Concise Report

Staphylococcal toxic-shock-syndrome-toxin-1 as a risk factor for disease relapse in Wegener’s granulomatosis


Objectives. Nasal carriage of *Staphylococcus aureus* constitutes a risk factor for disease exacerbation in Wegener’s granulomatosis (WG). We hypothesized that staphylococcal superantigens (SAg) are a determinant of *S. aureus*-related risk for disease relapse in WG.

Methods. In a retrospective longitudinal cohort study in 62 WG patients, we investigated the presence of the staphylococcal SAg genes *sea*, *seb*, *sec*, *sed*, *see*, *tsst-1* and *eta* in *S. aureus* strains isolated from WG patients during an observation period of seven years. Subsequently, we assessed whether relapses of WG were associated with the presence of SAg-positive staphylococci.

Results. Of 1718 swab cultures analysed, 709 (41.2%) were *S. aureus*-positive. Fifty-one patients carried *S. aureus*, of whom 37 (72.5%) patients carried at least one SAg-positive *S. aureus* strain. Of the 709 *S. aureus*-positive cultures, 326 (46%) contained at least one SAg gene. Except for *see*, all assessed SAg genes were detected. *sea* was found most frequently, followed by *sec*, *tsst-1* and *eta* and finally, by *sed* and *seb*. Using a multivariate, time-dependent Cox regression analysis we found that the presence of *S. aureus* was associated with relapses of WG (RR 3.2; 95% CI 1.2–8.4). The risk for relapse was modulated by the presence and type of SAg, with *tsst-1* being associated with an increased risk for relapse (RR 13.3, 95% CI 4.2–42.6).

Conclusion. The risk for relapse of WG increases with the presence of *tsst-1*-positive *S. aureus*. Eradication of *tsst-1*-positive *S. aureus* in WG may show whether disease relapses can be prevented.

Key words: Wegener’s granulomatosis, Vasculitis, *S. aureus*, Superantigens, Relapse, TSST-1, PCR.

Introduction

Wegener’s granulomatosis (WG) is a form of idiopathic small-vessel vasculitis, characterized by granulomatous inflammation of the respiratory tract, necrotizing small vessel vasculitis and glomerulonephritis. Both genetic and environmental factors are thought to contribute to the aetiology of the disease [1, 2]. Among the latter, microbial infections, mainly of the upper airways, have been shown to be associated with disease relapse [3–5]. Importantly, the antibiotic trimethoprim-sulphamethoxazole (co-trimoxazole) lowered the rate of disease exacerbations and even induced long-term complete remission [6–10], supporting the ‘infectious agent’ theory in WG.

Previously, we showed that the rate of chronic carriage of *Staphylococcus aureus* in WG patients is three times higher than in healthy individuals and constitutes a risk factor for relapse [11]. In view of the dysregulated immune balance in WG, we postulated that staphylococcal superantigens (SAg) may mediate *S. aureus* pathogenicity [1]. SAg are bacterial exotoxins with a strong immunostimulatory capacity [12], which can induce relapses of autoimmune diseases [13, 14]. The aim of the present study was to assess whether the presence of SAg-positive *S. aureus* forms a risk factor for relapse in WG patients.

Materials and methods

Patients

In this retrospective study, 62 consecutive patients (32 male, 30 female, mean age 54.7 years, range 20–80 years) with biopsy-proven WG and fulfilling the criteria of the American College of Rheumatology for the diagnosis of WG [15] were included (Table 1). All patients had anti-protease three anti-neutrophil cytoplasmic antibodies (ANCA). At each visit, usually every 4–6 weeks, patients were evaluated for symptoms of active WG and swab cultures of the anterior nares were performed to detect *S. aureus* [11]. Immunosuppressive treatment typically consisted of prednisolone, cyclophosphamide, azathioprine or a combination thereof [11]. Antibiotic treatment for presumed or proven infection episodes, usually of the upper airways, typically consisted of 1 to 2 week courses of co-trimoxazole (800/160 mg tablets, twice daily), mupirocin, doxycyclin or amoxycillin. Disease relapses were defined according to previously described criteria [16]. During the observation period, 35 patients had 64 relapses (median 1, range 0–6 relapses per patient) involving the ENT region (69%), eyes (31%), trachea (6%), lungs (20%), kidneys (54%), peripheral nervous system (26%), central nervous system (3%), joints (77%) and oral mucosa (14%). Fever and involuntary weight loss (>2 kg/month) occurred in 34% and 11% of the patients, respectively. The mean follow-up period was 52.5 months (range 2–83 months). The study was performed in accordance with the ethical standards of the institutional committee on human experimentation and the Declaration of Helsinki.

Detection of *S. aureus*

Nasal swabs were inoculated on 5% sheep-blood and salt mannitol agar and cultured overnight. *S. aureus* was identified by coagulase and DNase positivity. The *aureus* species was confirmed by PCR detection of the *S. aureus*-specific gene *nuclease-a* (nuc-a; see subsequently). Single *S. aureus* colonies
were isolated, cultured on blood agar plates and subsequently stored at –80°C until further usage.

Detection of SAg genes

DNA was isolated from *S. aureus* strains by the method of Boom *et al.* [17]. To detect the staphylococcal SAg genes *sea, seb, sec, sed, see, eta* and *tsst-1* we established a multiplex PCR that allows the simultaneous detection of two to three SAg genes (Fig. 1A). Primers (Life Technologies, Paisley, UK) have been previously described [18]. PCR was carried out in a volume of 25 µl (0.58 mM dNTPs, Pharmacia; 21% Super-Taq reaction buffer; 2.5 U SuperTaq, (Ambion, Austin, TX, USA), 0.025 mM MgCl₂, Merck; 25 pmol *sea* primers, 12.5 pmol *SEB* primers, 75 pmol *sed* primers, 50 pmol *sec, see*, *eta* and *tsst-1* primers, 10 pmol *mnt-a* primers, 50 ng DNA). PCR consisted of one pre-amplification cycle (5 min 94°C, 3 min 45°C, 3 min 72°C), 2 cycles (10 s 94°C, 15 s 45°C, 30 s 72°C) and 37 cycles (10 s 94°C, 15 s 55°C, 30 s 72°C). In order to ascertain that the absence of SAg genes in the multiplex PCR was not due to technical failure, all PCRs were also performed on a positive control (Fig. 1A).

Statistical analysis

The relative risk of WG patients who carried a SAg-positive *S. aureus* strain to develop disease exacerbations was determined
Presence of SAg-positive individual SAg genes. (Associated with the presence or absence of staphylococcal SAg are shown relative to absence of the SAg confirm the strain species (arrowhead). To illustrate the method and to serve as a positive control, total DNA from sed and see (lane 3) and sed and eta (lane 4) were detected in the same reaction. Additionally, the S. aureus-specific nuclease a (nuc-a) gene was coamplified in order to confirm the strain species (arrowhead). To illustrate the method and to serve as a positive control, total DNA from S. aureus sed and see (lane 3) and sed and eta (lane 4) were detected in the same reaction. Additionally, the S. aureus-specific nuclease a (nuc-a) gene was coamplified in order to confirm the strain species (arrowhead). To illustrate the method and to serve as a positive control, total DNA from sed and see (lane 3) and sed and eta (lane 4) were detected in the same reaction. Additionally, the S. aureus-specific nuclease a (nuc-a) gene was coamplified in order to confirm the strain species (arrowhead).

Analyses were adjusted for other risk factors possibly associated with disease relapses in WG, i.e. age, previous relapses, disease extent, as well as for use of immunosuppressive and antibiotic treatment.

Results

S. aureus carriage

Definitions for S. aureus carriage have been previously formulated [11]. Eleven of 62 (17.7%) WG patients were non-carriers, as defined by the absence of S. aureus in all cultures. Fifty-one of the 62 patients (82.2%) had at least one S. aureus-positive culture. Eighteen of these patients (35.3%) were intermittent carriers, as defined by the presence of S. aureus in less than 75% of the cultures. Thirty-three of the 51 patients (64.7%) were chronic carriers, as defined by the presence of S. aureus in 75% or more of the cultures (Table 1). In 31 patients, S. aureus was present despite antibiotic treatment (Table 1). Out of 1718 swabs, 709 (41.2%) were S. aureus-positive (mean 11, range 1–43 cultures per patient). All S. aureus strains were positive for the S. aureus-specific nuc-a gene.

Detection and typing of SAg genes in S. aureus

Thirty-seven (72.5%) of the 51 S. aureus-positive patients carried a SAg-positive S. aureus strain at least once. Of the 709 S. aureus-positive cultures, 326 (46%, mean 8.7, range 1–28 cultures per patient) were positive for at least one SAg gene. Except for see, all assessed SAg genes were detected (Fig. 1B). sea was the most frequent SAg, followed in frequency at cohort level by sec, tsst-1 and eta and finally, by sed and seb (Fig. 1B). Frequently, S. aureus cultures simultaneously contained two or more SAg genes in various combinations (Table 1).

Relative risk of presence of SAg-positive S. aureus for the development of disease relapses

We first tested the hypothesis that the presence of SAg-positive S. aureus in WG patients is a risk factor for disease exacerbation, irrespective of the type of SAg.

In 27 of the 35 first relapses occurring during the observation period, S. aureus had been cultured in the 3-month period preceding diagnosis of the relapse. The presence of S. aureus was associated with these relapses with an RR of 3.2 (95% CI 1.2–8.4). As we have previously shown that non-carriers of S. aureus have a lower risk of relapse compared with carriers [11], we re-analysed our data in the group of 51 patients with at
least one S. aureus-positive nasal culture. The risk for relapse associated with the preceding presence of S. aureus was comparable in this subgroup with the risk in the whole group.

When occurrence of a relapse within 3 months from detection of a S. aureus strain was handled as a criterion, the presence of SAg-negative S. aureus was associated with an RR for relapse of 2.2 (95% CI 0.9–5.1) (Fig. 1C), as compared with the absence of S. aureus. The presence of SAg-positive S. aureus was associated with an RR for relapses of 2.9 (95% CI 1.1–7.0), as compared with absence of S. aureus. Thus, the presence of SAg-positive or SAg-negative S. aureus in WG patients were both associated with a comparable RR to develop disease relapses. Moreover, the presence of both SAg-positive and SAg-negative S. aureus were associated with an increased risk for disease relapse compared with absence of S. aureus.

Relative risk of individual SAg for the development of disease relapses

Subsequently we assessed the RR of S. aureus containing sea, sec, eta, tsst-1 and seb/sec (pooled due to small numbers) for the development of disease relapses.

No relapses were detected in the 3-month period following detection of seb-positive or sed-positive S. aureus (Fig. 1C). The presence of sea- positive or sea- positive S. aureus was not significantly associated with a relapse of WG (Fig. 1C) when compared with absence of S. aureus. In contrast, the presence of S. aureus positive for tsst-1 showed the strongest association with disease relapse (RR 13.4, 95% CI 4.2–42.6) (Fig. 1C).

Discussion

In the present retrospective study, we confirmed and extended the observation that S. aureus is a risk factor for relapse in WG [11]. Since the mechanism of S. aureus pathogenicity in WG is unknown, we hypothesized that staphylococcal SAg may be involved in relapses and found that tsst-1 was associated with the highest relapse risk.

SAg are potent immunomodulatory molecules that are thought to contribute to disease pathogenesis in autoimmune disorders by activating the immune system. This activation is initiated at the level of T cells, which can bind SAg via specific V-beta chains [12], and as a consequence, produce pro-inflammatory cytokines [19, 20] and enhance production of (auto)antibodies [21]. It is conceivable that in WG, similar mechanisms may lead to disease reactivation. However, in a previous study we were unable to document an association between the presence of S. aureus carrying SAg genes and expansion of responsive peripheral blood T cell subsets [22]. Moreover, the present study shows that the presence of SAg-positive S. aureus is associated with the same RR for relapse as SAg-negative strains, with a risk of approximately three compared with the absence of S. aureus. Therefore, we assessed the association between individual SAg genes and relapses of WG. The presence of tsst-1 was associated with a higher RR for relapses than that of other SAg or SAg-negative S. aureus. TSST-1 has been implicated in expansions of V-beta 2-positive T cells and active disease episodes in yet another form of vasculitis, Kawasaki disease [23]. In contrast to Kawasaki disease, we previously found no association between the presence of tsst-1-containing staphylococci and T cell expansions in patients with WG [22]. The mechanism by which this SAg may affect the course of the disease remains unknown, although it is conceivable that it may influence local lymphocyte activity and cytokine profiles. Importantly, tsst-1-positive S. aureus strains produce lower amounts of haemolysin [24], a virulence factor that confers S. aureus the capacity to damage endothelial cells. Since strains that produce less haemolysin could have a survival advantage within endothelial cells (so-called ‘small colony variants’) [25], we postulate that tsst-1-positive strains may be more difficult to eradicate and are therefore related to relapses.

A limitation of this study was the fact that, due to its retrospective character, and thus the absence of biopsies, SAg proteins could not be detected in vivo. Moreover, the presence of SAg proteins are difficult to assess in vivo, since they can be cleared by the humoral defence or scavenged by binding to specific cells. For example, it is known that SAg can be bound by the MHC class II molecule of antigen presenting cells and by specific V-beta chains of the T cell receptor. However, previous in vitro data demonstrated that staphylococcal SAg genes are expressed in approximately 95% of the cases [18] indicating that gene expression may also occur in vivo.

In the past, several risk factors for relapse have been identified in WG, among which S. aureus carriage. The presence of tsst-1 in staphylococcal strains may be added to this list and monitoring of patients for this parameter may be useful for the prediction of disease relapses. Treatment aiming at the eradication of TSST-1-positive S. aureus in WG may have therapeutic advantage.

Rheumatology key messages

- S. aureus is a risk factor for relapse in WG.
- The risk for relapse of WG increases with the presence of tsst-1-positive S. aureus.

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