Seropositivity for Antibodies to DRS-G, a Virulence Factor from *Streptococcus dysgalactiae* subsp. *equisimilis*, Is an Independent Risk Factor for Poststreptococcus Glomerulonephritis and Chronic Kidney Disease in Mumbai, India

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The disease spectrum caused by *Streptococcus dysgalactiae* subsp. *equisimilis* resembles that of *S. pyogenes* (group A streptococcus [GAS]). These two bacterial species are closely related and possess many common virulence characteristics. While some GAS strains express virulence factors called streptococcal inhibitor of complement (SIC) and distantly related to SIC (DRS), some *S. dysgalactiae* subsp. *equisimilis* isolates express an orthologue of DRS, which is referred to as DRS-G. We reported previously that seropositivity for either anti-SIC or anti-DRS antibodies (Abs) is associated with poststreptococcal glomerulonephritis (PSGN). However, only seropositivity for anti-SIC Abs is associated with chronic kidney disease (CKD). We now extend the study to test whether seropositivity for anti-DRS-G Abs is also associated with these renal diseases. Stored serum samples collected for our previous study were tested by an enzyme-linked immunosorbent assay (ELISA) for Abs to DRS-G. The samples represented sera from 100 CKD adult patients, 70 adult end-stage renal disease (ESRD) patients, 25 PSGN pediatric patients, and corresponding age-matched control subjects. The proportion of PSGN, CKD, and ESRD patients who showed seroreaction to anti-DRS-G Abs was significantly higher than that of the corresponding age-matched controls, who in general exhibited seropositivity rates commensurate with the isolation rate of *drsG*-positive *S. dysgalactiae* subsp. *equisimilis* in the community during this study period. Since higher rates of seropositivity for anti-DRS-G Abs in the renal disease categories are resultant of previous infections with DRS-G-positive *S. dysgalactiae* subsp. *equisimilis* strains, we conclude the seropositivity is an additional risk factor for these renal diseases. In this regard, anti-DRS-G Abs have attributes similar to those of the anti-SIC Abs.

*S. dysgalactiae* subsp. *equisimilis* was often not regarded as a human pathogen in the past, but it is now being recognized as a significant human pathogen, with an increasing frequency of reports of epidemiological observations (1–4). *S. pyogenes* (group A streptococcus [GAS]) and *S. dysgalactiae* subsp. *equisimilis* are associated with similar disease spectra, including the immune system-mediated postinfectious sequelae poststreptococcal glomerulonephritis (PSGN) (1, 5). Comparative genomic studies revealed that these two pathogens are genetically related, with many common virulence factors (6, 7).

Among the virulence factors produced by *S. dysgalactiae* subsp. *equisimilis* is a secretory protein called DRS-G. This protein has limited primary sequence homology with SIC (streptococcal inhibitor of complement) and DRS (distantly related to SIC) in GAS (6). As might be expected, DRS-G exhibits limited functional overlap with SIC and DRS. For instance, unlike SIC, DRS-G does not inhibit complement-mediated cell lysis. In this regard, DRS-G resembles DRS. However, like DRS and SIC, DRS-G inhibits the antimicrobial peptide LL37 (8).

Several studies (9–11) have shown that positive seroprevalence for anti-SIC or anti-DRS antibodies (Abs) is associated with PSGN. PSGN in turn is an established risk factor for chronic kidney disease (CKD) and end-stage renal disease (ESRD) (12). Our recent studies in the Mumbai, India, population (13) revealed that seroprevalence for anti-SIC Abs, unlike that for anti-DRS Abs, is positively associated with the presence of CKD and ESRD. Moreover, among the anti-SIC antibody-positive patients, the prognosis of CKD is poor.

Associations between DRS-G and PSGN, CKD, and ESRD have not been studied. Here we clearly demonstrate that anti-DRS-G antibody positivity is associated with PSGN, CKD, and ESRD in the population of Mumbai, a city where streptococcal infections and diseases are endemic. We conclude that both anti-DRS-G Abs and anti-SIC Abs are positively associated with chronic renal diseases and are independent risk factors.
PCR, and Southern blot analyses of genomic DNA of isolates from the same population in this study period. The rate of the

**RESULTS**

The rate of the *drsg* distribution in *S. dysgalactiae* subsp. *equisimilis* isolates recovered from the Mumbai population was similar to that of the *sic* or *drs* distribution in GAS isolates from the same population in this study period. Examination of draft genome sequences of *S. dysgalactiae* subsp. *equisimilis*, screening by PCR, and Southern blot analyses of genomic DNA of isolates from different regions (8, 14, 15) showed that the rates of *drsg* gene distribution could range between 12% and 17% of the rate seen with *S. dysgalactiae* subsp. *equisimilis* isolates of different *emm* types. In the current study, we tested for the presence of the *drsg* gene in 48 isolates recovered from the Mumbai region during 2 years of this study period (Table 1). These isolates belong to 24 *emm* types, with a recovery rate of 1 to 6 isolates per type. Of these, we found that only 4 isolates belonging to 2 *emm* types were positive for *drsg*.

We found earlier that less than 5% of GAS isolates recovered from the same community possessed the *sic* gene and that a similar proportion possessed the *drsg* gene (13). The overall seroprevalence of anti-SIC or anti-DRS Abs in healthy subjects in the Mumbai community is commensurate with this isolation rate. Since the recovery rate of *drsg*-positive *S. dysgalactiae* subsp. *equisimilis* isolates (~8%) is not significantly different from that of *sic* or *drsg*-positive GAS isolates in this population, we expect that a similar proportion of healthy subjects living in this community is likely to be seropositive for Abs for each of these three antigens. Indeed, only 3 to 4% of the control groups whose members had no evidence of renal diseases were seropositive for anti-DRS-G Abs (see below; Fig. 1).

**Anti-DRS-G Ab seroprevalence is significantly higher in renal disease patients than in the age-matched control subjects in Mumbai.** Panels A and C of Fig. 1 show the spread of raw data (OD values) for all the disease categories and the corresponding controls. Statistically significant differences between the OD values for PGN and the corresponding age-matched control and between CKD or ESRD cohorts and the corresponding age-matched control were found. In both the controls, the values for the third quartile are lower than the respective means + 2× SD, whereas 24 to 36% of serum samples from the kidney disease patients had OD values greater than this cutoff. For instance, among the 25 pediatric PGN patients, 24% were positive for...
anti-DRS-G Abs (Fig. 1B). In contrast, only 4% were positive among the age-matched controls ($P = 0.0380$). Likewise, 27% and 36% were positive for anti-DRS-G Abs among the CKD ($n = 100$) and ESRD ($n = 70$) cohorts, respectively (Fig. 1D), whereas the proportion for the corresponding control subjects is only 3% ($P = 0.0114$ and 0.0002 for CKD and ESRD, respectively).

In the chronic renal disease cohorts, there are subjects who are positive for anti-SIC and anti-DRS-G Abs or anti-DRS and anti-DRS-G Abs. We have carried out competitive ELISA to show that the double seropositivity was not due to cross-reactivity of anti-DRS-G Abs with the other antigens (Fig. 2), as only homologous competitors effectively competed with the antibody binding. Moreover, as shown in Table 2, 31%, 26%, and 7% of the combined CKD-plus-ESRD cohorts were positive for Abs to a single antigen, i.e., DRS-G, SIC, and DRS, respectively. Based on these values, the expected rate of double seropositivity for anti-SIC plus anti-DRS-G Abs is estimated to be 8% (31% × 26%) and that for anti-DRS plus anti-DRS-G Abs is estimated to be 2% (31% × 7%). These expected values are similar to the corresponding observed rates (6.5% and 0.6%, respectively) (Table 2). Together,

![FIG 1](http://cvi.asm.org/)

FIG 1 Seropositivity of anti-DRS-G Abs in PSGN, CKD, and ESRD patients. Recombinant DRS-G protein was coated for plating in ELISA. Panel A shows OD values for PSGN and control 1 ($n = 25$ each). Panel C shows OD values for CKD ($n = 100$), ESRD ($n = 70$), and control 2 ($n = 70$). The dot plots show medians (the longer cross lines), the first and the third quartiles (the two shorter lines), and calculated cutoff values (mean + 2x the standard deviation [SD]) (dotted lines). Samples with values equal to or above the cutoff values were taken as positive. Panels B and D show percent seropositive samples for anti-DRS-G Abs within each group and compared with respective controls. Lines with asterisks (**, $P = 0.0003$; ***, $P < 0.0003$) indicate statistically significant differences between the means (A and C) or between the proportions of seropositives (B and D).

![FIG 2](http://cvi.asm.org/)

FIG 2 Competitive ELISAs. Serum samples positive for antibodies to SIC only, DRS only, and DRS-G only were preincubated with homologous (A) or heterologous (B) antigens at 0 µg, 10 µg, and 50 µg prior to incubation. The plates were coated with the corresponding antigen for the respective seropositive sera.
these results suggest that rate of seroprevalence for anti-DRS-G Abs is independent of past exposure to SIC or DRS antigen and also is an independent risk factor for CKD/ESRD.

**DISCUSSION**

The overlap of the disease spectrum of GAS and *S. dysgalactiae* subsp. *equisimilis* and the high frequency of recovery of *S. dysgalactiae* subsp. *equisimilis* from the throats of children in some communities makes *S. dysgalactiae* subsp. *equisimilis* an important opportunistic pathogen. The two species share many common virulence factors, including M protein, streptolysin O, streptolysin S, streptokinase, C5a peptidase, DNase, fibronectin binding proteins, and NADase (6). M1 and M57 GAS express a major secretory protein called SIC; M12 and M55 GAS express the related DRS. SIC inhibits complement function, binds to various host proteins, and inhibits various antimicrobial peptides. Anti-SIC antibodies are associated with PSGN, CKD, and ESRD (9–11, 13, 16–23). Compared to SIC, DRS seems to possess restricted functions. Although it binds to several complement proteins, DRS does not inhibit their function (17). DRS, however, inhibits the antimicrobial activity of LL37. While seropositivity for anti-DRS Abs is associated with PSGN, no association was found with CKD or ESRD (13). In Table 3, functional attributes of SIC, DRS, and DRS-G and disease associations of their antibodies are summarized. DRS-G has at best only limited sequence similarity to either SIC or DRS. Despite this, like SIC and DRS, DRS-G inhibits LL37 function (8). But like DRS, DRS-G does not act as an inhibitor of complement function. We now show that, like anti-SIC Abs, anti-DRS-G Abs are associated with PSGN, CKD, and ESRD. Furthermore, our results show that these two antibodies are independent risk factors for CKD and ESRD.

Interestingly, the three antigens elicit distinct antibody responses in humans, as these antibodies do not cross-react with heterologous antigens, as shown by competition ELISA results. Nonetheless, these antibodies show partial overlapping spectra of associations with diseases, namely, pyoderma, PSGN, CKD, and ESRD (Table 3). Hence, we conclude that no single common epitope that elicits a major antibody response in humans is responsible for the common attributes of these antibodies in the renal disease association.

Because *S. dysgalactiae* subsp. *equisimilis* is recovered more often than GAS from throat swabs in schoolchildren in Mumbai (24), our current observation emphasizes the need to monitor for seropositivity for anti-DRS-G antibody in this population so that early interventions could be offered to prevent or delay progression to CKD and ESRD. As summarized in Table 2, 26% of CKD-ESRD patients are seropositive for anti-SIC Abs. By inclusion of a test for anti-DRS-G Abs, the proportion of these patients positive for either of the Abs significantly increased to 50% (*P* = 0.0079). These results clearly suggest that anti-DRS-G Abs are independent risk factors for the chronic renal diseases. We therefore propose that, in regions of streptococcal disease endemicity, regular monitoring of children and young adults for these anti-SIC and anti-DRS-G Abs may identify the at-risk individuals, to whom educational and medical intervention could be offered to delay or avoid the onset of late-stage CKD.

Of note, the mean ages of the members of the two control cohorts in this study (pediatric and adult control subjects) are different. Nevertheless, the results of this cross-sectional study show that the seroprevalence of anti-DRS-G Abs is independent of the mean age of the members of a given population. The results suggest that exposure to these antigens early in life may be a determining factor.

As PSGN is a consequence of streptococcal infection, in our recent study (9) we tested whether anti-SIC and anti-DRS Abs are responsible for a greater predilection for GAS pyoderma. This was found to be so, and we proposed that the increased infection rate is akin to that seen with antibody-dependent enhancement (ADE) of skin infection. We could not test this feature for anti-DRS-G Abs because we did not have an *S. dysgalactiae* subsp. *equisimilis* pyoderma cohort. Be that it may, we tested for anti-DRS-G Abs in the same set of sera from pyoderma and control subjects used in the earlier study mentioned above (9). Figure 3 shows that no “cross-species” ADE was attributable to the presence of anti-DRS-G Abs.

In summary, this report highlights the importance of monitoring for anti-DRS-G Abs among patients post-*S. dysgalactiae*

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**TABLE 2** Sera from chronic renal disease (CKD plus ESRD) patients seropositive for single and double antibodies (anti-SIC, anti-DRS, and anti-DRS-G Abs)

| Parameter | Anti-SIC Abs | Anti-DRS-G Abs | Anti-DRS Abs | Anti-SIC Abs + anti-DRS-G Abs | Anti-DRS Abs + anti-DRS-G Abs | Anti-SIC Abs or anti-DRS-G Abs |
|-----------|-------------|---------------|-------------|-----------------------------|-------------------------------|-------------------------------|
| No. of CKD + ESRD patients (n = 140) | 44 | 52 | 12 | 11 | 1 | 85 |
| % seropositive | 26 | 31 | 7 | 6.5 | 0.6 | 50 |
| No. of expected double positives | | | | | | |

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**TABLE 3** Comparison of functional attributes of SIC, DRS, and DRS-G and disease associations of their antibody positivity

| Activity/disease association | SIC or anti-SIC Abs | DRS or anti-DRS Abs | DRS-G or anti-DRS-G Abs |
|-----------------------------|---------------------|---------------------|------------------------|
| C6/C7 binding               | Yes                 | Yes                 | ND                     |
| SIC activity                | Yes                 | No                  | No                     |
| BD2/BD3 inhibition          | Yes                 | Yes                 | ND                     |
| SLPI binding                | Yes                 | Yes                 | ND                     |
| SLPI inhibition             | Yes                 | No                  | ND                     |
| Lysozyme inhibition         | Yes                 | No                  | ND                     |
| LL-37 binding               | Yes                 | Yes                 | Yes                    |
| LL-37 inhibition            | Yes                 | Yes                 | Yes                    |
| Association with PSGN       | Yes                 | Yes                 | Yes                    |
| Association with CKD/ESRD   | Yes                 | No                  | Yes                    |
| Association with GAS pyoderma | Yes               | Yes                 | No                     |

*The information in this table is a summary of results from this and several previous studies (9–11, 13, 16–23). C6/C7, complement proteins; BD, human beta defensin; SLPI, secretory leukocyte protease inhibitor; LL-37, cathelicidin (antimicrobial peptide); ND, not done.*
subsp. equisimilis infection and of following up those who give positive results to identify subjects at risk for CKD.

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