Supplementary figure 1.- Modulation of SAg-induced human IL-2 response by the bacterial cell wall. PBMC (5 x 10^6 cells/group) were stimulated with the indicated number (2.5 x 10^8 or 2.5 x 10^9) of heat-killed S. epidermidis or B. subtilis without or with SEE (at 10ng ml^-1), in a final volume of 2 ml, for 18 h at 37°C. Supernatants were collected, and IL-2 (left graphs) and IFN-γ (right graphs) accumulation was measured by ELISA. - : no bacteria or SEE added to the culture. Each group result represents mean ± standard deviation of triplicate samples. ***: P<0.001 compared to SEE stimulation alone. These data are representative of at least 3 independent experiments using PBMC from 3 different donors.
Supplementary figure 2.- The IL-2 response of T cells to SEE requires interaction with the TCR. Stable TCR Vβ5.1 transfectants of the beta-chain deficient Jurkat JRT3-T3.5 cells (10^5 cells/well) were stimulated with APCs (2x10^4 cells/well) and either wildtype SEE or SEE mutated at the indicated residues, at 10 ng ml⁻¹ final concentration. Data represent mean ± standard error of the mean.
Supplementary figure 3.- Bacteria do not sequester SEE and do not interfere with the detection of IL-2. (a) Heat-killed B. subtilis, S. aureus and S. epidermidis were added at a final concentration of 1.25 x 10^9 bacteria ml⁻¹ to selected wells of a 96-well plate. Biotinylated SEE was added at concentrations of 100 µg ml⁻¹ in complete RPMI (standard R10) media. Samples were incubated at 37°C for 16 h. Bacteria were then pelleted at 3000 r.p.m. for 10 m, sample supernatants were removed from the wells and loaded on a 12% SDS PAGE gel and immunoblotted with streptavidin-conjugated IR800Dye antibodies (Rockland Labs). (b) S. aureus, S. epidermidis, or B. subtilis were plated at the indicated numbers in the absence or in the presence of known amounts of recombinant human IL-2, for 30 m. Supernatants were collected and IL-2 in the samples was determined by ELISA. Data are presented as IL-2 measured for each amount of IL-2 added to the culture. Each point result represents mean ± standard deviation of triplicate samples.
Supplementary figure 4.- Presence of lipoproteins in S. aureus membranes is abolished in lgt mutants. Two strains of S. aureus (RN6390 and Newman) either in wildtype background (WT) or isogenic lgt-disrupted background (lgt) were assessed for expression of two representative membrane lipoproteins HtsA and SirA by Western blot analysis with antisera raised against these iron-regulated lipoproteins. WC: whole cell lysates; sup: culture supernatants; mem: Triton X 114-soluble membrane fractions. Lipoproteins in the lgt mutants are absent in the membrane fraction (mem lanes) but are still produced as pre-lipoproteins and secreted into the culture media (sup lanes).
**Supplementary figure 5.** - Staphylococcal PGN preparations also down-regulate the T cell response to CD3/CD28 ligation. Human PBMCs (5 x 10^6 cells/group) were stimulated with OKT3 (an antibody against human CD3ε, 10µg ml⁻¹) and anti-CD28 antibodies (2µg ml⁻¹) or with SEE (0.1ng ml⁻¹), in the absence or presence of staphylococcal PGN preparation (10µg ml⁻¹) for 24 h. IL-2 in the supernatant was measured by ELISA, and normalized as percentage of maximal IL-2 production in the assay. ***: P<0.001.
**TLR2 Ligands on the Staphylococcal Cell Wall Down-regulate Superantigen-Induced T Cell Activation and Prevent Toxic Shock Syndrome**

by

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**Supplementary figure 6.** - The modulatory effect of staphylococcal cell wall is due to signalling through TLR2 likely in complex with TLR6. Human PBMC (2x10^6 cells/group) were stimulated with increasing concentrations of SEE in the absence (solid line, squares) or presence (dotted line, triangles) of PGN preparation (10µg ml^{-1}) (containing TLR2 ligands), Pam3Csk4 (1µg ml^{-1}) (a TLR2/1 agonist), Zymosan (10µg ml^{-1}) (a TLR2/6 agonist), Murabutide (1µg ml^{-1}) and MDP (10µg ml^{-1}) (both NOD agonists) for 18 h. IL-2 in the culture supernatant was measured by ELISA. Each group result represents mean ± standard deviation of triplicate samples. These data are representative of at least 3 independent experiments with 3 different donors.
Supplementary figure 7.- Detection of TLR2 expression on PBMCs and on LG2 APCs. (a) TLR2 in human PBMCs is mostly expressed by non-T cells. Two-color FACS for CD3 expression and TLR2 expression on PBMC (right contour plot) and APC-labelled and PE-labelled isotype controls (left contour plot). (b) LG2 APCs were stained with an anti-TLR2 mAb (solid black line) or with a PE-labeled isotype control (dotted grey line) and examined by flow cytometry.
**Supplementary Figure 8.**- Staphylococcal PGN down-regulates CD86 expression and induces apoptosis of monocytes and macrophages. (a) PBMC were cultured without (top row) or with PGN (100µg ml⁻¹) (bottom row) overnight and stained for CD14, HLA-DR and CD86. For cells expressing CD14 and HLA DR (monocytes and macrophages), the expression of CD86 (black solid line) was measured by flow cytometry. Dotted line represents PE-labelled isotype control. The gates represent the percentage of cells expressing high levels of CD86 on the cell surface. We observed a drop of this percentage from 79.8 to 26.1%. (b) Peripheral blood mononuclear cells were cultured for 3 h with SEE and zymosan (TLR2 and 6 agonist) or murabutide (NOD agonist) at the indicated concentrations, and stained with annexin-V, an indicator of cell apoptosis. The percentage of CD14-positive cells that were annexin-V positive were determined by flow cytometry. Data in this figure are representative of at least three independent experiments using 3 different donors.