**Characterisation of teicoplanin-specific T-cells from drug naïve donors expressing HLA-A\*32:01.**

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ABSTRACT: Teicoplanin is a glycopeptide antibiotic deployed to combat gram-positive bacterial infection and has recently been associated with development of adverse drug reactions, particularly following previous exposure to vancomycin. In this study, we generated teicoplanin-specific monoclonal T-cell populations from healthy volunteers expressing HLA-A\*32:01 and defined pathways of T-cell activation and HLA allele restriction. Teicoplanin-responsive T-cells were CD8+, HLA class I-restricted and cross-reacted with the lipoglycopeptide daptomycin in proliferation and cytokine/cytolytic molecule (granzyme B, Perforin and FasL) release assays. These data show that teicoplanin activates T-cells, which may play a role in the pathogenesis of teicoplanin-induced adverse events, in HLA-A\*32:01 positive donors.

Hypersensitivity to otherwise efficacious antibiotics is an area of concern to patients, clinicians and researchers in the field of drug development. Prediction of such reactions is often difficult due the elicitation of adverse events arising outside of a drug’s known pharmacology. Although rare, reactions of this nature have been associated with activation of the adaptive immune system, with T-cells implicated in the pathogenesis of severe cutaneous adverse reaction, including drug-reaction with eosinophilia and systemic symptoms (DRESS)[1](#_ENREF_1). Glycopeptide antibiotics, such as teicoplanin, have been utilised for over 30 years with strong efficacy demonstrated against gram-positive bacterial infection, including β-lactam resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *Clostridium difficile*[2](#_ENREF_2). Teicoplanin is typically administered as a 2nd line treatment option and as an alternative to vancomycin. Despite the incidence of adverse drug reaction (ADR) associated with teicoplanin being substantially lower (13.9% vs 21.9%[3](#_ENREF_3)) compared to vancomycin, the drug still poses a significant risk to patient safety. A recent GWAS has shown an association between vancomycin-induced DRESS and HLA-A\*32:01 in European populations[4](#_ENREF_4). Case studies have reported clinical cross-reactivity and subsequent teicoplanin-induced DRESS following initial vancomycin hypersensitivity[5](#_ENREF_5), [6](#_ENREF_6). Preliminary *in vitro* studies using vancomycin-responsive T-cells generated from HLA-A\*32:01 positive healthy donor PBMC have already demonstrated low levels of cross-reactivity with teicoplanin[7](#_ENREF_7). Cross-reactivity has been illustrated further in patients presenting with suspected vancomycin or teicoplanin-induced DRESS, with *ex* *vivo* data suggesting complex patterns of immunogenicity within the context of HLA class II presentation[8](#_ENREF_8). The aim of the present study was to investigate the intrinsic immunogenic potential of teicoplanin in terms of evoking T-cell responses in healthy donors (HD), in addition to further exploring patterns of cross-reactivity to structurally related glycopeptides.



Figure 1. Proliferation of TCC generated from HLA-A\*32:01 positive donors following exposure to teicoplanin. T-cell populations were positively enriched for either CD4+ or CD8+ T-cells via magnetic bead separation (Miltenyi Biotec, UK). TCC were rechallanged with 250 µM teicoplanin or cell culture medium for 48 h in the presence of autologous antigen presenting cells (Epstein-Barr virus-transformed B-cells; APCs). [3H]thymidine was added for the final 16 h of incubation to measure proliferation and clones with a stimulation index (SI) > 2 were deemed to be drug-responsive.

Teicoplanin-specific TCC, generated by serial dilution[9](#_ENREF_9), were identified in 3 healthy donors positive for HLA-A\*32:01 expression (Figure 1). TCC generated from CD8+ enriched populations proliferated to a greater degree (HD-2, 3; SI > 40) and frequency (HD-1; 118/216 TCC SI > 2) than CD4+ enriched. The presence of drug-reactive T-cells that proliferated in a dose-dependent manner to teicoplanin (data not shown) were restricted to monoclonal populations enriched for CD8+ T-cells as upon expansion CD4+ TCC did not respond to teicoplanin following confirmatory dose-response tests. Drug-responsive clonal populations that exclusively expressed a CD8+ phenotype and were expanded via mitogen driven stimulation for further functional analysis.

Following pre-treatment of both APCs and T-cells with anti-HLA blocking antibodies, proliferation of CD8+ TCC was unaffected after HLA class II blockade (HLA-DP, HLA-DQ and HLA-DR). However, proliferation was found to be inhibited in the presence of MHC class I blocking antibodies (Figure 2A) indicating T-cell responses to teicoplanin are driven primarily by MHC class I complexes. Autologous APCs pulsed with teicoplanin (30 min, 1 h, 4 h and 24 h) displayed no proliferative response following co-culture with teicoplanin-reactive TCC (Figure 2B). After fixation of APCs with glutaraldehyde and subsequent attenuation of peptide processing pathways, drug-responsive T-cells exhibited the capacity for proliferation after exposure to a co-culture of fixed APCs and teicoplanin. These data suggest teicoplanin is able to activate CD8+ TCC in a processing independent manner in which direct pharmacological interactions with MHC, concordant with the p-i concept, evoke T-cell responses to drug.



Figure 2. HLA restriction and activation pathway of teicoplanin-responsive CD8+ TCC from HD-3. A) Proliferation in response to teicoplanin (250 µM) was measured following blocking of HLA complexes present on the surface of both APCs and TCC using anti-HLA antibodies (BD Pharmingen, San Jose, USA) at a concentration of 10 µg/mL. B) Autologous APCs were either pulsed with teicoplanin for multiple time-points and extensively washed to remove unbound drug or fixed with glutaraldehyde to inhibit APC peptide processing. TCC were then incubated for 48 h with pulsed APCs or fixed APCs plus teicoplanin (250 µM), with unmodified autologous APCs used as a positive control. [3H]thymidine was added for the final 16 h of incubation to measure proliferative responses. Data is shown for representative TCC (n=3) and statistical significance was determined using Mann-Whitney U test (\*p< 0.05, \*\*p< 0.01, \*\*\*\*p<0.0001).

Cytokine and cytolytic molecule secretion of teicoplanin-reactive TCC was assessed via ELISpot after drug rechallenge (Figure 3A). Clones were observed to secrete both Th1 (IFN-γ) and Th2 (IL-5 and IL-13) cytokines. However, the secretion of Th17 and Th22 associated cytokines such as IL-17A and IL-22 were not present (data not shown). Interestingly, secretion of cytolytic molecules were detected in all TCC profiled. Most notably, increased secretion of granzyme B, perforin and FasL indicating involvement of cytotoxic T-cell responses and potential for activation of pro-apoptotic pathways. Cross-reactivity study of clones initially primed and exhibiting proliferative responses to teicoplanin revealed that memory T-cell responses to teicoplanin were associated with a greater degree of proliferation. Interestingly, TCC exhibited cross-reactivity with the cyclic lipoglycopeptide, daptomycin, at graded concentrations. However, no cross-reactive T-cells were identified after exposure to vancomycin (Figure 3B).



Figure 3. Cytokine/cytolytic molecule secretion profile and glycopeptide cross-reactivity of CD8+ teicoplanin-reactive TCC from HD-3. A) Drug-responsive clones were incubated with autologous APCs and either teicoplanin (250 µM) or cell culture medium for 48 h (representative TCC shown). T-cell secretion of cytokines (IFN-γ, IL-5 and IL-13) and cytolytic molecules (granzyme B, perforin and FasL) was visualised via enzyme-linked immunospot (ELISpot) assay using an ELISpot plate pre-coated for the cytokines of interest and developed according to the manufactures instructions (Mabtech, Sweden). B) Cross-reactivity of teicoplanin-responsive T-cells to glycopeptides (vancomycin and daptomycin) was measured via proliferation assay as previously described in Figures 1 and 2. Statistical significance was determined using a non-parametric *t*-test (\*\*\*p< 0.001, \*\*\*\*p<0.0001).

In summary, teicoplanin-responsive T-cells displaying a CD8+ phenotype were generated from 3 drug-naïve healthy donors expressing the HLA-A\*32:01 allele, recently associated with cases of vancomycin-induced DRESS. Therapeutics concentrations associated with glycopeptide administration are typically between 10-20 µM, substantially lower than the optimal doses using within this study to elicit maximal T-cell responses for functional analysis. However, we have observed that glycopeptide-specific TCC are capable of eliciting proliferative responses at lower, more therapeutically relevant doses in line with concentrations found within the blood plasma of patients. The identification of TCCs that proliferate and secrete both cytotoxic and DRESS related cytokines such as IL-5 suggest T-cell involvement within the pathogenesis of teicoplanin-induced DRESS syndrome[10](#_ENREF_10).

Mechanistic T-cell assays revealed a processing independent mechanism of activation that hinges on drug presentation via direct interaction with HLA class I molecules. These data are concordant with previous mechanistic findings relating to T-cell responses to vancomycin for which it has been hypothesised glycopeptide compounds possess the capacity to displace and mimic native HLA peptides[7](#_ENREF_7). Proliferative T-cell cross reactivity of teicoplanin-responsive TCC generated from healthy volunteers to daptomycin highlights the complex patterns of reactivity encountered within clinical settings. The observed *in vitro* T-cell cross-reactivity may be explained by structural similarities between both teicoplanin and daptomycin, specifically the presence of a hydrophobic lipid chain. Conversely, vancomycin’s structure comprises a heptapeptide chain that crucially contains a disaccharide, composed of vancosamine and glucose, instead of the lipid tail found on both teicoplanin and daptomycin molecules. This potentially explains why some teicoplanin-specific T-cells are able to proliferate in the presence of daptomycin but not vancomycin. One intriguing avenue to explore the nature of these cross-reactive responses involves the study of cellular energetic parameters, such as glycolysis, which may provide greater sensitivity for the determination of T-cell activation thresholds upon antigen presentation. However, to investigate the specificity of teicoplanin for HLA-A\*32:01, additional cloning experiments focusing on individuals negative for HLA-A\*32:01 expression will need to be conducted. Further genetic studies and functional T-cell analysis following HLA-glycopeptide binding will be required to determine the full pathway of glycopeptide cross-reactivity in addition to the extent of interactions with HLA-A\*32:01 in order to predict potential susceptibility to severe cross-reactivity and improve patient safety.

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ABBREVIATIONS

ADR, adverse drug reaction; APCs, antigen presenting cells; DRESS, drug-reaction with eosinophilia and systemic symptoms; PBMC, peripheral blood mononuclear cells; HLA, human leukocyte antigen; SI, stimulation index; TCC, T-cell clone.

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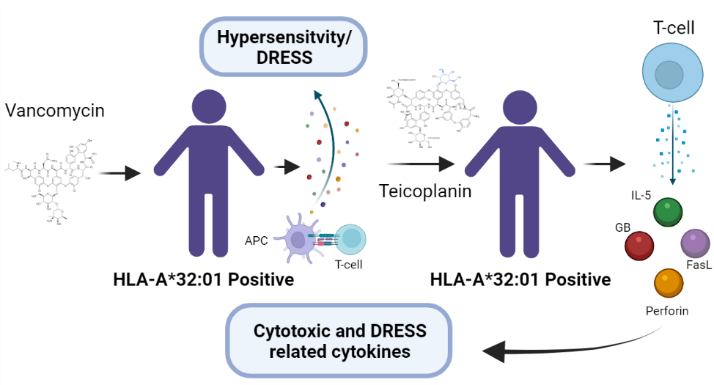
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