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Published in: Scientific Reports

DOI: 10.1038/srep08188

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Document Version
Publisher's PDF, also known as Version of record

Publication date: 2015

Link to publication in University of Groningen/UMCG research database

*Citation for published version (APA):*
https://doi.org/10.1038/srep08188

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Low anti-staphylococcal IgG responses in granulomatosis with polyangiitis patients despite long-term *Staphylococcus aureus* exposure

Corinna Glasner1 *, Mirjan M. van Timmeren2 *, Tim Stobernack1, Till F. Omansen1, Erwin C. Raangs1, John W. Rossen1, Marcus C. de Goiffou1, Jan P. Arends1, Greetje A. Kampinga1, Denny G. A. M. Koedijk1, Jolanda Neef1, Girbe Buist1, Mehri Tavakol3, Willem J. B. van Wamel3, Abraham Rutgers4, Coen A. Stegeman5, Cees G. M. Kallenberg4, Peter Heeringa6 & Jan Maarten van Dijl1 *

1Department of Medical Microbiology, University of Groningen, University Medical Center Groningen, Hanzeplein 1, P.O. Box 30001, 9700 RB Groningen, The Netherlands, 2Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen, Hanzeplein 1, P.O. Box 30001, 9700 RB Groningen, The Netherlands, 3Department of Medical Microbiology and Infectious Diseases, Erasmus MC, ’s Gravendijkwal 230, 3015 CE Rotterdam, The Netherlands, 4Department of Rheumatology and Clinical Immunology, University of Groningen, University Medical Center Groningen, Hanzeplein 1, P.O. Box 30001, 9700 RB Groningen, The Netherlands, 5Department of Internal Medicine, Division of Nephrology, University of Groningen, University Medical Center Groningen, Hanzeplein 1, P.O. Box 30001, 9700 RB Groningen, The Netherlands.

Chronic nasal carriage of the bacterium *Staphylococcus aureus* in patients with the autoimmune disease granulomatosis with polyangiitis (GPA) is a risk factor for disease relapse. To date, it was neither known whether GPA patients show similar humoral immune responses to *S. aureus* as healthy carriers, nor whether specific *S. aureus* types are associated with GPA. Therefore, this study was aimed at assessing humoral immune responses of GPA patients against *S. aureus* antigens in relation to the genetic diversity of their nasal *S. aureus* isolates. A retrospective cohort study was conducted, including 85 GPA patients and 18 healthy controls (HC). Humoral immune responses against *S. aureus* were investigated by determining serum IgG levels against 59 *S. aureus* antigens. Unexpectedly, patient sera contained lower anti-staphylococcal IgG levels than sera from HC, regardless of the patients’ treatment, while total IgG levels were similar or higher. Furthermore, 210 *S. aureus* isolates obtained from GPA patients were characterized by different typing approaches. This showed that the *S. aureus* population of GPA patients is highly diverse and mirrors the general *S. aureus* population. Our combined findings imply that GPA patients are less capable of mounting a potentially protective antibody response to *S. aureus* than healthy individuals.

Granulomatosis with polyangiitis (GPA) is a systemic autoimmune disease characterized by small-vessel vasculitis and chronic necrotizing granulomatous inflammation with a predilection for the upper and lower respiratory tract and kidneys1. GPA is further characterized by the presence of anti-neutrophil cytoplasmic antibodies (ANCA) against proteinase 3 (PR3). Although the etiopathogenesis of GPA has been studied extensively and various genetic and environmental factors are known to contribute to inflammation, the primary cause of this disease is still debated2–5. However, upper airway infections have been repeatedly linked to GPA2,3,6–9.

Approximately 60–70% of GPA patients are chronic nasal carriers of the opportunistic pathogen *Staphylococcus aureus*, and nasal *S. aureus* carriage is associated with an increased risk of relapse6,10. Consistent with these findings, anti-bacterial treatment with co-trimoxazole reduces the risk of relapse11,12. To date, the precise mechanism by which *S. aureus* could exert a pathophysiological role in GPA has remained enigmatic. In view of the persistent activation of circulating T cells, staphylococcal superantigens (SAGs) were invoked as chronic stimuli of aberrant immune responses13. Indeed, it was shown that GPA patients carrying *S. aureus* positive for the superantigen toxic shock syndrome toxin-1 (TSST-1) have an increased risk for relapse, although earlier studies had not revealed a correlation between the presence of SAG genes and the expansion of specific T cell subsets in peripheral blood14,15.
S. aureus carriage, occurring in 20–30% of the general human population, is usually asymptomatic. However this bacterium can cause serious infections. Epidemiological studies have shown that certain clonal lineages of S. aureus attain a geo-spatial predominance, but clear associations of specific S. aureus types with specific diseases have not been reported. Nevertheless, it is known that virulence factors, like TSST-1 and exfoliative toxins, cause particular disease phenotypes, such as toxic shock syndrome and staphylococcal scalded skin syndrome, respectively.

Information on anti-staphylococcal immune responses in GPA patients and in-depth genetic analyses of their S. aureus isolates have so far been lacking. Hence, it was unknown to which extent particular S. aureus antigens or types may contribute to GPA. To address these questions, we performed a retrospective study in 85 GPA patients. We first investigated the humoral immune response against S. aureus by determining serum antibody levels against a comprehensive set of S. aureus antigens. Subsequently, the S. aureus isolates were genetically characterized to investigate whether specific S. aureus types colonize GPA patients.

Results

Low levels of anti-staphylococcal antibodies in GPA patients. Serum IgG levels against 59 S. aureus antigens were measured in 35 GPA patients (21 carriers, 14 non-carriers) and 18 healthy control (HC) individuals (10 carriers, 8 non-carriers) by bead-based Luminex flow cytometry. The overall antibody responses showed broad variability in both groups (Figure 1A). The highest median antibody titers were observed against several secreted proteins. In GPA patients, the IgG responses per antigen appeared overall lower than in HC, and this reached statistical significance for several surface proteins (ClfA, ClfB, FnbpA, and SdrE) and secreted proteins (Atl-2, CHIPS, Efb, Lipase, Nuc, Scin, Sen, Seo, SSL3 and TSST-1). For HC, multiple sera from different time points were measured, but serum IgG levels against S. aureus proteins did not
Table 1 | Clinical data of GPA patients and HC whose serum samples were included in the multiplex S. aureus antibody assay

<table>
<thead>
<tr>
<th>Group</th>
<th>S. aureus carriership</th>
<th>No. of subjects</th>
<th>No. of sera</th>
<th>No. of male/female</th>
<th>Age (years) mean ± SD</th>
<th>No. receiving vasculitis treatment/antibiotics*</th>
<th>BVAS Median (range)</th>
<th>Total IgG (g/L) mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPA Carrier</td>
<td>24</td>
<td>11/10</td>
<td>51.6 ± 16.3</td>
<td>0/2</td>
<td>14 (4–32)</td>
<td>15.8 ± 5.9*</td>
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<td></td>
</tr>
<tr>
<td>Diagnosis</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td>13/16</td>
<td>0 (0–2)</td>
<td>11.0 ± 2.4</td>
<td></td>
</tr>
<tr>
<td>Remission</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td>9/14</td>
<td>6 (3–16)</td>
<td>10.5 ± 2.9</td>
<td></td>
</tr>
<tr>
<td>GPA Non-carrier</td>
<td>14</td>
<td>9/5</td>
<td>54.3 ± 17.4</td>
<td>4/4</td>
<td>21 (8–28)</td>
<td>10.7 ± 2.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnosis</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td>9/8</td>
<td>0 (0–17)</td>
<td>8.5 ± 2.1</td>
<td></td>
</tr>
<tr>
<td>Remission</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td>2/2</td>
<td>14.5 (11–19)</td>
<td>10.1 ± 1.0</td>
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<tr>
<td>Relapse</td>
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<td></td>
<td></td>
<td></td>
<td>4/4</td>
<td>21 (8–28)</td>
<td>10.7 ± 2.1</td>
<td></td>
</tr>
<tr>
<td>HC Carrier</td>
<td>10</td>
<td>4/6</td>
<td>33.6 ± 11.8*</td>
<td>None</td>
<td>n.a.</td>
<td>11.3 ± 1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC Non-carrier</td>
<td>8</td>
<td>1/7</td>
<td>40.1 ± 12.2</td>
<td>None</td>
<td>n.a.</td>
<td>11.6 ± 1.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Vasculitis treatment consisted of azathioprine, (methyl) prednisolone, methotrexate, cyclophosphamide or mycophenolate mofetil.

# Change in time (data not shown). For GPA patients, two to three sera were included from the time of diagnosis, remission and/or relapse, but no differences were observed between the different disease states (data not shown). Despite the broad inter-individual variability, some clear differences were observed between S. aureus carriers and non-carriers in both patients and HC. As expected, overall higher responses were found in S. aureus carriers than non-carriers (Figure 1B). Amongst the S. aureus carriers, serum IgG levels against the surface proteins CIFA, CIB and SdrE, and the secreted proteins EfB, Nuc, Pro-Atl, SEN, SEQ, SSL3 and TSST-1, were lower in patients than in HC (Figure 1C) irrespective of the immunosuppressive and/or corticosteroid treatment of the patients (data not shown). Furthermore, we also measured total IgG in a subset of sera from patients and HC. This showed that the patient sera contained equal or even higher total IgG levels than HC (Table 1). Altogether, these findings show that GPA patients have lower levels of IgGs against many staphylococcal antigens than HC, irrespective of the patients’ treatment.

Detection of antigen-encoding genes in S. aureus isolates from GPA patients. To determine whether there is a direct connection between IgG responses to particular S. aureus antigens and the bacterial production of these antigens, we assessed the presence of the corresponding genes in bacterial production of these antigens, we assessed the presence of the corresponding genes in S. aureus isolates from 21 of the investigated patients (75 isolates) and 10 HC (18 isolates) (Supplementary Table 5) by DNA microarray-based genotyping. The genes for the surface proteins CIFA, CIB, FnbpA and the secreted nuclease were present in all isolates from patients and HC. Interestingly, the genes for the superantigens TSST-1, SEN and SEO were less frequently detected in patient than HC isolates (5% vs 44%, 24% vs 72%, and 24% vs 72%, respectively) corresponding to the lower IgG levels against these antigens in patients, while the gene for the superantigen SEB was only detected in patient isolates (33%) corresponding to the higher IgG levels against SEB in patients. Otherwise, the S. aureus isolates from patients and HC had, overall, a comparable gene repertoire. Therefore, the decreased IgG responses against particular proteins (e.g. CIFA, CIB, FnbpA) in GPA patients cannot be attributed to a lower abundance of the corresponding genes in their S. aureus isolates.

The S. aureus population in GPA patients mirrors the general S. aureus population structure. The genetic diversity of the colonizing S. aureus isolates was determined using two complementary typing methods, namely spa-typing and multiple-locus variable number tandem repeat fingerprinting (MLVF)23. For this purpose, the S. aureus collection was extended to 210 isolates from 71 GPA patients. The single-locus spa-typing approach yielded 55 different spa-types, ranging in length between 3 (t026) and 14 (t328) repeats. Additionally, five novel spa-types were identified, and two isolates (Vas103 and Vas106) were not spa-typable. Thirty-one spa-types were represented by ≥2 isolates (184 isolates in total), while 24 spa-types were represented by single isolates. The most frequent spa-types were t064 (n = 46, 21 patients), t084 (n = 26, 16 patients), t091 (n = 19, 8 patients), t012 (n = 10, 7 patients) and t021 (n = 10, 7 patients), covering 52.4% of the investigated patient isolates. Intriguingly, the prevalence of four predominant spa-types showed a shift over time; t084 and t012 were solely found between 1990–2003, while t064 and t091 were predominantly found since 2000 (Figure 2A). Of the 58 patients who provided ≥1 S. aureus isolate, 39 carried isolates with different spa-types over time, whereas isolates from the 19 other patients showed the same spa-type over time. Analysis of the S. aureus population structure in GPA patients with the BURP algorithm revealed the respective spa clonal complexes (spa-CCs; Figure 2B)24. The 18 HC isolates yielded 12 different spa-types that partly overlapped with the spa-types of patient isolates (Supplementary Table 3).

Typing of the 210 patients’ isolates by MLVF identified 95 different MLVF banding patterns. Fifty-one patterns were represented by one isolate, whereas 44 patterns were represented by ≥2 isolates. Notably, two MLVF patterns were represented by 18 and 27 isolates, respectively. The highest concordance (Adjusted Rand’s Coefficient 0.671) between MLVF and spa-typing was found with a 66% similarity cut-off value, resulting in 30 clusters (Figure 3). Six clusters contained single isolates whereas 24 clusters contained ≥2 isolates. Four clusters contained ≥12 isolates (61 isolates [C17, 32 [C26], 18 [C16] and 12 [C3]) and were derived from 27, 21, 7, and 8 patients respectively. Of the 58 patients who provided ≥1 isolate, 33 carried S. aureus belonging to different MLVF clusters over time, whereas the remaining 25 patients carried S. aureus belonging to the same MLVF cluster. The isolates from 18 of the latter 25 patients also had the same spa-type. Altogether, the combined typing data suggest that the S. aureus population structure in GPA patients is highly diverse, and that it has changed over time.
Figure 2 | Spa-types of the 210 S. aureus isolates from GPA patients presented as (A) the five most frequent identified spa-types displayed by year and number and (B) spa clonal complexes. (A) The frequencies of the 5 predominant spa-types, i.e. t012 (dark blue), t084 (light blue), t064 (red), t091 (orange), t021 (white), and all other spa-types (black) found amongst the 210 S. aureus isolates from GPA patients are shown throughout the whole collection period (1990–2012). (B) The clustering of the 210 S. aureus isolates from GPA patients into clonal lineages was performed by BURP analysis. spa clonal complexes (spa-CCs) were composed of ≥2 related spa-types. A spa-type not clustered into any spa-CC was regarded as non-clonal (singleton). spa-types defined as founders of particular clusters are indicated in blue. The circle size is proportional to the number of isolates. The intensity of connecting lines indicates the evolutionary relationship. One hundred and forty nine isolates (71% of all isolates) were clustered in 5 spa-CCs (CC064, CC084, CC012, CC330/180 and CC062) and 4 groups without founder. Fifty isolates (24% of all isolates) comprising 17 spa-types (30% of all spa-types) were identified as singletons. Nine isolates (4% of all isolates) comprising two spa-types (t026 and t842, 4% of all spa-types) were excluded and two isolates (Vas103 and Vas106, 1% of all isolates) were not spa-typable.
Figure 3 | MLVF dendrogram of the 210 S. aureus isolates from GPA patients. An MLVF dendrogram of the 210 S. aureus isolates from GPA patients was generated by the UPGMA algorithm. Isolate clusters were delineated with a 66% similarity cut-off value, since this showed the highest concordance between MLVF and spa-typing (Adjusted Rand’s Coefficient 0.671). Additionally to the 210 studied S. aureus isolates from GPA patients, also 22 control samples of the control isolate M2 were included in this delineation. The names of clusters are indicated at the right side of the dendrogram.
Relationship between the shift in spa-CCs over time and antibiotic resistance profiles. The resistances to 18 different antibiotics and the antibiotic resistance genotypes of all 210 S. aureus isolates from GPA patients are shown in Table 3. While these isolates were susceptible to most antibiotics, resistance to penicillin, co-trimoxazole and ciprofloxacin was observed for, respectively, 72.7%, 41.4%, and 26.7% of the isolates. Notably, the spa-CC064 and t091 isolates collected after 2000 showed increased resistance to co-trimoxazole and ciprofloxacin compared to spa-CCs/SPA-types isolated before 2000. This increased resistance seems to coincide with the increased treatment of patients with co-trimoxazole (Figure 4). Accordingly, co-trimoxazole-resistant isolates were only obtained from patients treated with this antibiotic, and co-trimoxazole-resistance was not observed for HC isolates (Table 3).

**Discussion**

Although S. aureus carriage has been linked to relapses in GPA for many years, it had yet to be determined to which extent different S. aureus antigens or types could contribute to GPA. The present study was therefore undertaken to investigate the humoral immune responses of GPA patients against S. aureus antigens in relation to...
the genetic diversity of their S. aureus isolates. For this purpose, we studied S. aureus antigen-specific serum IgG levels in a large cohort of GPA patients, who were monitored for over 20 years at our hospital, in combination with extensive genotyping of their S. aureus isolates.

Bead-based Luminex flow cytometry of 59 S. aureus antigens revealed that GPA patients had circulating antibodies against many staphylococcal antigens and that antibody levels in individual patients were constant over time, irrespective of their disease state. Patients carrying S. aureus had overall higher anti-staphylococcal IgG levels than patients not carrying S. aureus, confirming previous observations. The exact role of anti-staphylococcal antibodies is still debated. On the one hand, they could reflect the properties of the colonizing S. aureus type and/or infection episodes while, on the other hand, they could protect against colonization and/or infection. Persistent carriers of S. aureus have an increased risk of developing staphylococcal infections, which are in 80% of the cases caused by the endogenous strain. In spite of this, persistent carriers have a lower risk of death by bacteremia compared to non-carriers. This reduced risk could be the consequence of increased levels of protective antibodies against S. aureus that may accumulate due to long-term exposure to the colonizing strain. Unexpectedly, all GPA patients, irrespective of treatment with corticosteroids and/or immunosuppressives, had overall lower levels of anti-staphylococcal IgG than HC, while their total IgG levels were comparable. Moreover, we have previously shown that antibody responses following influenza vaccination in GPA patients and HC are similar, suggesting that the S. aureus-specific IgG response of GPA patients is aberrant. The exact causes for the lower anti-staphylococcal IgG levels in GPA patients are yet unknown. Most likely, this relates to the S. aureus-specific immune response in GPA patients, since all patient isolates contained the genes for important host colonization factors, like ClfA, ClfB and FnbpA, against which their hosts showed lower IgG levels than HCs.

To explore the diversity of S. aureus carried by GPA patients, a large number of isolates sampled between 1990 and 2012 was characterized by spa-typing and MLVF. This revealed 55 different spa-types, with 5 predominant spa-types covering more than 50% of the isolates. The subsequent MLVF analysis revealed a considerable diversity with 95 different banding patterns. A comparison of the spa-types from the present collection with the Ridom Spa Server (October 2014) comprising 13881 different spa-types submitted by 107 countries with isolation dates from 2003 onwards, revealed that four of the predominant spa-types of our patient isolates are among the 20 most common spa-types. Additionally, a recent study on the diversity of 206 methicillin-resistant S. aureus isolates collected at our hospital between 2006 and 2012 revealed a similar diversity (107 MLVF banding patterns, 66 spa-types) as observed for our GPA isolates. Taken together, these observations imply that the present GPA S. aureus collection mirrors the general S. aureus population structure after 2003.

While the Ridom Spa Server includes S. aureus typing results for different patient populations, other studies focused on particular diseases associated with increased S. aureus carriage rates, such as epidermolysis bullosa (EB) and cystic fibrosis (CF). A recent study from our hospital revealed that S. aureus isolates from EB patients were highly diverse and that these patients carried different types that fluctuated over time. Surprisingly, the spa-types identified in the EB patient population did not overlap with the spa-types from the present GPA collection. A German multicenter study investigating the genetic diversity of S. aureus isolates from 195 CF patients identified 269 different spa-types among ~4000 isolates collected between 2008 and 2011 (ECCMID 2014 Abstract No. ep166). Two of the four most prevalent spa-types, 0884 and 091, overlapped with the dominant spa-types in the present collection, underscoring the dominancy of these spa-types during the past decade.

In the present study, we have for the first time correlated spa-CCs/types with antibiotic resistance profiles. Although the overall antibiotic resistance of the patient isolates was very low, the abundance of co-trimoxazole-resistant isolates was higher amongst the more recent isolates, coinciding with an increase in co-trimoxazole treatment. This suggests that prolonged co-trimoxazole treatment either induced or selected for co-trimoxazole resistance. Interestingly,

Note: The table below shows the antibiotic resistance profiles of S. aureus isolates from GPA patients and HC in relation to the dominant spa-CCs/types.

<table>
<thead>
<tr>
<th>spaCC/type</th>
<th>All</th>
<th>spaCC084</th>
<th>spaCC128</th>
<th>spaCC062</th>
<th>spaCC064</th>
<th>f091</th>
<th>Others</th>
<th>HC</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of isolates (%)</td>
<td>210+ (100)</td>
<td>37 (17.6)</td>
<td>28 (13.3)</td>
<td>13 (6.2)</td>
<td>53 (25.2)</td>
<td>19 (9.0)</td>
<td>60 (28.6)</td>
<td>18 (100)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Chlomphenicol</td>
<td>1.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6c</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>26.7</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>94</td>
<td>11</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Clindamycin (const.)</td>
<td>6.1</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>9e</td>
<td>11e</td>
<td>7e</td>
<td>10</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>41.4</td>
<td>30</td>
<td>11d</td>
<td>23d</td>
<td>94</td>
<td>84d</td>
<td>7d</td>
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<tr>
<td>Erythromycin</td>
<td>10.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>42e &amp; mp</td>
<td>13e</td>
<td>16e &amp; mp</td>
<td>7e</td>
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<tr>
<td>Fosfomycin</td>
<td>0.5</td>
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<td>0</td>
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<td>Fusidic acid</td>
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<td>0</td>
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<td>0</td>
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<td>85kn</td>
<td>86kn</td>
<td>42kn</td>
<td>96kn</td>
<td>21kn</td>
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</tbody>
</table>

*For 197 S. aureus isolates the antibiotic resistance profile to 18 different antibiotics was determined using the VITEK2 system, and for one isolate using the standard disk diffusion assay. Twelve S. aureus isolates grew neither in the VITEK2 system nor in the standard disk diffusion assay, these isolates were thymidine-dependent, resulting in co-trimoxazole resistance. Numbers are percentages. The presence of antibiotic resistance genes in all 210 S. aureus isolates was determined by the DNA microarray system (Alere Technologies GmbH, Jena, Germany) and is indicated with letters. Abbreviations of the resistance genes: β-lactamase, a = aadA, b = dfrA, i = emmC (& emmD), m = msrA, mmp = mpbBM, k = cat, l = tetK, f = G66GDS0, m = mpur. Capital letters indicate that in nearly all isolates (95%) the observed resistance phenotype is explained by the presence of the resistance gene. Small letters indicate that only in a fraction (5–50%) of the isolates the resistance phenotype is explained by the resistance gene. ND = not determined.

Table 3 | Antibiotic resistance profiles of the S. aureus isolates from GPA patients and HC in relation to the dominant spa-CCs/types
Ciprofloxacin resistance was almost solely associated with spa-CC064 and mupirocin resistance with spa-type 1091, two spa-CCs/types that were predominant in the later years of isolation. These associations between years of isolation, spa-types, antibiotics resistance and anti-biotic therapy are highly relevant not only in relation to GPA patient treatment and the prevention of emerging antibiotic resistance, but also for other S. aureus infections. In addition, the abundance of identified co-trimoxazole-resistant S. aureus isolates warrants further investigations on the efficacy of prolonged co-trimoxazole treatment in GPA patients.

Superantigens, like TSST-1, cause non-specific activation of T cells resulting in polyclonal T cell proliferation and massive cytokine release33. Previous studies have shown that GPA patients carrying tst-1-positive S. aureus isolates have an increased risk for disease relapses33. Although the present study revealed only 23/210 (10.1%) tst-1-positive S. aureus isolates, all GPA patients, both carriers and non-carriers, had high IgG levels against TSST-1. This suggests that all patients encountered tst-1-positive S. aureus strains during their life. Potential associations between S. aureus and other autoimmune diseases, namely rheumatoid arthritis (RA) and multiple sclerosis (MS), have previously been investigated. RA patients were shown to carry different S. aureus types compared to HC and had higher IgG levels against TSST-134. More recently, relapsing MS patients were shown to carry S. aureus isolates positive for the SAG gene sea more frequently than non-relapsing MS patients35. However, in the present study no apparent associations between clinical data of GPA patients and particular S. aureus types were found.

In conclusion, the present study investigated for the first time a large cohort of GPA patients and their S. aureus isolates over an extended time period. On the host side, we show that GPA patients have overall lower anti-staphylococcal IgG responses than HC. On the pathogen side, we show that GPA patients carry S. aureus types that are widely represented amongst the general S. aureus population. We therefore conclude that GPA is not associated with a particular S. aureus genotype, but rather with a lower ability of GPA patients to mount potentially protective antibody responses to S. aureus, despite their long-term exposure to this pathogen. Notably, the fact that we do not find a particular S. aureus type associated with GPA does not exclude a role for S. aureus carriage or the expression levels of particular S. aureus virulence factors in the GPA disease pathogenesis. We consider our findings important since they may lead to a full definition of the role of S. aureus in GPA. Accordingly, we believe that this lead will be relevant to the research community that investigates the role of bacterial pathogens in the onset and relapse of autoimmune diseases, and the clinicians who treat patients with such pathogen-related autoimmune diseases in general and GPA in particular.

Methods

GPA patients and HC. This retrospective study included 85 GPA patients (71 nasal S. aureus carriers and 14 non-carriers) and 18 HC (10 nasal S. aureus carriers and 8 non-carriers). All patients were PR3-ANCA positive, fulfilled the Chapel Hill Consensus Conference definitions for the diagnosis of GPA and regularly visited the University Medical Center Groningen (UMCG, The Netherlands)36. The patients were selected based on availability of stored S. aureus isolates and/or serum samples, but formed a representative cohort of all GPA patients from our hospital. From 21 S. aureus-carrying GPA patients and all 14 non-carriers serum samples from two to three different time points (diagnosis, remission, relapse) were included in the multiplex S. aureus antibody assay, as well as previously collected and described sera from the 18 HC37,38. From each HC at least two sera from different time points were included. Clinical data of the patients and HC, whose sera were used, are summarized in Table 1 and detailed in Supplementary Table 1. Clinical characteristics and information on the respective S. aureus isolates included in DNA typing from all S. aureus-carrying patients and HC are summarized in Table 2 and detailed in Supplementary Tables 2 and 3. This study was approved by the Medical Ethics Committee of the UMCG and conducted in accordance with the guidelines of the Declaration of Helsinki. Written informed consent was obtained from all patients.

Multiplex S. aureus antibody assay. The relative amounts of serum IgGs against 59 S. aureus antigens were determined by bead-based Luminex flow cytometry (xMAP®, Luminex Corporation, Austin, Texas, USA) as previously described (Supplementary Table 4)33,34.

Bacterial isolates. From 71 GPA patients, a total of 210 S. aureus nasal isolates (1–8 per patient, median 3) with isolation dates between 1990 and 2012 were included (Supplementary Table 3). Seventy-five of these isolates belonged to 21 patients whose anti-staphylococcal serum IgG levels were assayed. In addition, 18 S. aureus isolates (1–3 per HC, median 2) from the previously described HC with isolation dates between 2007 and 2012 were included as controls (Supplementary Table 3)37,38.

S. aureus DNA typing. spa-typing and MLVF were performed as previously described39,40. To determine the clonal relatedness of the S. aureus population, the based upon repeat patterns (BURP) algorithm was applied (Ridom StaphType software 2.2.1)40.

Antibiotic susceptibility testing. Antibiotic susceptibility was determined using the VITEK 2 system (bioMérieux, Marcy l’Etoile, France) with AST P633 cards, according to the manufacturer’s protocol. The VITEK 2 minimum inhibitory concentration (MIC) results were interpreted using the VITEK 2 Advanced Expert System following EUCAST guidelines (www.eucast.org).

DNA microarray-based genotyping. The presence of genes for staphylococcal virulence factors or antibiotic resistance in S. aureus isolates from patients and HC was determined with the Clondiag S. aureus Genotyping Kit 2.0 following the manufacturer’s instructions (Alere Technologies GmbH, Jena, Germany)41,42.

Statistical analyses. Statistical analyses were performed with GraphPad Prism (Version 6, La Jolla, California) or SPSS 20 (Chicago, USA). Differences between groups were tested for statistical significance using one-way ANOVA in case of a parametric variable and Mann-Whitney-U or the Kruskal-Wallis test in case of a non-parametric variable. A two-sided p value < 0.05 was considered to be statistically significant. Parametric variables are given as means ± SD. Non-parametric variables are given as median with range.


Acknowledgments
We thank the participants who took part in the study. Benita Jansen is acknowledged for her excellent work in performing the Alere ClonDiag microarray experiments. The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007–2013) under grant agreement no. 261382.

Author contributions
C.G., M.v.T., P.H. and J.v.D. initiated and designed the study. C.G. and M.v.T. directed the study, prepared tables and figures and wrote the manuscript. C.G., T.S. and T.O. performed Luminex analyses. A.R., C.S. and C.K. produced antigens for the Luminex analyses. A.R., C.S. and C.K. saw the patients and processed and stored of samples and laboratory management. D.K., J.N. and G.B. performed statistical analyses. E.R. and J.R. performed the DNA typing. J.A. and G.K. contributed to the processing and storage of samples and laboratory management. D.K., J.N. and G.B. produced antigens for the Luminex analyses. A.R., C.S. and C.K. saw the patients and collected samples and clinical data. M.T. and W.v.W. collected sera from healthy individuals and performed the Luminex analyses. P.H. and J.v.D. supervised the study and reviewed the manuscript. All authors have read and approved the final manuscript.

Additional information
Supplementary information accompanies this paper at http://www.nature.com/scientificreports

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Glasner, C. et al. Low anti-staphylococcal IgG responses in granulomatosis with polyangiitis patients despite long-term Staphylococcus aureus exposure. Sci. Rep. 5, 8188; DOI:10.1038/srep08188 (2015).

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