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Protection from Systemic *Candida albicans* Infection by Inactivation of the Sts Phosphatases

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The human fungal pathogen *Candida albicans* causes invasive candidiasis, characterized by fatal organ failure due to disseminated fungal growth and inflammatory damage. The suppressor of TCR signaling 1 (Sts-1) and Sts-2 are two homologous phosphatases that negatively regulate signaling pathways in a number of hematopoietic cell lineages, including T lymphocytes, mast cells, and platelets. Functional inactivation of both Sts enzymes leads to profound resistance to systemic infection by *C. albicans*, such that greater than 80% of mice lacking Sts-1 and -2 survive a dose of *C. albicans* (2.5×10^5 CFU/mouse) that is uniformly lethal to wild-type mice within 10 days. Restriction of fungal growth within the kidney occurs by 24 h postinfection in the mutant mice. This occurs without induction of a hyperinflammatory response, as evidenced by the decreased presence of leukocytes and inflammatory cytokines that normally accompany the antifungal immune response. Instead, the absence of the Sts phosphatases leads to the rapid induction of a unique immunological environment within the kidney, as indicated by the early induction of a proinflammatory cytokine (CXL10). Mice lacking either Sts enzyme individually display an intermediate lethality phenotype. These observations identify an opportunity to optimize host immune responses toward a deadly fungal pathogen.

Changes in medical care are creating increasing opportunities for lethal systemic infections by the human fungal pathogen *Candida albicans* (1, 2). In recent years, *C. albicans* has become the fourth leading cause of hospital-acquired bloodstream infections, with close to 50,000 cases being reported yearly in the United States alone, and global mortality rates have not decreased in the last 20 years, in spite of advances in antifungal therapy (2–4). One underlying reason is that *C. albicans* ordinarily inhabits the skin and mucosa as a benign commensal. New medical procedures, such as cancer chemotherapy or organ transplantation, that reduce host immunity allow *C. albicans* to escape normal physiological barriers and disseminate to deeper tissues. In addition, factors that permit the overgrowth of *C. albicans*, including the use of broad-spectrum antibacterial antibiotics or biofilm formation on catheters and indwelling medical devices, allow this fungus to infect immunocompetent individuals (5). Better antifungal therapies are needed to address this escalating problem (6).

During the course of infection, kidneys are highly favorable niches for both fungal proliferation and the morphogenetic switch of *C. albicans* to a hyphal state (7, 8). Recent analysis has drawn attention to the intensity of the immune response following systemic infection. On the one hand, the innate immune system that is critical for host protection is rapidly activated. In particular, fungal cell wall constituents are recognized by Toll-like receptors (TLRs) and C-type lectin receptors (CLRs) on the surface of phagocytes within the kidney, promoting the release of cytokines and chemokines that lead to the infiltration of additional waves of monocytes and neutrophils into the kidney (9, 10). On the other hand, it has become appreciated that host inflammatory responses targeting *C. albicans* lead to detrimental and often fatal collateral tissue damage (11). Indeed, in a well-characterized mouse model of systemic candidiasis that mimics the clinical progression of disseminated candidiasis in humans, progressive sepsis accompanied by renal failure has been identified as the cause of death (12). Thus, destructive inflammatory responses leading to pathological tissue destruction is a significant clinical problem.

Current antifungal medications suffer from a number of drawbacks, including high cost, toxicity, and a narrow spectrum of activity (13). These limitations are compounded by difficulties in making a rapid and accurate disease diagnosis (14). The emergence of strains resistant both to fluconazole and to newer members of the echinocandin class of compounds is now considered a major threat by the CDC (15). Because no effective *C. albicans* vaccine is currently available, developing strategies for enhancing host immune responses that synergize with current medications holds great promise (16). Indeed, attempts to develop combination therapies, such as the coadministration of antifungal monoclonal antibodies or adjuvant immunotherapy with recombinant cytokines to boost a patient's immune response, are ongoing (17–19). The development of combination therapies that are most effective will require a thorough understanding of the host antifungal immune response.

To gain new insight into the mechanisms that control the host response to fungal pathogens, we examined the role of the suppressor of TCR signaling 1 (Sts-1) and Sts-2, two homologous proteins that negatively regulate signaling pathways in a number of hematopoietic cell lineages, including T lymphocytes, mast

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cells, and platelets (20–23). These evolutionarily conserved proteins share 50% amino acid identity and appear to carry out overlapping functions. However, their expression patterns differ markedly, in that *Sts-1* has been found to have a widespread pattern of expression in numerous tissues, while *Sts-2* has a more limited pattern of expression, mainly in cells of hematopoietic origin. Critical to Sts function is a distinctive type of phosphatase domain in which the nucleophilic activity is mediated by a conserved histidine (24, 25). Other conserved features include a ubiquitin association (UBA) domain that can bind mono- and polyubiquitin and an SH3 domain that interacts with proline-rich segments of other polypeptides. Current models of Sts function suggest that these protein interaction domains play key roles in either localizing Sts enzymatic functions to specific intracellular regions or targeting the phosphatase domain to specific intracellular substrates (23). Of the two, Sts-1 is the more active enzyme *in vitro*, although the bona fide *in vivo* substrate(s) of both enzymes has not been established definitively (24, 25). The important role of Sts-1 and Sts-2 in controlling cellular responses is underscored by the hypersensitivity to TCR stimulation of T cells that lack both proteins. However, *Sts-1/2*$^{-/-}$ mice do not display an overt autoimmune phenotype, suggesting that other mechanisms of lymphocyte regulation can compensate for the absence of Sts function (20). We report here that functional inactivation of Sts activity has a profound effect on the ability to resist invasive candidiasis and ameliorate the destructive immunopathophysiology that accompanies the disease.

MATERIALS AND METHODS

Mice. The generation of mice containing the Sts mutations, backcrossed 10 generations onto the C57/B6 background, has been described previously (26). Mice were housed and bred in the Stony Brook University Animal Facility under specific-pathogen-free conditions. Mice used for experiments were 8 weeks old. All mice were maintained in accordance with Stony Brook University Division of Laboratory Animal Resources (DLAR) guidelines. All animal experiments were approved by the Stony Brook University Institutional Animal Care and Use Committee (IACUC).

Infection assays. Mouse infections were carried out as described previously (27). *C. albicans* wild-type (WT) strain SC5314 was grown overnight at 30°C in yeast extract-peptone-dextrose (YPD) medium with 80 μg/ml uridine, reinoculated into fresh medium, and incubated again overnight. Cells were harvested by centrifugation, washed twice in phosphate-buffered saline (PBS), counted, and diluted to the appropriate density with PBS. The cell counts were confirmed by plating dilutions onto solid YPD medium. Female mice were inoculated via the lateral tail vein and monitored for 28 days. If a mouse became moribund, humane euthanasia was performed. Graphing and statistical analysis of survival after infection were carried out using a log-rank test (Mantel-Haenszel test) with Prism (version 6) software (GraphPad Software, Inc., La Jolla, CA).

Analysis of numbers of CFU. Organs were excised from the mice, placed in 5 ml PBS, and homogenized with a tissue homogenizer (Pro Scientific, Inc., Oxford, CT). The number of CFU per gram of tissue was determined by plating serial dilutions of the homogenates onto YPD medium plates and incubating at 30°C. Time course analysis of the numbers of CFU was performed using groups of mice preassigned to specific time points.

Calcofluor white staining. Kidneys were homogenized in PBS, to which KOH was added to 20%. Following incubation at 37°C for 3 h, lysates were spun at 13,000 × *g* for 10 min. The pellet was washed 3 times with PBS, resuspended in a small volume of PBS, and stained with calcofluor white. Hyphae were visualized by fluorescence microscopy, and total hyphal clusters present in each homogenate were counted.

Histology. Tissues were fixed in neutral buffered formalin, paraffin embedded, and sectioned at 5 μm. Sections were counterstained with either hematoxylin and eosin (H&E) or periodic acid-Schiff (PAS), the former at the Stony Brook Histology Core and the latter at McClain Laboratories (Smithtown, NY).

Generation of single-cell organ suspensions. Kidney leukocyte suspensions were prepared as described previously (28). Briefly, kidneys were disassociated in PBS with 2% fetal calf serum and treated with 50 U/ml DNase (Roche) and 0.2 mg/ml Liberase TL enzyme (Roche) for 30 min at 37°C. Following erythrocyte lysis by addition of ACK buffer (150 mM NH_4Cl, 1 mM $KHCO_3$, 0.1 mM EDTA), the debris was removed by straining and centrifuged, and the resulting pellet was suspended in PBS with 2% fetal bovine serum and centrifuged at 1,000 × *g* at room temperature for 30 min over a 40% Ficoll cushion. The cell pellet was washed, filtered, and suspended in buffer for flow cytometric analysis.

Flow cytometry. Cell suspensions were incubated for 15 min with FC block prior to staining with fluorochrome-conjugated antibodies to the following for 20 to 30 min: T cell receptor β (TCRβ; clone H57-597; BioLegend), CD19 (clone 6D5; BioLegend), NK1.1 (clone PK136; BioLegend), CD45 (clone 30-F11; BD Biosciences), Ly6C (clone AL-21; BioLegend), CD11c (clone N418; BD Biosciences), Ly6G (clone 1A8; BioLegend), F4/80 (clone BM8; BioLegend), CD11b (clone M1/70; BioLegend), CD4 (clone RM4-5; BD Biosciences), CD8 (clone 53-6-7; BD Biosciences), and I-A/I-E (clone M5/114.15.2; BioLegend). The total numbers of kidney-infiltrating leukocytes were quantified as the number of $CD45^+$ cells in the live-cell gate. Flow cytometric analysis was conducted with a BD LSR Fortessa flow cytometer.

Cytokine analysis. Kidneys were homogenized using a PRO250 homogenizer (ProScientific) in buffer containing 10 mM Tris, pH 7.8, 200 mM NaCl, 5 mM EDTA, 10% glycerol, and 1× Roche protease inhibitor cocktail. Clarified homogenates were analyzed by enzyme-linked immunosorbent assay (ELISA; for interleukin-6 [IL-6] and tumor necrosis factor alpha [TNF-α], R&D Systems; for alpha interferon [IFN-α], eBioscience, Inc.) or multiplex analysis (Millipore, Inc.). Data were analyzed with a Molecular Devices Spectramax M5 (ELISAs) or a Bio-Plex 200 (Bio-Rad) system.

RESULTS

***Sts-1/2*$^{-/-}$ mice are highly resistant to systemic *C. albicans* infection.** The role of Sts-1 and Sts-2 in regulating the host response to a fungal pathogen was examined by testing the susceptibility of *Sts-1/2*$^{-/-}$ mice to systemic infection by *C. albicans*. Infections were initiated by injecting wild-type strain SC5314 via the tail vein, and then the mice were monitored over a 28-day period. As expected, nearly 100% of wild-type mice became moribund at the three doses evaluated, with the median time to death being delayed by approximately 5 days with the lowest dose (10^5 CFU/mouse) relative to that with the highest dose (5×10^5 CFU/mouse) (Fig. 1A). In contrast, mice lacking the Sts proteins exhibited strikingly enhanced survival following induction of systemic candidiasis. Specifically, *Sts-1/2*$^{-/-}$ mice were almost uniformly resistant to an inoculum of 10^5 CFU of *C. albicans*, and at the higher doses they displayed a significantly delayed onset of disease and less than half became moribund (Fig. 1A).

Because lethality is caused in part by inflammation-mediated damage to the kidneys, we examined histological sections of kidneys (8, 11). Kidneys harvested from uninfected WT and *Sts-1/2*$^{-/-}$ mice were similar in appearance (see Fig. S1 in the supplemental material). Following intravenous *C. albicans* administration (2.5×10^5 CFU/mouse), the kidneys from wild-type mice infected for 6 days showed the expected pathogenic effects resulting from the invasive growth of *C. albicans* combined with an inflammatory immune response. In particular, many misshapen tubules with large, open lumens were evident throughout the cortex region

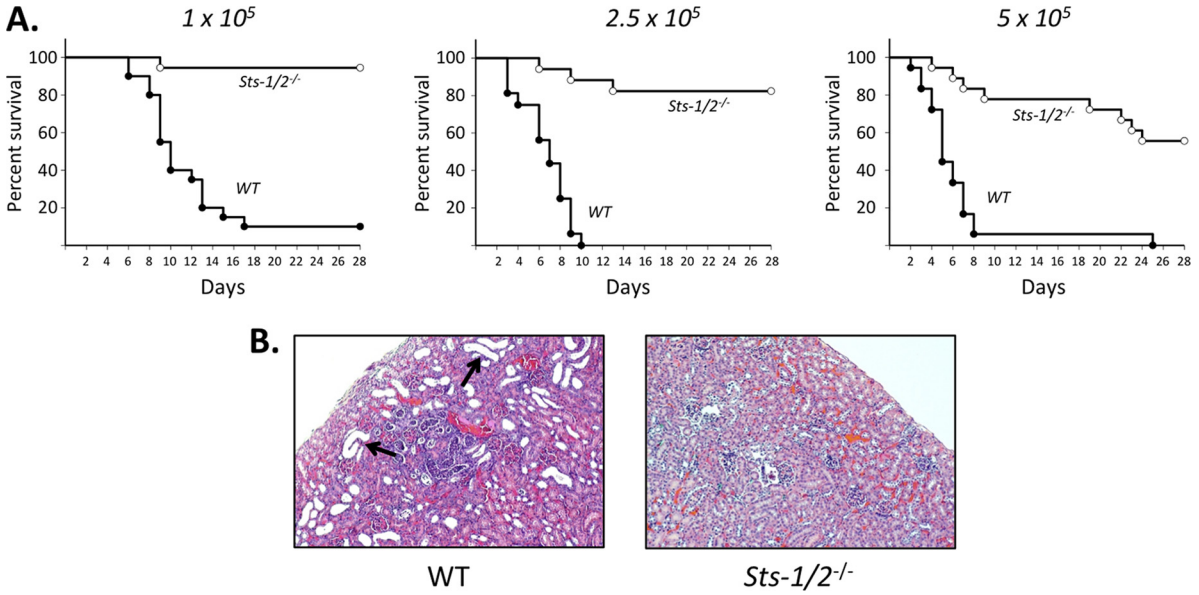

FIG 1 Resistance of *Sts-1/2*$^{-/-}$ mice to systemic infection by *C. albicans*. (A) Mice were infected with the indicated doses of *C. albicans* (number of CFU per mouse) and monitored for 28 days. *Sts-1/2*$^{-/-}$ mice demonstrated significantly enhanced survival ($P < 0.0001$ by the log-rank Mantel-Cox test) at all doses tested. For each dose, results are for a total of 16 to 20 mice analyzed in three independent experiments. (B) Representative histological analysis of wild-type and *Sts-1/2*$^{-/-}$ kidneys 6 days after infection with a dose of 2.5×10^6 CFU. Staining was with H&E. Arrows, large misshapen tubules indicative of pathological damage. Magnifications, ×100.

(Fig. 1B, left; see also Fig. S1 in the supplemental material) (11). In some cases, the lumenal regions contained large numbers of mononuclear leukocytes. In contrast, kidneys isolated from *Sts-1/2*$^{-/-}$ mice 6 days after infection appeared undamaged (Fig. 1B, right; see also Fig. S1 in the supplemental material). Thus, the widespread damage to the kidneys that is a hallmark of *C. albicans* systemic infection did not occur in the absence of the Sts proteins.

Individual contributions of Sts homologues to the host response to *C. albicans*. To dissect the individual contributions of Sts-1 and Sts-2 to the regulation of the host response to systemic *C. albicans* infection, we compared the corresponding single mutant mice to the *Sts-1/2*$^{-/-}$ mice. When infected with 1×10^5 CFU/mouse, both *Sts-1*$^{-/-}$ and *Sts-2*$^{-/-}$ mice displayed resistance to *C. albicans* similar to that of *Sts-1/2*$^{-/-}$ mice (Fig. 2, top). However, when infected at a dose of 2.5×10^5 CFU/mouse, mice with an individual deletion of either *Sts-1* or *Sts-2* demonstrated an intermediate susceptibility to infection relative to that of wild-type and *Sts-1/2*$^{-/-}$ mice (Fig. 2, bottom). Thus, despite interesting functional differences between the Sts proteins, including their *in vitro* phosphatase activities and their patterns of tissue expression, they appear to carry out redundant functions in regulating the host response to *C. albicans* infection.

Reduced fungal burden in mice lacking Sts proteins. The enhanced ability of *Sts-1/2*$^{-/-}$ mice to survive a lethal systemic challenge of *C. albicans* suggested that they could be more effective than wild-type mice at either tolerating a higher kidney fungal burden or disabling pathogen virulence mechanisms and clearing the infection, or a combination of both. Following the introduction of *C. albicans* into the mouse bloodstream, the fungus spreads rapidly to a wide range of tissues, where it is brought under control by organ-specific resident and/or infiltrating leukocytes (8, 28). However, for reasons that are not completely understood, within the kidney there is rapid fungal growth accompanied by a mor-

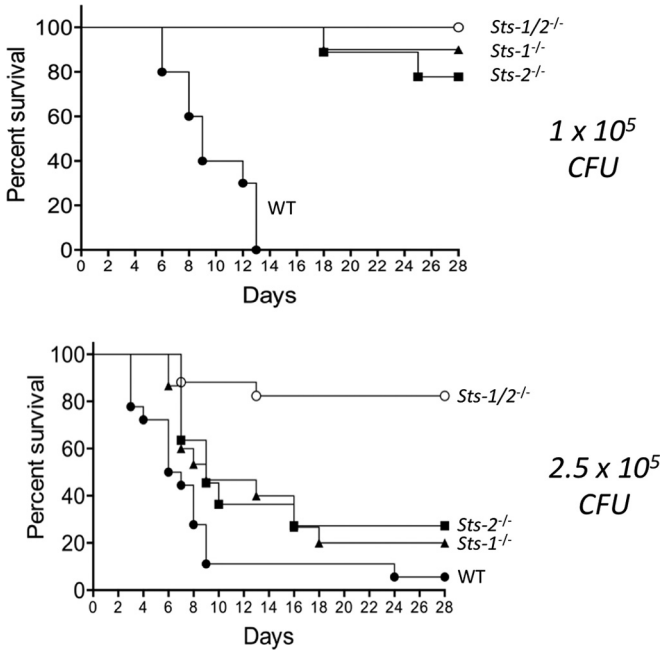

FIG 2 Resistance of *Sts-1*$^{-/-}$ and *Sts-2*$^{-/-}$ mice to systemic infection by *C. albicans*. Mice with an individual deletion of either *Sts-1* or *Sts-2* were infected intravenously and monitored for 28 days. Each result is for a total of 14 to 15 mice from two independent experiments. (Top) For a dose of 1×10^5 CFU/mouse, P was <0.01 (by log-rank Mantel-Cox analysis) for *Sts-1*$^{-/-}$ versus WT mice and *Sts-2*$^{-/-}$ versus WT mice. (Bottom) For a dose of 2.5×10^5 CFU/mouse, P was <0.01 (by log-rank Mantel-Cox analysis) for *Sts-1*$^{-/-}$ versus *Sts-1/2*$^{-/-}$ mice and *Sts-2*$^{-/-}$ versus *Sts-1/2*$^{-/-}$ mice.

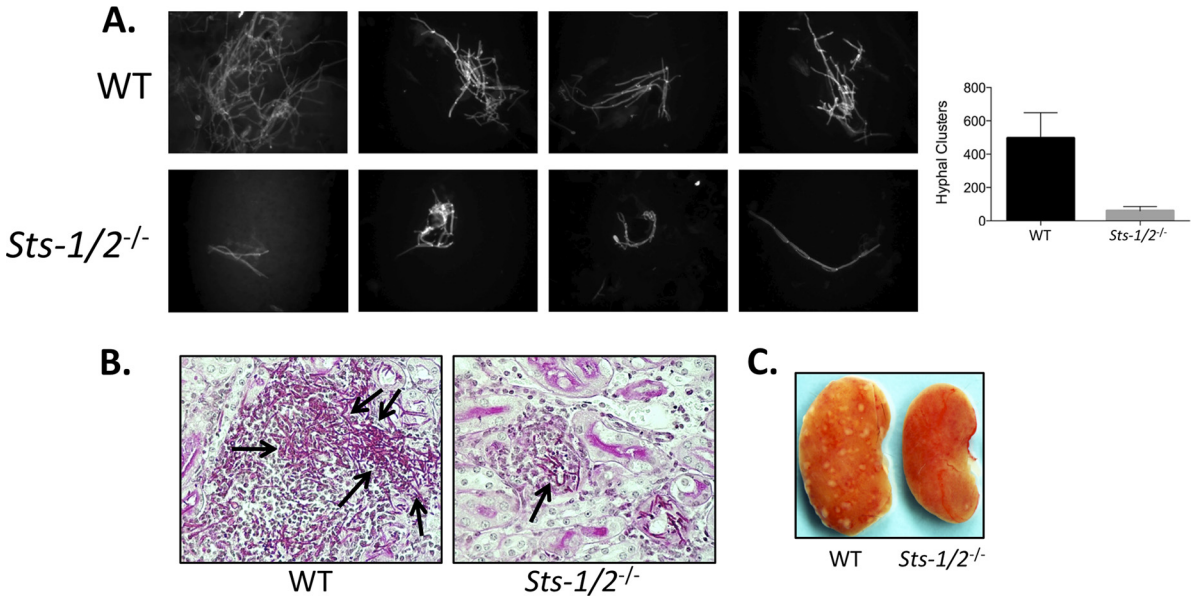

FIG 3 Attenuated fungal growth in *Sts-1/2*$^{-/-}$ kidneys relative to wild-type kidneys. (A) Hyphae in kidney homogenates were visualized with calcofluor white stain at 24 h after infection. (Left) Representative hyphal clusters; (right) total number of hyphal cells per kidney homogenate observed microscopically from two independent experiments (3 mice per group). *P* was <0.01 (by Mann-Whitney analysis) for *Sts-1/2*$^{-/-}$ versus WT mice. (B) At 48 h postinfection, representative kidney histological sections were stained with PAS to visualize *C. albicans* hyphae. Black arrows, hyphae. Magnifications, ×400. (C) Multiple inflammatory foci appear on the surface of wild-type kidneys but not on *Sts-1/2*$^{-/-}$ kidneys. Kidneys were harvested 3 days after infection with 2.5×10^5 CFU.

phogenic switch to the hyphal state. This extensive fungal proliferation appears to overwhelm the host immune response, eventually leading to kidney destruction and mortality. To determine whether the mutant mice were better at tolerating or clearing the pathogen, we infected mice and assessed the kidney fungal load when mice became moribund or at the end of 28 days. Uniformly, the numbers of CFU in wild-type mouse kidneys were significantly greater than those in *Sts-1/2*$^{-/-}$ mouse kidneys (see Fig. S2 in the supplemental material). Indeed, most of the *Sts-1/2*$^{-/-}$ mice survived to the end of the 28-day period, and most appeared to clear the infection, as there were no detectable CFU. Importantly, the few *Sts-1/2*$^{-/-}$ mice that did not survive also displayed a significant reduction in kidney fungal burden (see Fig. S2 in the supplemental material). Thus, the absence of the Sts proteins appeared to promote elimination of the pathogen from the kidney.

This conclusion is supported by several additional observations. First, the number and size of *C. albicans* hyphal clusters observed in *Sts-1/2*$^{-/-}$ kidney homogenates 24 h after infection were significantly diminished relative to those observed in wild-type kidney homogenates (Fig. 3A). Second, histological analysis indicated that while concentrated foci of filamentous *C. albicans* were readily apparent within the cortex of wild-type kidneys 2 days after infection, only small clusters of *C. albicans* extending hyphae that were not easily discernible could be found in the cortex of *Sts-1/2*$^{-/-}$ kidneys (Fig. 3B). Finally, in contrast to wild-type kidneys, which were moderately swollen and displayed a large number of infectious foci on their surface 3 days following inoculation, *Sts-1/2*$^{-/-}$ kidneys appeared normal, with the surface being devoid of similar infectious foci (Fig. 3C).

***Sts-1/2*$^{-/-}$ mice suppress *C. albicans* growth within the kidney more readily than wild-type mice.** To more precisely define the kinetic parameters underlying pathogen clearance from *Sts-1/2*$^{-/-}$ kidneys, we infected mice and assessed the numbers of kidney CFU along a time course beginning at 4 h after infection. Importantly, we noted equivalent seeding in both wild-type and mutant mouse strains, indicating that a lack of the Sts proteins does not affect dissemination to the kidney. As expected, fungal growth in both the right and left wild-type kidneys was robust, achieving a 100-fold increase in numbers of CFU within 48 h (Fig. 4A). After 48 h, the numbers of CFU in the wild-type kidney were sustained at similarly high levels for the next 4 days. In contrast, by 24 h the numbers of CFU in both the right and left *Sts-1/2*$^{-/-}$ kidneys were significantly lower than those in wild-type organs, and they remained at levels significantly lower than those in wild-type kidneys for the duration of the time course of analysis. Interestingly, the kidney was the only organ for which the number of fungal CFU differed dramatically between wild-type and mutant mice. No differences in fungal burdens or rates of clearance were observed in the spleen, liver, lungs, or brain (Fig. 4B). Thus, rather than causing a global effect on *C. albicans* survival, these data indicate that inactivation of the Sts proteins transforms the mammalian kidney from an environment in which *C. albicans* can readily thrive to one that is hostile to the growth of the pathogen.

Altered cellular immune response induced by *C. albicans* in *Sts-1/2*$^{-/-}$ mice. The survival of *Sts-1/2*$^{-/-}$ mice after inoculation with lethal doses of *C. albicans* suggested that their immune response to the fungal pathogen within the kidney was altered relative to the response of wild-type mice. Because immunopathology and organ failure resulting from lethal systemic *C. albicans* infection are associated with a large influx of inflammatory cells into the kidneys during the initial stage of the disease, we examined hematopoietic cell infiltrates into the kidney at regular time intervals after infection (8, 9). During the first 24 h, we observed an equivalent increase in the total numbers of CD45$^+$ cells within the kidneys of both wild-type and *Sts-1/2*$^{-/-}$ mice (Fig. 5A). During the next 48 h, however, the responses of the two strains differed

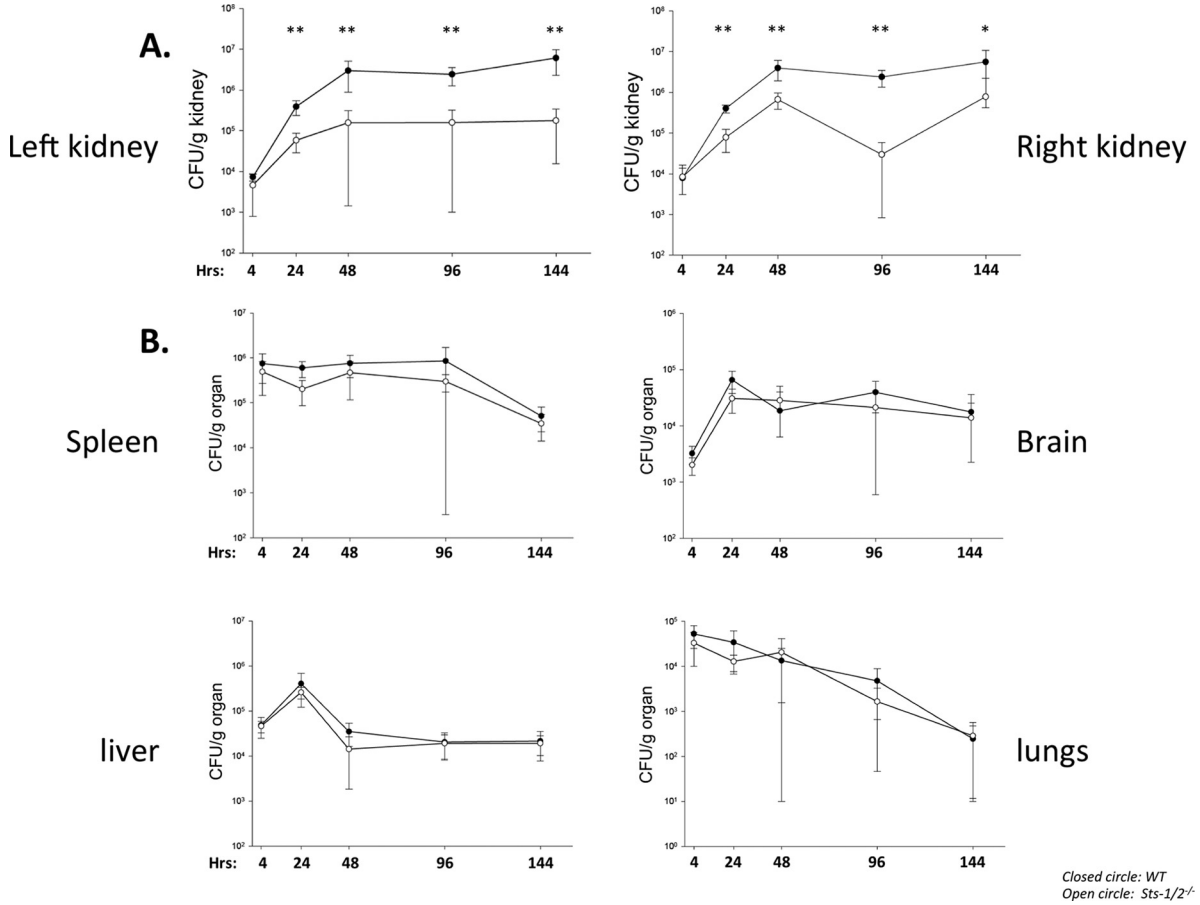

FIG 4 Organ-specific reduction in fungal burden within *Sts-1/2*$^{-/-}$ mice. A time course analysis of fungal burden in kidneys (A) or additional peripheral organs (B) from mice infected with 2.5×10^5 CFU. Results represent the average of two independent experiments each carried out with three mice per group. **, $P < 0.01$ by Mann-Whitney analysis; *, $P < 0.05$ by Mann-Whitney analysis.

dramatically. Within wild-type kidneys, the leukocyte population continued to expand markedly. In striking contrast, within *Sts-1/2*$^{-/-}$ kidneys the number of leukocytes began to decline after 24 h following infection. The number, size, and cellularity of the inflammatory foci in the cortex of infected kidneys further demonstrated the attenuated response of *Sts-1/2*$^{-/-}$ mice. Histological analysis revealed that whereas a large number of dense, highly concentrated H&E-positive leukocyte abscesses were clearly evident throughout the wild-type kidneys 2 days after *Candida* infection, the few foci that were visible in *Sts-1/2*$^{-/-}$ kidneys were thinly populated and diffuse (Fig. 5B).

We then evaluated specific subsets of responding cells. We detected similar steady-state levels of kidney-resident leukocyte subpopulations prior to infection, with both mouse strains having low levels of CD45$^+$ CD11b$^+$ Ly6C^{hi} monocytes and CD45$^+$ CD11b$^+$ Ly6C^{med} neutrophils (Fig. 6) (8). In wild-type kidneys, the percentages of monocytes peaked at 24 h and began to decline thereafter, while the number of neutrophils continued to grow rapidly. However, within *Sts-1/2*$^{-/-}$ kidneys, the numbers and ratios of both monocytes and neutrophils exhibited a substantial decline after 24 h following infection (see representative plots and plot summaries in Fig. 6). Thus, while the initial stage of innate immune effector cell recruitment into the kidney appeared to be identical in wild-type and *Sts-1/2*$^{-/-}$ mice, the sustained influx of immune cells into the kidney that normally occurs was significantly attenuated in mice lacking the Sts proteins.

Altered cytokine profile induced by *C. albicans* in *Sts-1/2*$^{-/-}$ mice. To gain more insight into the attenuated cellular immune response observed in *Sts-1/2*$^{-/-}$ mouse kidneys following systemic infection with *C. albicans*, we monitored the levels of inflammatory cytokines during the early stages of the infection (29–31). As expected, within wild-type kidneys the levels of TNF-α and IL-6 increased significantly during the first 48 h (Fig. 7A). *Sts-1/2*$^{-/-}$ kidney TNF-α and IL-6 levels were also increased at 24 h, but then the levels declined, such that by 48 h they were significantly lower than the corresponding wild-type kidney cytokine levels (Fig. 7A). The kidney levels of four chemokines that play an important role in recruiting monocytes and neutrophils to sites of infection, CCL2 (monocyte chemotactic protein 1), CCL3, CXCL1 (keratinocyte-derived chemokine), and CXCL2, also trended lower in *Sts-1/2*$^{-/-}$ mice than in wild-type mice by 24 h (Fig. 7B). In contrast, the levels of a variety of other cytokines were unchanged between the two strains (see Fig. S4 in the supplemental material). Thus, we observed reduced levels of specific inflammatory factors in *Sts-1/2*$^{-/-}$ kidneys relative to wild-type kidneys during the time frame that corresponded to both a reduction in the numbers of fungal CFU and reduced cellular immune responses.

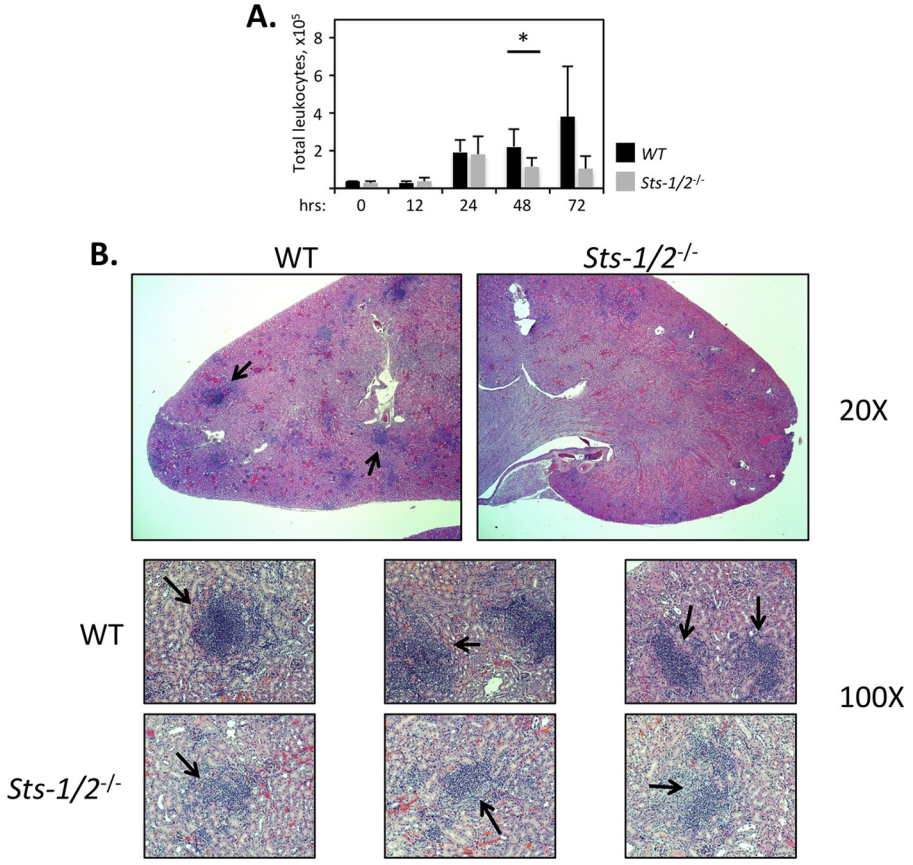

FIG 5 Attenuated leukocyte response in the absence of Sts-1 and -2. (A) Comparison of total kidney-infiltrating leukocytes ($CD45^+$). Hematopoietic cells were isolated from the kidneys of mice infected with 2.5×10^5 CFU for the indicated times. Results are from two independent experiments with 3 mice for each time point. *, $P < 0.05$ by Mann-Whitney analysis. (B) Inflammatory foci (black arrows) formed within the kidney cortex, visualized by H&E staining of histological sections of kidneys harvested 2 days after infection with 2.5×10^5 CFU. Foci within *Sts-1/2*$^{-/-}$ kidneys were reduced in size and cellularity. Magnifications, ×20 (top) and ×100 (bottom). Images with a higher magnification are presented in Fig. S3 in the supplemental material.

The sharply reduced fungal burden in *Sts-1/2*$^{-/-}$ kidneys within 24 h after infection suggested that mice lacking the Sts proteins could mount a rapid immune response able to neutralize early fungal growth. To identify evidence of a more potent early inflammatory response in *Sts-1/2*$^{-/-}$ mice, we assessed the levels of cytokines and chemokines within the first 12 h following infection. Of 15 common cytokines and chemokines examined, including TNF-α, IL-6, CCL2, and CXCL1, only the level of CXCL10 was increased significantly at 12 h (Fig. 7C). However, antibody-mediated neutralization of either CXCL10 or its receptor, CXCR3, failed to abrogate the protective phenotype evident in *Sts-1/2*$^{-/-}$ mice (data not shown), suggesting that CXCL10 is not solely responsible for the resistance phenotype. Collectively, these observations suggest that a potent and directed innate inflammatory response that favors the elimination of freshly seeded fungal cells is rapidly generated within *Sts-1/2*$^{-/-}$ kidneys. By allowing the host-pathogen balance to be tipped quickly in favor of the host, functional inactivation of the negative regulatory Sts proteins could thereby lead to reduced inflammation and host survival in the face of an overwhelming *C. albicans* infection.

DISCUSSION

Mice lacking Sts-1 and -2 are significantly protected from death due to systemic infection by *Candida albicans*. In this disease model, the kidney is the organ most acutely affected, leading wild-type mice to succumb to pyelonephritis within days of infection (7). The reduction in kidney fungal burden and the maintenance of organ homeostasis that take place in *Sts-1/2*$^{-/-}$ mice are associated with sharply decreased levels of many inflammatory molecules beginning at 24 h postinfection. This phenotype is accompanied by a concomitant reduction in kidney leukocyte infiltrates after 24 h and an absence of inflammatory lesions. It is possible that the reduced inflammation evident in *Sts-1/2*$^{-/-}$ mice follows directly from the reduction in the numbers of fungal CFU that occurs within 24 h. Importantly, mice deficient in either Sts-1 or Sts-2 individually display an intermediate phenotype, suggesting a redundant effect.

Previous studies have demonstrated a functional role for the Sts proteins as negative regulators of TCR signaling in T cells, FcεRI receptor signaling in mast cells, and GPVI signaling in platelets (23). However, since the predominant cell types associated with the early innate immune response to *C. albicans* infection within the kidney are phagocytes, including monocytes and neutrophils (8), our results suggest that the Sts proteins have important negative regulatory functions in a range of cell types broader than that previously identified. Alternatively, the enhanced functions of *Sts-1/2*$^{-/-}$ T cells could directly or indirectly stimulate the candidacidal behavior of responding phagocytes

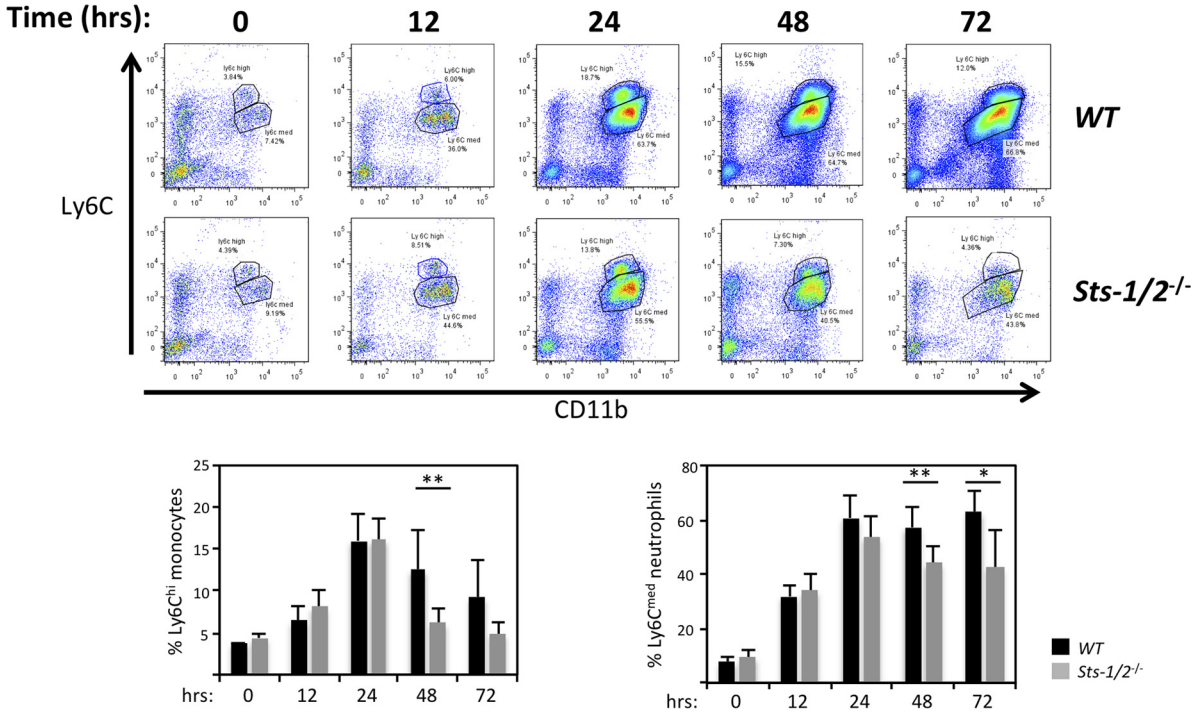

FIG 6 Reduced kidney infiltration of Ly6C$^+$ monocytes and Ly6C^{med} neutrophils in the absence of Sts-1 and -2. (Top) Representative fluorescence-activated cell sorter plots illustrating the levels of CD11b$^+$ Ly6C^{hi} cells (monocytes) and CD11b$^+$ Ly6C^{med} cells (neutrophils) in the kidney at time points following fungal infection. (Bottom) Summary of fluorescence-activated cell sorter plots with total flow cytometric data from two independent experiments (3 mice per group) analyzed. **, $P < 0.01$ by Mann-Whitney analysis; *, $P < 0.05$ by Mann-Whitney analysis.

within *Sts-1/2*$^{-/-}$ mice, for example, through increased production of specific cytokines. In this regard, however, it is important to point out that we saw no significant differences in the levels of several important T cell cytokines within wild-type and mutant kidneys, with IFN-γ and IL-17 being among them (see Fig. S4 in the supplemental material). Interestingly, previous studies have demonstrated that the absence of T cells has an overall beneficial effect on the host response to systemic *C. albicans* infection, suggesting that T cell-inhibitory effects predominate (32). Studies are under way to address the *in vivo* role of *Sts-1/2*$^{-/-}$ hyperresponsive T cells on the protective phenotype.

This study yields important insight into the physiological functions of the Sts proteins, stemming from the observation that antifungal effects were the strongest in the kidney. It is currently unclear why this is the case, although we speculate that the internal architecture of the organ and the functional properties of resident phagocytes could be important determinants, along with the relative ratio of resident and/or incoming phagocytes to freshly seeded fungal cells. Although the number of kidney CFU increased through 48 h in both strains of mice, the fungal burden remained consistently lower in *Sts-1/2*$^{-/-}$ mice than wild-type mice. Simultaneously, the levels of kidney cytokines and chemokines were significantly lower, consistent with a reduced fungal burden. These data suggest a model in which the loss of Sts inhibitory functions leads to activation of innate immune responses important for fungal clearance in the kidney. Analysis of cytokine expression indicates that the absence of the Sts proteins creates a novel type of immunological environment in the kidney. For example, although there was no detectable increase in IFN-γ, TNF-α, or IL-6 expression in the *Sts-1/2*$^{-/-}$ kidney relative to that in the wild-type kidney at either 12 or 24 h postinfection, the level of the proinflammatory chemokine CXCL10 was significantly increased in *Sts-1/2*$^{-/-}$ kidneys at 12 h postinfection. CXCL10 is a T lymphocyte recruitment factor, and increased circulating levels of CXCL10 have been correlated with different inflammatory conditions (33, 34). CXCL10 is also known to be upregulated by a number of different cell types in response to various cytokines, and in many physiological situations it is thought to promote an immune response (35, 36). Because antibody neutralization of CXCL10 and its receptor, CXCR3, has no effect on the resistance phenotype of *Sts-1/2*$^{-/-}$ mice, it is likely that additional inflammatory mediators are also involved in protection. Altogether, these results demonstrate that a lack of Sts proteins results in a distinct inflammatory environment in the kidney that is more capable of restricting the growth of *C. albicans*.

The remarkable ability of *Sts-1/2*$^{-/-}$ mice to withstand lethal doses of *C. albicans* is all the more striking because fungal clearance does not occur in the context of an overall hyperinflammatory response. This contrasts with previous reports of increased resistance to *C. albicans* in mice lacking a functional receptor for type I interferons (*Ifnar1*$^{-/-}$ mice) or lacking the important neutrophil chemokine receptor CCR1 (11, 31). While both of these mutant mouse strains survived longer after they received low inocula because of decreased inflammatory damage, their kidney fungal burden remained very high during the entire course of infection. In both cases, decreased lethality was primarily associated with the absence of an important component of the inflammatory response rather than a decreased fungal burden, under-

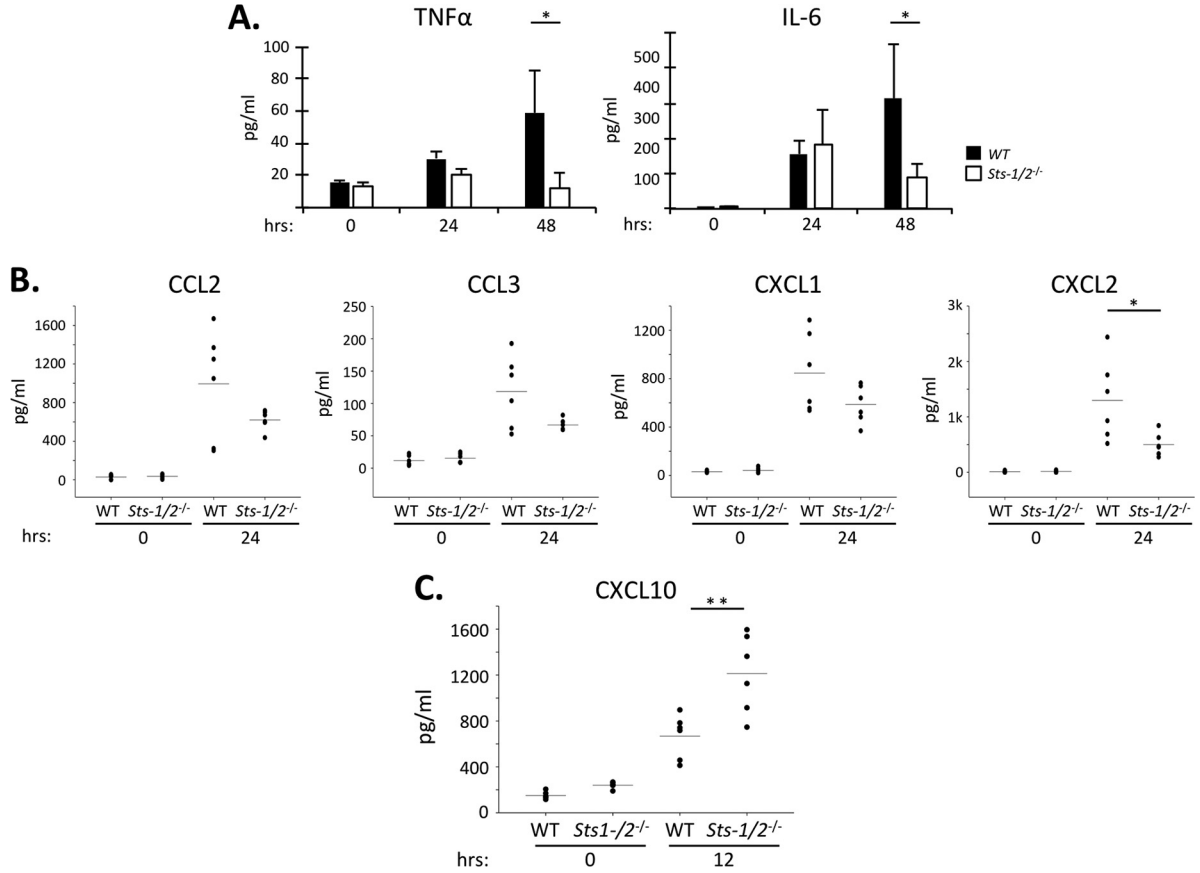

FIG 7 Altered inflammatory cytokine environment in the absence of Sts-1 and -2. Mice were infected with 2.5×10^5 CFU, and kidneys were harvested at the indicated time points and analyzed. (A) Levels of inflammatory cytokines TNF-α and IL-6 within the kidney. The results of one of two independent experiments with similar results (6 mice per group) are displayed. *, $P < 0.05$ by Mann-Whitney analysis. (B) Levels of kidney chemokines were determined by multiplex analysis. Scatter plots display the results of one of two independent experiments (each with 6 mice per group) with similar results. *, $P < 0.05$ by Mann-Whitney analysis. (C) Levels of kidney CXCL10 at 12 h postinfection. The results of one of two independent experiments (each with 6 mice per group) with similar results are displayed. **, $P < 0.01$ by Mann-Whitney analysis. Horizontal line represents the average of values.

scoring the deleterious nature of collateral tissue damage that can often occur during an overwhelming immune response. In contrast to these mouse models, mice lacking the CD37 tetraspanin were more resistant to *C. albicans* because they displayed more efficient fungal clearance from the kidney (37). However, their ability to resist infection occurred in the context of significantly increased levels of serum IgA and proinflammatory IL-6 by day 3 postinfection, both of which were concluded to aid in the clearance of *C. albicans*. CD37 associates with Dectin 1, a pattern recognition receptor that recognizes fungal cell wall β-glucan, and inhibits IL-6 production (38). Tellingly, another study demonstrated that elevated serum IgA levels in the absence of CD37 led to glomerular IgA deposition and increased levels of inflammatory phagocytes within the kidney, making *CD37*$^{-/-}$ mice prone to spontaneous nephropathy (39). This stands in marked contrast to the findings for *Sts-1/2*$^{-/-}$ mice, which displayed no spontaneous autoimmunity.

In sum, our results underscore the important role of the Sts proteins in regulating host responses to a deadly fungal pathogen and have the potential to contribute to the development of much needed new antifungal therapies. Importantly, the antifungal protection evident in *Sts-1/2*$^{-/-}$ mice is not accompanied by an overt hyperinflammatory response that would cause tissue damage. Indeed, mice tolerate Sts deficiency well, as it does not lead to deleterious side effects. Therefore, our studies identify the Sts proteins to be potential molecular targets for inhibitory molecules that could be used prophylactically in high-risk patients. We have also demonstrated a novel inflammatory environment within *Sts-1/2*$^{-/-}$ kidneys that promotes the early clearance of *C. albicans*. Further understanding of this environment could aid in the development of tailored immunological approaches that could be combined with current antifungal medications to achieve synergistic effects.

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