The Relation between *Staphylococcus aureus* and Wegener’s Granulomatosis: Current Knowledge and Future Directions

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**Abstract**

To date, in the investigation of the role of *S. aureus* in WG, we face a paradoxical situation. On the one hand, clinical results obtained from treatment of WG patients with co-trimoxazole and studies assessing the impact of *S. aureus* on disease relapses strongly suggest that this bacterium contributes to disease pathophysiology. On the other hand, laboratory investigation of the possible mechanisms by which *S. aureus* is involved in WG is scarce, despite the fact that knowledge and tools to study this microorganism are abundant. In the present review, we discuss recent works investigating the possible pathophysiologic contribution of *S. aureus* to WG. Moreover, we propose a number of possibly relevant pathways of interaction of this bacterium with lymphoid and non-lymphoid cells of the WG host.

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**Key words:** Wegener’s granulomatosis, *S. aureus*, autoimmunity, superantigens

**Introduction**

Within the group of small vessel vasculitides—a cluster of conditions characterized by inflammation of vessel walls that can lead to systemic organ damage—Wegener’s granulomatosis (WG) occupies a distinctive place since it has been particularly well studied from a clinical but also a mechanistic point of view. Frequently commencing with general, non-specific symptoms in combination with a chronic respiratory tract infection, WG proceeds in many cases as an autoimmune disorder, hallmarked by the presence of autoantibodies specific for granulocytic enzymes, such as proteinase 3 and myeloperoxidase (1–4). If untreated, the disease eventually affects the vasculature of various organs, among which the kidney is the most prominent target.

The immune system has been the major target of medical intervention in WG and treatment with immunosuppressive drugs such as prednisolone, cyclophosphamide, azathioprine, and, more recently, methotrexate and mofetil mycophenolate is meanwhile established (5). Moreover, our group has proposed treatment with the antibiotic trimethoprim-sulfamethoxazole (co-trimoxazole) as a means of reducing the incidence of disease relapses in WG (6). Together with our findings that the incidence of chronic nasal carriage of the gram-positive bacterium *Staphylococcus aureus* was significantly higher in WG patients than in healthy individuals, and that chronic carriage of *S. aureus* constitutes a risk factor for disease exacerbation in WG (7), our study postulated a significant role for *S. aureus* in disease (re)activation in WG. In the present review, we will discuss recent work investigating the possible pathophysiologic contribution of *S. aureus* to WG. Since studies on the involvement of *S. aureus* in WG are yet scarce, we also propose a number of possibly relevant pathways of interaction of this bacterium with lymphoid and non-lymphoid cells of the WG host.

**S. aureus: Epidemiology**

*Staphylococcus aureus* is a gram-positive bacterium that is most consistently isolated from the anterior nares (8, 9). In various settings and conditions, carriage of *S. aureus* is elevated; a prominent group at risk for increased *S. aureus* carriage is patients who regularly use needles, such as dialysis patients, diabetes patients, but also patients with skin lesions, AIDS or undergoing surgery (reviewed in 10). In these patients carriage can be associated with the development of clinical *S. aureus* infections.

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**S. aureus in WG: Clinical Evidence**

Friedrich Wegener was the first to postulate that a microbial infection or hyperreaction to external pathogens may cause WG. Wegener based his hypothesis on the observation that the presence of granulomatous inflammation, which is typical of these patients, is generally associated with infections (11, 12). The importance of *S. aureus* in WG was later noted in a prospective study in 85 WG patients, conducted by Fauci et al over a period of 21 years. This group found that virtually every WG patient had secondary infections of the paranasal sinuses, caused mainly by *S. aureus* and responding to anti-staphylococcal treatment (13). Furthermore, Pinching et al reported that in 9 out of 20 WG patients relapses were triggered by microbial infections, among others with *S. aureus* (14). Other groups confirmed a link between infections and disease relapses and postulated that bacterial infections may trigger disease activity in WG (15, 16).

Therapeutic results obtained by the introduction of co-trimoxazole for treatment of WG patients supported this postulate (5). DeRemee et al reported on treatment of 12 WG patients with co-trimoxazole, resulting in clinical improvement in 11 patients (17, 18). This improvement was observed in patients who had received monotherapy with co-trimoxazole, as well as when this antibiotic was added to conventional immunosuppressive therapy. The efficacy of co-trimoxazole as a monotherapy for WG patients with limited disease was recently assessed in a prospective study by our group (manuscript in preparation). We concluded that co-trimoxazole treatment led to partial or complete remission in a substantial proportion of patients presenting with limited, local disease. Reports from smaller studies have confirmed the beneficial effect of co-trimoxazole on the achievement of partial or complete remission (19–23).

These clinical studies, however, did not formally demonstrate a relationship between antibiotic treatment, eradication of bacterial populations and disease activity. DeRemee pointed out that, apart from its antibiotic properties, co-trimoxazole may actually influence the disease course in WG by virtue of its anti-inflammatory effect (18). Co-trimoxazole exerts its antibiotic effect by antagonizing folic acid metabolism, a mechanism that could affect immunocompetent cells (24).

In order to determine whether *S. aureus* is implicated in disease exacerbation in WG, we conducted a prospective study, assessing nasal carriage of *S. aureus* and its impact on disease reactivation (7). Chronic nasal carriage of *S. aureus* was defined as the presence of >75% of nasal cultures positive for this bacterium (cultures were obtained every 4–6 weeks). By this criterion, 36/57 patients (63%) were chronic nasal carriers of *S. aureus*, a rate approximately three times higher than in healthy controls. Relapses occurred predominantly in chronic carriers of *S. aureus* (relative risk of carriage for ensuing relapse, 7.16).

In the following sections we will review mechanisms by which this bacterium may be implicated in the pathogenesis of this form of vasculitis.

**Impact of *S. aureus* on T Cells in WG**

The main influence of *S. aureus* on T cells is exerted by its exotoxins. Three classes of exotoxins have been described to date, comprising the staphylococcal enterotoxins (SE), exfoliative toxins (ET) and toxic-shock syndrome toxin 1 (TSST-1). Although initially described as toxic agents responsible for the development of food poisoning (SE) (25), skin exfoliation (ET) and toxic shock (TSST-1) (26), staphylococcal exotoxins were soon recognized as extremely potent immunostimulatory molecules, and therefore termed “superantigens” (SAg) (27).

Staphylococcal SAg are bifunctional molecules that bind to MHC class II molecules on antigen presenting cells, outside the peptide-binding groove (28), and simultaneously to conserved regions of T-cell receptor V-beta chains (TCR Vβ) (27), independently of ligand specificity of T cells (Fig. 1). Virtually all T cells expressing a SAg-binding TCR Vβ chain are stimulated (29, 30).

Stimulation with a SAg results in a biphasic response of SAg-reactive T cells. In the initial phase these T cells proliferate (29, 31, 32), causing skewing of the TCR Vβ repertoire. Moreover, activated T cells produce pro-inflammatory cytokines, such as IL-2, IFN-γ, TNF-α and IL-12 (33–36), which are responsible for the pyrogenic effect of SAg in vivo. In the second phase, the T-cell response to SAg is contained and terminated. This phase is dependent on chronic or restimulation with the original SAg and is modulated by the production of the immunosuppressive cytokine IL-10 (37–39). An anergic state sets in, marked by the inability of previously stimulated T cells to produce IL-2 and to proliferate upon restimulation with the same SAg (40–42). In addition to anergy, SAg can also induce clonal deletion of activated T cells, in a process involving Fas-mediated apoptosis of activated T cells (43, 44).

In human autoimmune diseases a role for SAg has been postulated (45, 46), although evidence for the implication of SAg in these diseases has so far been circumstantial. Kawasaki disease, a form of large to medium vessel systemic vasculitis, has for some time been the only vasculitis in which the presence of T-cell skewing (47–50) and SAg-reactive T cells are critically assessed (51–53), demonstrating the correlation between the presence of TSST-1 producing *S. aureus*, the presence of corresponding Vβ+ T cells expansions and concomitant vasculitic disease activity.

In WG, the increased incidence of chronic nasal carriage of *S. aureus* (24), together with the finding that circulating T cells are chronically activated (54, 55) support the hypothesis that a persistent stimulus, possibly a staphylococcal SAg, may contribute to T cell activation and thus to disease reactivation (54). We have addressed this hypothesis by typing SAg genes in 42 *S. aureus* strains obtained from WG patients (56, 57). TSST-1 (present in 19% of the strains), SEA (14%), SEC (10%) and ETA (10%) were the most frequently
Relation between S. aureus and WG

Figure 1. S. aureus and T cells. SAg can bridge antigen-presenting cells (APC) and T cells by simultaneously binding to the MHC II molecule on APC and SAg-responsive T cell receptor V-beta chains on T cells (A). This interaction results in proliferation of the respective V-beta T cell subset and cytokine production. T cells without a SAg-responsive TCR V-beta chain cannot be bound by SAg and will not proliferate (B).

Table 1. Occurrence of SAg-ene Positive S. aureus in WG Patients.

<table>
<thead>
<tr>
<th>SAg</th>
<th>patients (n=37)</th>
<th>cultures (n=326)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEA</td>
<td>20 (54%)</td>
<td>157 (48.1%)</td>
</tr>
<tr>
<td>SEB</td>
<td>3 (8.1%)</td>
<td>13 (3.9%)</td>
</tr>
<tr>
<td>SEC</td>
<td>19 (51.3%)</td>
<td>62 (19%)</td>
</tr>
<tr>
<td>SED</td>
<td>22 (6.7%)</td>
<td>6 (1.6%)</td>
</tr>
<tr>
<td>SEE</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TSST-1</td>
<td>18 (48.6%)</td>
<td>119 (36.5%)</td>
</tr>
<tr>
<td>ETA</td>
<td>7 (18.9%)</td>
<td>61 (18.7%)</td>
</tr>
</tbody>
</table>

present SAg in these strains, although their presence was not significantly more frequent than in S. aureus strains obtained from healthy individuals. However, in this preliminary study, SAg-positive S. aureus did identify a subgroup of WG patients with a higher risk for the development of disease relapses (57). Very recently we have extended our study to comprise 709 S. aureus strains isolated from 63 WG patients over a time period of 6 years. Typing of SAg genes in these strains by PCR confirmed our previous finding of SEA, TSST-1, SEC and ETA being the most frequently occurring SAg (Table 1).

Analysis of the time-dependent relation between the occurrence of relapses and SAg-carriage identified TSST-1 as a risk factor for disease exacerbation (E.R.P, manuscript in preparation).

In order to investigate the possible involvement of staphylococcal SAg in WG and other vasculitides, a number of studies have analyzed the presence of T cell repertoire skewing as an indication for potential SAg activation. Giscombe et al (58) detected expansions of T cell subsets expressing SAg-reactive Vβ chains in 10/11 patients with WG and PAN, but found no obvious preferential skewing of any particular Vβ subset. Similarly, Simpson et al reported on skewed expression of Vβ3, Vβ9, Vβ13, Vβ14, and Vβ15 and particularly marked skewing of Vβ2.1 in WG patients (59). In support of a hypothetical stimulation of T cells by SAg, these authors also demonstrated polyclonality of the expanded T cell subsets (59, 60).

To investigate the association between the presence of SAg-positive S. aureus and the presence of T cell skewing in WG we hypothesized that the presence of staphylococcal SAg is accompanied by expansion of SAg-reacting T cell subsets. In a cross-sectional and a longitudinal study we assessed the association between seven staphylococcal SAg
genes (typed by PCR), eight SAg-binding Vβ chains and four SAg-nonbinding Vβ chains (assessed by flow-cytometry). Both studies showed that T cell expansions were present at a significantly higher rate in WG patients than in healthy individuals, but were not associated with the presence of either S. aureus or SAg. Moreover, T cell expansions were generally of small extent, and did not simultaneously appear in both CD4 and CD8 subsets, suggesting that they had not been induced by SAg (manuscript in preparation).

Being the first dramatic manifestation of SAg stimulation, T cell proliferation has received special attention as an in vivo reflection of SAg stimulation. Other effects of SAg on cellular immunity in vitro and in vivo certainly deserve further exploration in vasculitides. As previously mentioned, one of these effects consists in the induction of IL-10 mediated T cell anergy by chronic or repeated stimulation with the same SAg. Chronic exposure of T cells to SAg is possible in the case of chronic carriage of SAg-positive S. aureus strains. Since the upper airways are the major niche for S. aureus colonization in humans, it would be interesting to study the relationship between S. aureus carriage, SAg production and cytokine patterns in infiltrating T cells in these lesions. Moreover, it would be of interest to study whether resolution of active localized disease correlates with a switch to a Th2 cytokine production pattern, dominated by IL-10.

One of the unresolved puzzles of SAg-mediated T cell activation is the impact of the biphasic T cell response to SAg on the antigen-specific T cell response. A consequence of the initial SAg-induced T cell activation phase could be proliferation of autospecific T cells, expressing a SAg-responsive Vβ chain. Evidently, this could result in disease exacerbation (61). Studies in mouse models of influenza virus infection showed that challenge of mice that had recovered from influenza virus infection, with SEB resulted in the generation of a strong cytotoxic response of influenza virus-specific CTLs (62, 63). Moreover, infections with the influenza virus exacerbated subsequent T-cell responses to the SAg SEB (63). It is conceivable that a similar effect of SAg on (auto)antigen-specific CD4⁺ T cells may exist. On the other hand, if exposure of SAg to (auto)specific T cells persists, anergy and/or deletion of these T cells would improve the course of the disease.

Impact of S. aureus on B Cells in WG

The possible impact of S. aureus on B cells in WG or other vasculitides has not been addressed as yet and thus remains a matter of speculation. Previously, we discussed mechanisms by which infection might stimulate B lymphocytes (61). Two pathways by which staphylococcal products

![Figure 2. S. aureus and B cells. Interaction of SpA with membrane-bound Ig molecules encoded by Vh3 Ig genes has a mitogenic effect, stimulating proliferation of these B cells and Ig production. In WG, it is conceivable that binding of SpA to PR3-specific B cells expressing Vh3-encoded auto-Ig may lead to ANCA production. Alternatively, binding of SpA to soluble ANCA may lead to formation of immune depositions and complement activation.](image-url)
may interfere with the regulation of these cells are mediated by either staphylococcal protein A (SpA) (Fig. 2) or SAg, and will be briefly reviewed.

Staphylococcal protein A is a component of most clinical strains of *S. aureus* (64–66). SpA interacts with a variety of human IgG molecules, based on its affinity for the Fc portion of IgG (67, 68). In addition, SpA has additional alternative binding sites for V\_H\_3-encoded immunoglobulin heavy chains, which are structurally and functionally distinct from the Fc\_y binding site (69–71). Binding of F(ab’\_2) fragments by SpA can result in binding of 15-50% of human polyclonal IgM, IgG and IgA (70, 71), without affecting binding of a conventional antigen ligand (72).

The interaction of SpA with F(ab’\_2) fragments of membrane-bound Ig is essential for SpA-induced B cell mitogenicity and entails production of Ig (73). The potential pathologic significance of this process is suggested by in vitro and in vivo studies showing that, upon stimulation with SpA, B cells from healthy individuals or patients with rheumatoid arthritis can produce V\_H\_3\_kappa rheumatoid factor (74, 75). Interaction of SpA with soluble Ig can induce systemic complement activation (76), with potentially detrimental consequences, considering that up to 50% of circulating Ig can be bound by SpA.

In WG, the possible role of SpA can be conjectured from current information on the amino acid sequence of ANCA. Thus, the presence of ANCA with a V\_H\_3-encoded heavy chain would indicate potential binding capacity of SpA. Sibilia et al. found that three out of five IgM\^\_kappa, PR3-specific B cell lines obtained from a WG patient expressed V\_H\_3\_kappa-encoded ANCA. Two of these lines were clonally identical, suggesting that they had been derived from clonally expanded autospecific B cells (77). These findings imply that, in the presence of SpA, PR3-specific B cells could be induced to proliferate, resulting in an amplification of the pool of autoantibodies.

B cell activation and immunoglobulin production can also be mediated by classical staphylococcal T cell SAg. In vitro, high doses of SAg promote Ig production (78, 79). Importantly, T cell-dependent activation of B cells mediated by SAg can bypass cognate interaction with a specific antigen, ensuring that production of Ig takes place even in the absence of the specific antigen (80). Through this mechanism, SAg have been shown to stimulate T cell-dependent selective induction of rheumatoid factor by B cells (81). In WG, this could imply that, independent of the presence of the autoantigen PR3, SAg could facilitate ANCA production by bridging autospecific B cells and T cells expressing a SAg-specific TCR V\_\beta chain.

**S. aureus and the Endothelium**

Under normal conditions, i.e. in the presence of a functional immune defense and intact tissue barriers, *S. aureus* carriage has no pathological consequences for the host. Upon breaching of skin or mucosal barriers, however, the microorganism can gain access to underlying tissue or the blood stream, provoking infection and even septicemia.

*S. aureus* binds more readily to vascular endothelial cells than other bacterial species (82). Following binding, *S. aureus* is internalized by endothelial cells. Interestingly, the microorganism is able to persist intracellularly in phagosome-like vacuoles (82–85), as a small colony variant (86), without being degraded. In WG this stealth mechanism may provide a means to ensure recolonization of the patient after prolonged treatment with antibiotics. Indeed, in a recent study investigating the longitudinal clonality of *S. aureus* strains in relation to treatment with co-trimoxazole, we found that, after antibiotic treatment was discontinued, bacterial strains that were genetically identical to those isolated prior to antibiotic treatment, were present in the patient (ERP, manuscript in preparation). Thus, it may be relevant to investigate whether in WG the endothelium serves as a potential reservoir for immunologically inaccessible *S. aureus*.

From an immunopathogenic point of view, the interaction of *S. aureus* with the endothelium and penetration of the endothelium can have several outcomes. First, adhesiveness of leukocytes to endothelial cells is augmented by the increased expression of P-selectin and ICAM-1 (87, 88). Interestingly, killing of staphylococci by some antibiotics creates bacterial debris with a higher potential to induce endothelial adhesiveness (88), implying that antibiotic treatment may have repercussions on inflammatory processes.

As another consequence of endothelial interaction with *S. aureus*, endothelial production of chemoattractants, such as MCP-1 and IL-8 (88–90) and pro-inflammatory cytokines, such as IL-6 and IL-1\_beta (90) is upregulated. Recruitment of neutrophils and creation of an inflammatory milieu, have, in turn, consequences for endothelial integrity, since it has been postulated that in WG, vascular endothelial damage may result from the local production of proteolytic enzymes and reactive oxygen species by infiltrating neutrophils.

However, endothelial damage may not only be mediated in the course of inflammation, but can be induced directly by internalization of *S. aureus* (91, 92). Endothelial apoptosis is dependent on the presence of the pore-forming staphylococcal cytolsin a-toxin (92, 93).

Our group has directed its attention towards a novel staphylococcal product, the cationic molecule staphylococcal acid phosphatase (94). In vitro, this molecule can bind to HUVEC via charge-interactions, and endothelial bound staphylococcal acid phosphatase is recognized by IgG from patients with WG (95). Moreover, *in vivo* perfusion of rat kidneys with staphylococcal acid phosphatase after immunization with staphylococcal acid phosphatase was shown to induce glomerulonephritis (96, 97).

**The Impact of S. aureus on Neutrophils and Monocytes**

The natural manner to resolve bacterial infections is to remove pathogens by professional phagocytes. Uptake of
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Figure 3. Impact of S. aureus on PMNs. Uptake of opsonized S. aureus by PMN previously primed by pro-inflammatory cytokines IL-1 and TNF-α can lead to full activation of PMN, resulting in release of PR3 and reactive oxygen species (ROS). As a consequence, proteolytic and oxidative damage of the surrounding tissue can ensue, specifically of vascular endothelial cells engaged in contact with PMN via upregulated adhesion molecules. Moreover, released PR3 can boost the autopspecific B cell response.

Opsonized bacteria into phagocytes is mediated by Fc- and complement receptors. In the setting of WG it is particularly important that cross-linking of Fc-receptors on TNF-α primed neutrophils leads to neutrophil activation and membrane expression or secretion of the autoantigen PR3. Triggering of an activation signal through the Fc-receptor (FcR) has been demonstrated for PR3-ANCA (98) but can also be induced by S. aureus-binding Ig (Fig. 3). As a consequence of FcR cross-linking reactive oxygen species are shed (98), potentially affecting the integrity of underlying tissue. Combining our previous observation that activated neutrophils in WG express PR3 on their surface (99) and that neutrophil activation can be achieved by interaction of Fc-receptors with ANCA (98), it can be envisaged that binding of opsonized S. aureus to Fc receptors may induce PR3 expression and shedding by neutrophils (100). As a consequence, not only direct proteolytic destruction of surrounding tissue, but also stimulation of ANCA production by the released autoantigen would ensue. It is particularly the former consequence that may have a direct impact on the integrity of the vascular endothelium and thus on the progression of vascular damage. As mentioned above, whole S. aureus bacteria are able to adhere to endothelial surfaces, inducing upregulation of chemoattractant molecules and adhesion molecules. If in the sequel of this process, immunoglobulin with bacterial specificity are present, cross-linking of endothelium-bound bacterial agents and the Fc-receptors expressed on neutrophils could lead to local activation of neutrophils and release of proteolytic PR3 and reactive oxygen species. Thus, paradoxically, in an autoimmune disorder such as WG, attempts of the immune system to confront bacterial invasion can provoke exacerbation of autoimmunity.

Outlook

When we consider available and yet circumstantial data on the role of S. aureus in WG (Fig. 4) it becomes apparent that, despite the dramatic effects that this bacterium can have on an array of cell types of the host, these effects are not manifested in the patient. Most striking are our findings that carriage of staphylococcal strains that are positive for SAg genes by WG patients is not associated with the expected expansion pattern of SAg-reactive T cells. Obviously, the study of the pathophysiologic significance of S. aureus in vivo is encumbered by various factors, among which treatment of the patient with either antibiotics or immunosuppressives is one of the more important ones. Moreover, one of the natural reactions of the immune system upon confrontation with the microorganism is to mount a humoral immune response, which contributes a part of the defense against the pathogen (101–103). Frequently, these antibodies are directed against SAg and can inhibit the effect of these immunotoxins on T cells, as is seen in the case of preparations of pooled human Ig for intravenous administration (103, 104). Moreover, as mentioned above, study of S. aureus in vivo may be encumbered by the fact that the microorganism is able to avoid detection by surviving inside an array of host cells.

Clearly, a delicate balance exists between the various components of the immune system and the microorganism, and it should be realized that interaction of S. aureus or its products with one cell type may have functional implications for other cell types, as well. At this point we speculate that, in the setting of WG, the two roles of S. aureus most worthwhile of investigation are its activating and lytic effect on endothelial cells and on phagocytic cells. Such studies would give an insight into direct vascular damage by the bacterium...
as well as indirect damage by release of proteolytic enzymes and reactive oxygen radicals from neutrophils. Moreover, it would allow us to estimate whether *S. aureus* contributes to exposure of the autoantigen PR3 to the immune system (see section *S. aureus* and neutrophils).

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