Human Memory T Cells Avidly Respond to *Staphylococcus aureus* in Patients Recovering from Bloodstream Infection

AF Brown[1], TR Rogers[2], RM McLoughlin[1]

1. Host-Pathogen Interactions Group, School of Biochemistry & Immunology, Trinity College Dublin, Ireland
2. Department of Clinical Microbiology, Trinity College Dublin & St James's Hospital, Dublin, Ireland

Corresponding author: brownai@tcd.ie

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Introduction

Little is known about the normal human immune response to infection with *Staphylococcus aureus*. These bacteria are equipped with multiple mechanisms to evade the innate and adaptive immune system.

Exposure to this commensal organism may lead to the generation of specific humoral and cellular responses to some of its many polysaccharide and protein antigens. Anti-staphylococcal antibodies are present in the majority of the normal population but only appear to be protective against the rare staphylococcal toxic shock syndrome. To date, all completed anti-*S.aureus* vaccine trials that have centred on the generation of protective antibodies have failed, despite having shown promise in pre-clinical animal models. It is likely that generating humoral immunity alone may not be sufficient to confer protection against invasive *S.aureus* infection in humans.

It is unknown whether T cells are expanded following *S.aureus* exposure in humans, nor whether these cells can in fact respond to *S.aureus* antigens at all. Outside of the superantigens there are no known human *S.aureus* T cell epitopes. Future targeted vaccine design must firstly identify which cells are most important in recovery from natural infection, and secondly which bacterial antigens are the most potent T cell activators. This is the first study in humans aiming to answer these questions.

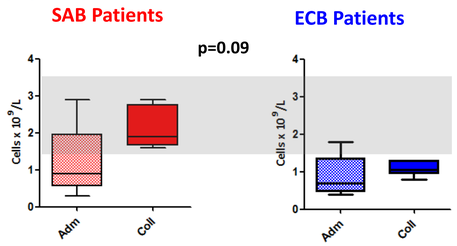
Methods

Immunocompetent adult inpatients recovering from *S. aureus* or *E. coli* bloodstream infections (SAB or ECB) and able to give informed consent were asked to participate.

Healthy volunteers were recruited as an additional control group.

Peripheral blood mononuclear cells (PBMCs) were isolated on a mean of day 7 (SD=3) post-bacteraemia and stained with CFSE before being cultured with heat-killed laboratory and clinical strains of *S.aureus* (1µg/ml – approx. 1 x 10^7 cfu/mL) for 10 days. Cells were then stained for extracellular markers (CD3, CD4, CD8, CD45RO) and intracellular cytokines (IFNγ, IL-17A, IL-10) and processed for flow cytometric analysis on a BD FACS-LSRFortessa®.

Figure 1. Lymphocyte Recovery in peripheral blood is greater in SAB than ECB



Shaded grey area shows normal range for peripheral blood lymphocytes.
Adm = admission date. Coll = collection date

Results

Nineteen eligible patients were recruited (12 SAB, 7 ECB) along with 19 healthy volunteers were recruited. Mean age in SAB patients was 55 years (SD=27), 66 years in ECB patients (SD=17, p=0.25). Eighty-three percent (n=10) of SAB patients recruited had complicated disease.

Recovery from SAB is associated with a greater expansion in lymphocytes over the initial 7 days than in ECB (0.9 vs 0.2 cells x 10^9/L increase, p=0.09) (Figure 1).

CD4+ T cells isolated from SAB patients demonstrate significantly increased levels of *S.aureus*-specific proliferation as compared to T cells isolated from ECB patients (Figure 2). The population of proliferating CD4+ cells was 33.1% vs 15.6% (p=0.03). The response in SAB patients was not significantly different to healthy volunteers, even though proliferative responses are known to be globally suppressed during acute illness. This indicates a very significant relative expansion of *S.aureus*-specific T cells.

Figure 2. SAB Patients' CD4+ cells show greater proliferation to *S.aureus* antigens

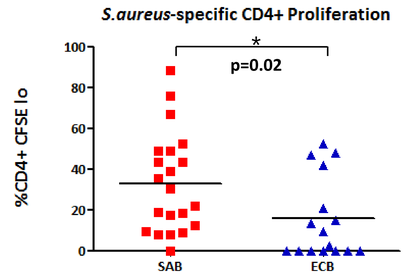
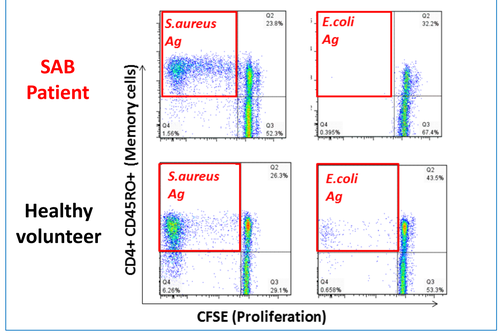


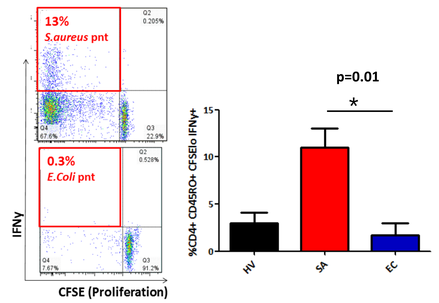
Figure 3. Most proliferation is by Memory T cells and these are also present in healthy volunteers



The majority of proliferating cells are CD45RO+, suggesting that they are circulating *S.aureus*-specific memory T cells expanded as a consequence of the recent exposure to the organism (Figure 3). Interestingly, these *S.aureus*-specific memory T cells were also present in healthy volunteers - likely due to a lifetime's exposure to *S.aureus* – although their responses are not nearly as marked as in recently infected patients.

These expanding cells exhibit a Th1-predominant phenotype – secreting IFNγ in response to bacterial antigen (11% vs 1.7%, p=0.01) (Figure 4).

Figure 4. *S.aureus*-specific memory CD4+ cells make more IFNγ in response to heat-killed bacteria in SAB patients



Conclusions

No study has previously been undertaken to profile which or, indeed, examine whether human T cells can specifically respond to *S.aureus* antigens. During recovery from *S.aureus* bloodstream infection, a population of antigen-specific Th1 memory cells are primed to expand and perform effector functions. This finding can be used in intelligent design of T cell-targeted anti-*S.aureus* vaccines.