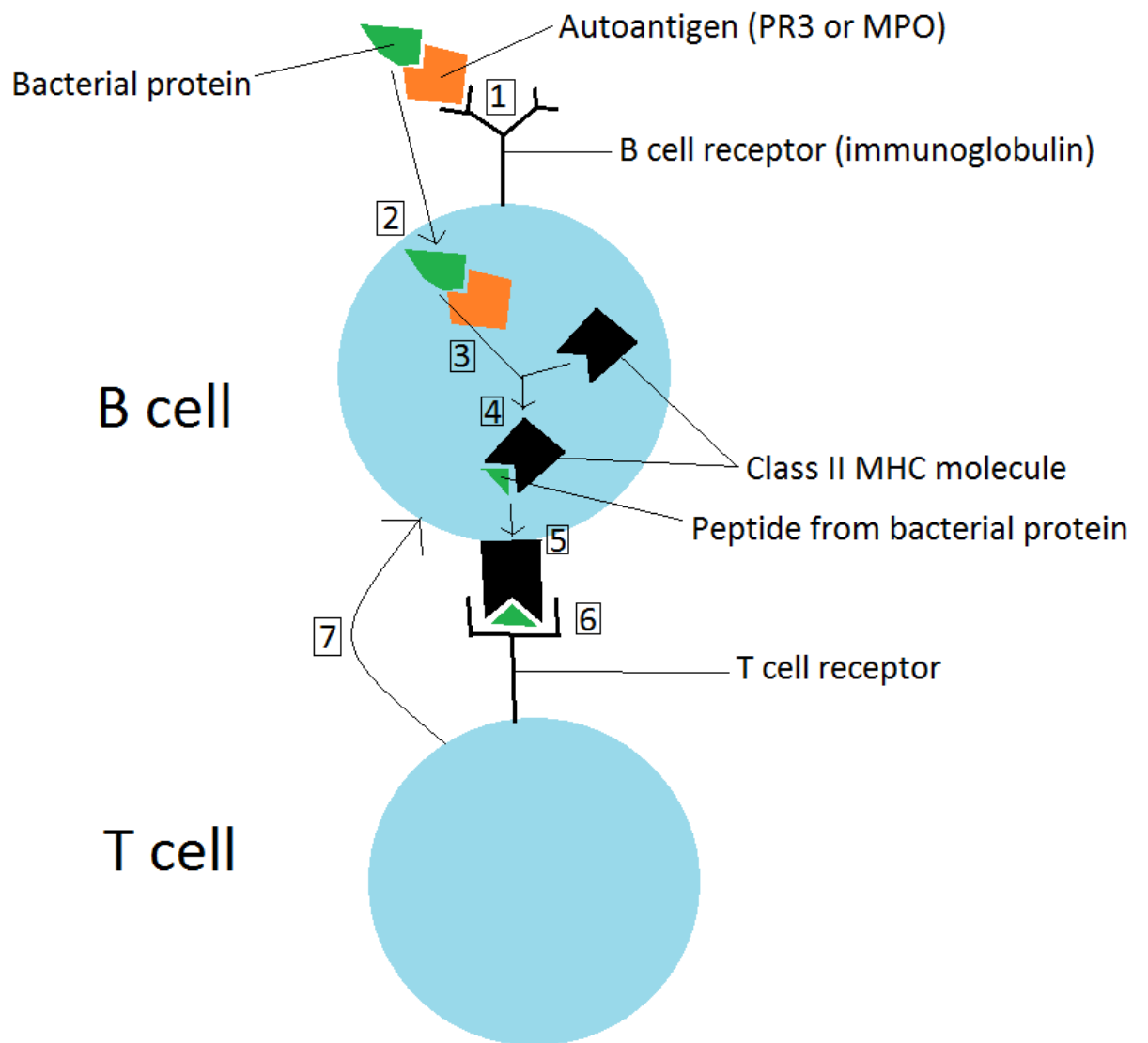


## Figure legend

Steps involved in the provision of linked help. 1: recognition by surface immunoglobulin of the autoantigen-bacterial protein complex. 2: internalisation of the complex. 3: processing of the bacterial protein. 4: loading of the resulting bacterial peptide onto MHC molecule. 5: display on B cell surface of the peptide-MHC complex. 6: recognition by T cell with specificity for peptide-MHC complex. 7: provision of help to B cell.

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Figure



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Conflict of interest

I have no conflicts of interest to declare

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