Staphylococcus aureus nasal carriage in rheumatoid arthritis: antibody response to toxic shock syndrome toxin-1

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Abstract

Objective—To determine the prevalence of Staphylococcus aureus nasal carriage and to compare antibody responses to two superantigens, staphylococcal toxic shock syndrome toxin-1 (TSST-1) and staphylococcal enterotoxin A (SEA), in rheumatoid arthritis patients and normal subjects.

Methods—88 rheumatoid arthritis patients and 110 control subjects were cultured for nasal carriage of S aureus; 62 isolates were bacteriophage typed. Twenty five patients and 11 spouses were tested for antibodies to TSST-1, SEA, and sonicate extracts of Bacteroides fragilis and Escherichia coli; 19 patients were HLA-DR typed.

Results—50% of patients and 33% of normal subjects were S aureus carriers. Bacteriophage typing of isolates suggested significant differences between strains isolated from the two groups. Patients showed higher IgG (P = 0.0025) and IgA (P = 0.0372) antibody levels to TSST-1 than normal spouses and these responses were not related to rheumatoid factor titres or HLA-DR type.

Conclusions—When compared to normals, rheumatoid arthritis patients more often carry S aureus in their nasal vestibule, carry a distinct subpopulation of S aureus strains, and have higher average antibody levels to TSST-1.

S aureus secretes up to 30 different protein antigens, including at least eight T cell superantigens: toxic shock syndrome toxin-1 (TSST-1) and staphylococcal enterotoxins A, B, C₁, C₂, C₃, D, and E. This makes S aureus one of the principal sources of currently identified T cell superantigens in the environment. T cell superantigens have a dual affinity for major histocompatibility complex (MHC) class II molecules and for a relatively invariant region of the variable portion of the β chain (Vβ) of the T cell antigen receptor to induce T cell activation in a Vβ restricted manner. Recent studies by Paliard et al. have shown such T cell antigen receptor restriction—compatible with an intense stimulation by a microbial superantigen—in patients with rheumatoid arthritis. In addition, the demonstration of increased susceptibility to the development of rheumatoid arthritis in patients with selected genetic variants of the β chain of the T cell receptor also makes exploration of immune responses to S aureus superantigens in rheumatoid arthritis of significant interest.

TSST-1 and staphylococcal enterotoxin A (SEA), which are commonly secreted S aureus T cell superantigens, can also act as conventional antigens and elicit antibodies which may protect individuals from their toxic effects. Approximately 91% of healthy subjects have significant antibody titres to TSST-1, and 46% have significant antibody titres to SEA, even though only 38% of S aureus strains can produce TSST-1 and about 23% are potential producers of SEA.

In addition to TSST-1, SEA, and the other T cell superantigens, S aureus also produces protein A, which is a known B cell superantigen. Protein A stimulates utilisation of the V₃₃ heavy chain gene and induces IgM RF production. Like TSST-1 and SEA, protein A can also act as a conventional antigen and has also been shown to induce an antibody response in 31% of rheumatoid arthritis patients.

Rheumatoid arthritis is an inflammatory arthritis of unknown etiology, and many different infectious agents have been suggested as being involved in the pathogenesis of the disease. S aureus is an attractive candidate because 33% of the normal population are nasal carriers of this ubiquitous organism and S aureus secretes both T cell and B cell superantigens. To evaluate S aureus as a possible causative agent in the pathogenesis of rheumatoid arthritis, we compared the antibody titres in rheumatoid arthritis patients and in normal spouses living in the same household to two commonly secreted S aureus superantigens (TSST-1 and SEA) and to crude extracts of two common fecal flora, Escherichia coli and Bacteroides fragilis. We show that rheumatoid arthritis patients have an exaggerated antibody response to TSST-1, but share with spouse controls a similar antibody response to SEA and to the antigens of two other micro-organisms to which they are uniformly exposed.

Methods

Patients and control subjects

All the rheumatoid arthritis patients included in this study fulfilled the American College of Rheumatology criteria for the diagnosis of rheumatoid arthritis and were seen as outpatients in the Arthritis Consultation Center at Presbyterian Hospital of Dallas. Cultures from high in the anterior nares were performed in 88
unselected rheumatoid arthritis patients (28 Caucasian men, 60 Caucasian women) with a mean age of 53.7 (SD 14.9) years (range 23 to 81) and a mean duration of disease of 8.8 (SD 7.4) years (range 0.3 to 35). Similar cultures were also taken from 110 unselected control subjects (62 Caucasian men, 48 Caucasian woman) with a mean age of 49.2 (SD 17.9) years (range 21 to 86). These 110 control subjects included non-laboratory hospital employees, friends of hospital workers, and household members of the rheumatoid arthritis patients. No clinical symptoms of local nasal infection were observed in any of the rheumatoid arthritis patients or control subjects.

Serum samples were obtained from 25 of the 88 rheumatoid arthritis patients at the time the nasal swabs were obtained. Many of the 25 patients (10 men, 15 woman) selected for further study had more severe, active rheumatoid arthritis than the total rheumatoid arthritis patient population as determined by physician global assessment. The mean age of the 25 patients was 53.3 (SD 12.5) years (range 24 to 79) and the mean duration of disease was 10.7 (SD 9.0) years (range 0.3 to 30). Nine (36%) of the patients were being treated with disease modifying antirheumatic drugs (DMARD) and 11 (44%) were treated with both DMARD and steroids. Serum samples were also obtained from 11 spouses (seven men, four woman) with a mean age of 61.9 (SD 8.8) years (range 45 to 74) of the 25 rheumatoid arthritis patients. Serum samples from spouses were chosen to control for environmental factors that could influence both exposure to and colonization of S. aureus in the matched rheumatoid arthritis patient. Serum samples from rheumatoid arthritis patients and spouse controls were tested for antibody responses to TSST-1, SEA, and sonicate extracts of E. coli and B. fragilis.

SERUM
Clotted blood samples were separated and the serum adjusted to 0.02% (wt/vol) sodium azide before storage at −20°C. A 1 ml aliquot of serum from each of 500 normal blood bank donors was pooled, treated with azide and stored at −20°C.

CULTURE METHODS AND MEDIA
To obtain the nasal culture, a moistened dual Rayon swab (Culturette, Marion Scientific Inc, Kansas City, MO, USA) was rotated high up in each anterior nostril, then streaked on sheep blood agar and mannitol salt agar plates (Difco, Detroit, MI, USA) which were incubated at 35°C for 48 hours. All Gram positive cocci which were positive for both catalase and coagulase production were identified as S. aureus. The two control organisms, B. fragilis and E. coli, were isolated from stool cultures of a rheumatoid arthritis patient.

BACTERIOPHAGE TYPING
Each of 62 isolates of S. aureus (37 isolates from rheumatoid arthritis patients and 25 from controls) was classified by bacteriophage typing using the procedure of Blair and Williams. A set of 19 bacteriophage types, representative of four bacteriophage groups, was obtained from the American Type Culture Collection (Rockville, MD, USA) and was used to generate a bacteriophage lysis profile for each S. aureus isolate. The following bacteriophage types were used to classify the isolates: group 1 (29, 52, 52A, and 80); group 2 (3A, 3C, and 55); group 3 (6, 42E, 47, 53, 54, 75, 77, 83A, 84, and 85); and group 4 (42D and 81). Viability and specificity of each bacteriophage was confirmed by demonstrating that the host strain (provided by the American Type Culture Collection) was lysed, while a non-host control strain of S. aureus was not lysed.

ANTIGENS
Extracts of B. fragilis and E. coli were obtained from cultures grown in thiorhodolate and brain/heart infusion broths (Difco, Detroit, MI, USA), respectively. B. fragilis was grown for 48 hours and E. coli was grown overnight, both at 35°C. Cultures were centrifuged at 5000 g and washed three times with 0.1 M phosphate-buffered saline (PBS). Each bacterial mass was sonicated for 35 minutes and then centrifuged at 5000 g for 20 minutes. The sediment was discarded, and the supernatant dialysed against 0.1 M PBS for 24 hours at 4°C, then filtered through a 0.2 μm membrane (Nalgene, Nalge Co, Rochester, NY, USA). The turbidity of each extract was read at 600 nm on a spectrophotometer (Gilford Instrument Laboratories, Oberlin, OH, USA) to allow use of comparable amounts of each bacterial sonicate. TSST-1 and SEA were purchased from Toxin Technology Inc, Sarasota, FL, USA.

ELISA
Ninety six well microtitre plates (Immulon 1, Dynatech Laboratories, Chantilly, VA, USA) were coated with 115 μl of 0.15 M PBS (pH 7.4) containing 5 μg ml⁻¹ of bovine serum albumin (BSA) (Sigma, St Louis, MO, USA), and 2 μg ml⁻¹ of either TSST-1 or SEA, and incubated at 4°C overnight. The next day the wells were aspirated and washed three times with 0.15 M PBS. The unbound sites were blocked with 1% (wt/vol) BSA in PBS (BSA-PBS) for 2 hours at room temperature. After washing three times, 100 μl of serum diluted 1:150 in BSA-PBS were added to each well, followed by incubation for 21 hours at room temperature. The wells were then washed for one hour with six changes and 100 μl of horseradish peroxidase-conjugated F(ab')₂ fragments of rabbit antihuman IgG-Fc or IgA-α-chain (Jackson ImmunoResearch, West Grove, PA, USA) diluted 1:10 000 in BSA-PBS were added to each well. The plates were left at room temperature for one hour and washed again six times as above. Finally, 100 μl of 3,3',5,5' tetramethyl benzidine (Microwell Peroxidase Substrate, Kirkegaard and Perry Laboratories, Gaithersburg, MD, USA) were added to each well and the difference in absorbance at 450 nm and 630 nm was read after one hour using a Dynatech MR 700 Microplate Reader (Dynatech Laboratories).
S aureus nasal carriage in rheumatoid arthritis

Figure 1 Percent S aureus isolates from rheumatoid arthritis patients and normal subjects lysed by bacteriophages in groups 1, 2, 3, and 4.

Figure 2 Comparison of antibody responses to TSST-1 between 25 rheumatoid arthritis patients and 11 spouses. Antibody levels are expressed as optical density units in the ELISA assay as described in Methods. (A) IgG values were determined using a serum dilution of 1:150 or were normalised to this dilution. (B) IgA values were determined using a serum dilution of 1:150. Rheumatoid arthritis patients had significantly higher IgG (P = 0.0025) and IgA (P = 0.0372) antibody responses than spouses. Means are indicated by horizontal lines. ◦, carrier; ○, non-carrier; △, not cultured.

HLA-DR Typing

Nineteen of the 25 rheumatoid arthritis patients studied for antibody responses were randomly selected and genetically typed for HLA-DRB1 alleles by the Tissue Transplantation Laboratory of the University of Texas Southwestern Medical Center at Dallas using allele-specific oligonucleotide probes to hybridise PCR amplified products.15

Statistical Methods

Data analysis was done by the Academic Computing Services at the University of Texas Southwestern Medical Center. Bacteriophage group lysis profiles were evaluated by a χ² test. Antibody titres were compared using the non-parametric Wilcoxon test for unpaired samples. In both comparisons a P value of < 0.05 was considered statistically significant. Data are presented as means (SD).

Results

Staphylococcal carriage and bacteriophage classification

When 88 unselected rheumatoid arthritis patients were cultured, 44 (50%) were found to harbour S aureus in the anterior nares. The majority of these 88 patients were early rheumatoid arthritis patients who had received limited treatment and would not be expected to be immunosuppressed. When 110 unselected normal control subjects were similarly cultured, only 36 (33%) were found to be S aureus carriers. Thirty seven isolates from rheumatoid arthritis patients and 25 isolates from normal subjects were randomly selected and tested for similarities or differences using bacteriophage typing.

The bacteriophage typing patterns of the 62 S aureus isolates from both groups indicated at least 49 different strains of S aureus with nine non-typable strains. Even among the matched rheumatoid arthritis patients and their spouses who were both carriers, only one of the five pairs shared the same S aureus strain, the isolates of which were lysed by bacteriophages 29, 53, 54, 75, and 83A. Three rheumatoid arthritis patients and no control subjects had an S aureus strain which was only lysed by bacteriophage 29, and two other rheumatoid arthritis patients had a strain which was lysed only by bacteriophages 3A, 3C, and 55. In all the other rheumatoid arthritis patients and non-rheumatoid arthritis control subjects S aureus strains were cultured which appeared to be different, based on this bacteriophage typing. The number of isolates lysed by at least one bacteriophage in a group was counted and percent lysis is shown in fig 1. When comparing the percentage of lysis in rheumatoid arthritis isolates with normal isolates, statistically significant differences were found with bacteriophage group 2 (P = 0.019) and group 4 (P = 0.011). In overview, the S aureus strains isolated from the anterior nares of rheumatoid arthritis patients were more likely to be lysed by bacteriophages in groups 2 and 4 than the S aureus isolates from the control subjects (fig 1).
IgG AND IgA ANTIBODIES TO TSST-1, SEA, AND TWO NON- S. AUREUS BACTERIAL SONICATES

Twenty five rheumatoid arthritis patients were further tested for their antibody response to TSST-1, SEA, and two control antigen preparations from filtered sonicates of B fragilis and E coli. Rheumatoid arthritis patients showed an average of 4.7 times more IgG antibody (P = 0.0025) and 5.6 times more IgA antibody (P = 0.0372) to TSST-1 than normal spouses. These results are presented in fig 2 and table 1. When the antibody response in 11 of these 25 rheumatoid arthritis patients was compared with their matched spouses, 9 of 11 patients had higher IgG and IgA antibody responses to TSST-1 than their spouses.

Unlike the antibody titres to TSST-1, there were no significant differences in the average IgG or IgA antibody titres to SEA (table 1) or to the sonicates of B fragilis and E coli between rheumatoid arthritis patients and normal spouses (table 2). Some of the rheumatoid arthritis patients did show very low responses to the B fragilis antigens when compared to spouse controls or pooled normal human sera, even though they had shown high responses to TSST-1. In both tables 1 and 2, the mean absorbance value of the pool of 500 normal sera was similar to the mean absorbance value of the spouse controls, showing that the average immune response in the sera of the spous controls was reflective of the immune response of the pool of 500 normal individuals.

The differences between the antibody responses to TSST-1 and SEA of rheumatoid arthritis S. aureus nasal carriers and non-carriers and of spouses who were carriers and non-carriers are presented in table 3. Rheumatoid arthritis patients who were nasal carriers showed an average of 2.2 times as much anti-TSST-1 (both IgG and IgA) as the rheumatoid arthritis patients who were not carriers at the time of testing, but the differences in antibody concentrations between carrier and non-carrier patients were not significant. When spouse carriers and non-carriers were compared, there was no significant differences in either IgG or IgA anti-TSST-1 levels. There were also no significant differences in the antibody responses to SEA among the groups (table 3).

Discussion

S. aureus may be cultured from asymptomatic normal subjects, not only from the nose (33%), but also from the vagina (4.6%),"14"13 perineum (22%),"16" and skin (12-20%)."5" The 33% nasal carriage rate of the 110 control subjects in this study corresponded well with previous studies."8""9""10" Since nasal S. aureus carriers may show persistent or transient positivity, the 33% carriage rate reflected the carrier status of the normal population at the time the nasal cultures were obtained. Transient positivity of nasal carriage and the possibility of carrying S. aureus undetected at other loci may help to explain why 91% of healthy subjects have significant antibody titres to TSST-1, even though only a third have detectable S. aureus in the anterior nares at any given time."10" A previous study of nasal carriage of S. aureus in non-rheumatoid arthritis patients"12" showed an enrichment for patients who were HLA-DR3. Another study"9" suggested that nasal carriage of S. aureus depends on the presence of a preferential binding site for the organism on the nasal epithelium, but that study did not show the site to be genetically determined. When 47 selected patients with toxic shock syndrome were studied they showed no identifiable HLA-A, B, C, or DR differences when compared to the known gene frequencies in a racially matched population."13"
It is not known why rheumatoid arthritis patients have an increased nasal carriage rate of *S. aureus*, but our work confirms an unpublished 1972 study in which 50% of 48 rheumatoid arthritis patients and 27% of 48 controls (matched for age, sex, and race) carried *S. aureus* in their upper nasal vestibule (A. McUsick, personal communication). In McUsick’s study, which was completed before immunotherapy was used in the treatment of rheumatoid arthritis, no obvious differences in disease severity or type of treatment were associated with the carriage of *S. aureus* in the rheumatoid arthritis patients. Therefore, this 1972 study suggests that the treatment of rheumatoid arthritis patients with immunosuppressive drugs does not increase colonization of the rheumatoid arthritis patients with *S. aureus*.

DNA fingerprinting or bacteriophage typing may be used to distinguish different strains of *S. aureus*. We used bacteriophage typing to examine the heterogeneity of *S. aureus* strains isolated in this study. Among the 62 *S. aureus* isolates (37 rheumatoid arthritis and 25 normal isolates) that were studied by this method, at least 49 different strains were identified and nine strains were non-typable. The variability and complexity of the lysis profiles and the number of unique strains that were identified suggest that a potentially large number of different *S. aureus* strains exist. One interesting finding of the bacteriophage typing was the prevalence of lysis by group 2 and group 4 bacteriophages among the *S. aureus* strains isolated from rheumatoid arthritis patients (fig 1). Another interesting finding was that approximately 50% of the isolates obtained from both the rheumatoid arthritis patients and the normal controls were lysed by group 1 bacteriophages. Since group 1 bacteriophages have been associated with a higher frequency of TSST-1 production, the data suggest that both rheumatoid arthritis and normal controls were exposed to strains secreting TSST-1.

Superantigens have been implicated in the clonal expansion of selected T lymphocytes in toxic shock syndrome, rheumatoid arthritis, and Kawasaki disease. Since *S. aureus* represents one of the main sources of currently known superantigen exposure for humans, we compared the serological response of rheumatoid arthritis patients and their spouses to two common *S. aureus* superantigens, TSST-1 and SEA, with the response to two non-*S. aureus* antigen mixtures from *B. fragilis* and *E. coli*. Figure 2 and table 1 show the substantial differences in the IgG and IgA antibody responses to TSST-1 between a group of the 25 rheumatoid arthritis patients and 11 of their spouses (controls for environmental factors that could influence both exposure to and colonization of *S. aureus* in the matched rheumatoid arthritis patient). Some subjects in each group were nasal *S. aureus* carriers and some were not carriers. The demonstration of an average of 7.7-fold greater IgG anti-TSST-1 level in rheumatoid arthritis patients who were *S. aureus* carriers compared to the level in the spouse controls who also carried *S. aureus* is impressive (table 3), and suggests at least four possible explanations: (1) a much more intense immune response to TSST-1 in rheumatoid arthritis than non-rheumatoid arthritis subjects; (2) a much greater average antigen exposure in rheumatoid arthritis due to increased carriage rate; (3) *S. aureus* strain differences in rheumatoid arthritis compared to non-rheumatoid arthritis carriers resulting in either a greater synthesis rate of TSST-1 or variations in the amount of TSST-1 released into the nasal mucosa of patients with micro-organism which shares antigen cross-reactivity with TSST-1. For example, Ramesh et al. identified a 20 amino acid peptide derived from TSST-1 which showed homology with the amino acid sequence 180-193 of the *Mycobacterium tuberculosis* heat shock protein 65 kDa (HSP-65 kDa). This shared segment of HSP-65 kDa was recognised by T cells isolated from rats with *M. tuberculosis* induced adjuvant arthritis and caused proliferation of peripheral blood mononuclear cells obtained from a majority of normal human donors. Increased immune responsiveness to the intact HSP-65 kDa protein has been shown in synovial fluid T cells and in the serum antibody titres of rheumatoid arthritis patients, but it is not known whether the specific amino acid sequence shared with TSST-1 elicits a humoral response.

Our study also showed that normal control subjects, whether *S. aureus* carriers or not, have similar levels of both IgG and IgA anti-TSST-1 antibodies (table 3). This latter result is surprising but consistent with earlier studies in toxic shock syndrome in which healthy persons who were vaginal *S. aureus* carriers were compared to other healthy controls not carrying *S. aureus*, and found to have similar levels of anti-TSST-1 antibody. The selective enhancement of the IgG and IgA antibody response to TSST-1 in rheumatoid arthritis patients was not matched by their response to SEA (table 1) or to the antigen mixtures from *B. fragilis* or *E. coli* (table 2). The observation that rheumatoid arthritis patients only had a raised immune response to one of the four antigens tested and the other three titres were in the normal range suggests that the raised response to TSST-1 was not confounded by other factors, such as concomitant treatment, immunosuppression, a polyclonal immunoglobulin response, or rheumatoid factor. In fact, the increased IgG and IgA titres to TSST-1 in rheumatoid arthritis patients did not correlate with the IgM rheumatoid factor titres to heat aggregated IgG, and antibody titres to TSST-1 were unchanged after IgM rheumatoid factor was removed from the serum by gel filtration (data not shown). A recent study that compared the serum IgM titres to staphylococcal enterotoxin B (SEB) in rheumatoid arthritis patients and normal controls also found no correlation between the increased titres of antibodies to SEB in rheumatoid arthritis patients and rheumatoid factor titres.
In conclusion, although it is not known why some rheumatoid arthritis patients have dramatically increased antibody titres to TSST-1, *S. aureus* remains an interesting organism to be considered as a causative agent in rheumatoid arthritis pathogenesis for several reasons. For example, TSST-1 has been shown to be arthritogenic in mice; in addition, *S. aureus* is ubiquitous and may be carried by humans at various sites either persistently or transiently; it makes up to 30 different protein antigens, including both T cell and B cell superantigens, and protein A can induce both IgM rheumatoid factor production and polyclonal B cell activation. The complexity of the bacteriophage lysis profiles suggests that a large number of *S. aureus* strains exists and rheumatoid patients appear to carry a distinct subpopulation of *S. aureus* strain in their nasal vestibule. If *S. aureus* was involved in the pathogenesis of rheumatoid arthritis, the potentially large number of unique strains, the possibility of variations in the synthetic profile of each strain, and the significant differences in the immune response patterns of individuals to specific antigens could explain why approximately one third of the population are *S. aureus* carriers but do not have rheumatoid arthritis. Additional studies are needed to determine why rheumatoid arthritis patients carry a unique population of *S. aureus* stains and have increased antibody titres to TSST-1.

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