Characterization 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 to 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). Glycopeptide antibiotics, such as teicoplanin, have been utilized 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. Teicoplanin is typically administered as a second 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%) 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. Case studies have reported clinical cross-reactivity and subsequent teicoplanin-induced DRESS following initial vancomycin hypersensitivity. Preliminary in vitro studies using vancomycin-responsive T-cells generated from HLA-A*32:01 positive healthy donor PBMCs have already demonstrated low levels of cross-reactivity with teicoplanin. 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. 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 (HDs), in addition to further exploring patterns of cross-reactivity to structurally related glycopeptides.

Teicoplanin-specific T-cell clones (TCCs), generated by serial dilution, were identified in 3 healthy donors positive for HLA-A*32:01 expression (Figure 1). TCCs 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) was restricted to monoclonal populations enriched for CD8+ T-cells, as upon expansion, CD4+ TCCs did not respond to teicoplanin following confirmatory dose–response tests. Drug-responsive clonal populations that exclusively expressed a CD8+ phenotype were expanded via mitogen driven stimulation for further functional analysis.

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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 coculture with teicoplanin-reactive TCCs (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 coculture of fixed APCs and teicoplanin. These data suggest teicoplanin is able to activate CD8+ TCCs in a processing independent manner in which direct pharmacological interactions with MHC, concordant with the p-i concept, evoke T-cell responses to drug.

Cytokine and cytolytic molecule secretion of teicoplanin-reactive TCCs was assessed via ELISpot after a 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 was not present (data not shown). Interestingly, secretion of cytolytic molecules was detected in all TCCs profiled. Most notably, increased secretion of granzyme B, perforin, and FasL indicated involvement of cytotoxic T-cell responses and potential for activation of pro-apoptotic pathways. A 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, TCCs 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).

In summary, teicoplanin-responsive T-cells displaying a CD8+ phenotype were generated from 3 drug-naive healthy donors expressing the HLA-A*32:01 allele, recently associated with cases of vancomycin-induced DRESS. Therapeutic concentrations associated with glycopeptide administration are typically between 10 and 20 μM, substantially lower than the optimal doses used within this study to elicit maximal T-cell responses for functional analysis. However, we have observed that glycopeptide-specific TCCs 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
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; PBMCs, peripheral blood mononuclear cells; HLA, human leukocyte antigen; SI, stimulation index; TCC, T-cell clone

**REFERENCES**

1. Pavlos, R.; Mallal, S.; Ostrov, D.; Baus, S.; Metushi, I.; Peters, B.; Phillips, E. T cell-mediated hypersensitivity reactions to drugs. Annu. Rev. Med. 2015, 66, 439–454.

2. Campoli-Richards, D. M.; Brogden, R. N.; Faulds, D. Teicoplanin. A review of its antibacterial activity, pharmacokinetic properties and therapeutic potential. Drugs 1990, 40, 449–486.

3. Wood, M. J. The comparative efficacy and safety of teicoplanin and vancomycin. J. Antimicrob. Chemother. 1996, 37, 209–222.

4. Konvins, K. C.; Trubiano, J. A.; Pavlos, R.; James, I.; Shaffer, C. M.; Bejan, C. A.; Schutte, R. J.; Ostrov, D. A.; Pilkinson, M. A.; Rosenbach, M.; Zwerner, J. P.; Williams, K. B.; Bourke, J.; Martinez, P.; Rwandamuriye, F.; Chopra, A.; Watson, M.; Redwood, A. J.; White, K. D.; Mallal, S. A.; Phillips, E. J. HLA-A*32:01 is strongly...
associated with vancomycin-induced drug reaction with eosinophilia and systemic symptoms. *Journal of allergy and clinical immunology* 2019, **144**, 183−192.

(5) Kwon, H. S.; Chang, Y. S.; Jeong, Y. Y.; Lee, S. M.; Song, W. J.; Kim, H. B.; Kim, Y. K.; Cho, S. H.; Kim, Y. Y.; Min, K. U. A case of hypersensitivity syndrome to both vancomycin and teicoplanin. *J. Korean Med. Sci.* 2006, **21**, 1108−1110.

(6) Hsiao, S. H.; Chen, H. H.; Chou, C. H.; Lin, W. L.; Liu Yeh, P. Y.; Wu, T. J. Teicoplanin-induced hypersensitivity syndrome with a preceding vancomycin-induced neutropenia: a case report and literature review. *J. Clin Pharm. Ther* 2010, **35**, 729−732.

(7) Ogese, M. O.; Lister, A.; Gardner, J.; Meng, X.; Alfirevic, A.; Pirmohamed, M.; Park, B. K.; Naisbitt, D. J. Deciphering adverse drug reactions: in vitro priming and characterization of vancomycin-specific T-cells from healthy donors expressing HLA-A*32:01. *Toxicol. Sci.* 2021, **183**, 139.

(8) Nakkam, N.; Gibson, A.; Mouhtouris, E.; Konvinse, K.; Holmes, N.; Chua, K. Y.; Deshpande, P.; Li, D.; Ostrov, D. A.; Trubiano, J.; Phillips, E. J. Cross-reactivity between vancomycin, teicoplanin and telavancin in HLA-A*32:01 positive vancomycin DRESS patients sharing an HLA-Class II haplotype. *J. Allergy Clin. Immunol.* 2021, **147**, 403.

(9) Mauri-Hellweg, D.; Bettens, F.; Mauri, D.; Brander, C.; Hunziker, T.; Pichler, W. J. Activation of drug-specific CD4+ and CD8+ T cells in individuals allergic to sulfonamides, phenytoin, and carbamazepine. *J. Immunol.* 1995, **155**, 462−472.

(10) Choquet-Kastylevsky, G.; Intrator, L.; Chenal, C.; Bocquet, H.; Revuz, J.; Roujeau, J. C. Increased levels of interleukin 5 are associated with the generation of eosinophilia in drug-induced hypersensitivity syndrome. *Br J. Dermatol.* 1998, **139**, 1026−1032.