

Supplementary Data

A multivalent T-antigen-based vaccine for Group A Streptococcus

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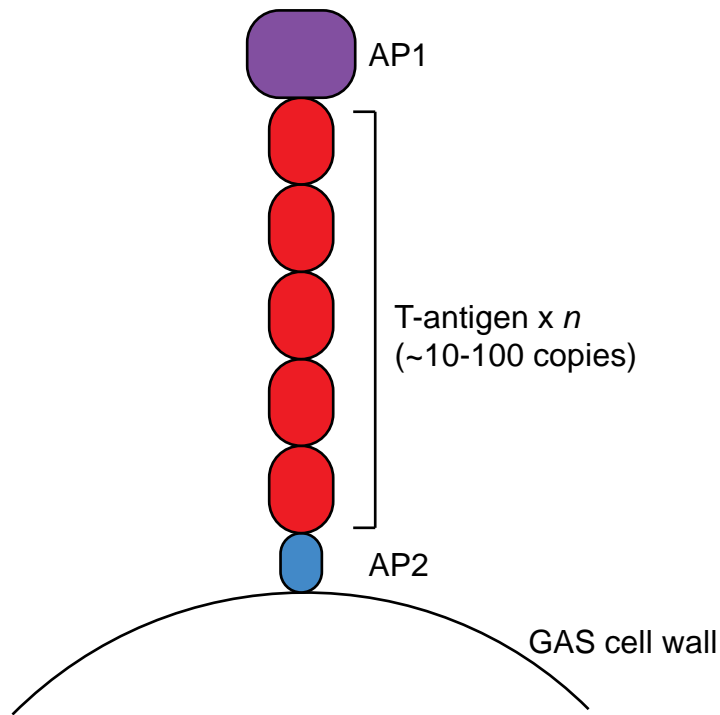


Fig. S1. Schematic diagram of the GAS pilus showing the T-antigen targeted for vaccination by TeeVax.

The GAS pilus is formed by covalent attachment of multiple pilus subunits to the cell wall. Strains express either one or two adaptor proteins (AP1 and AP2). Polymerisation of multiple copies of the T-antigen forms the pilus shaft.

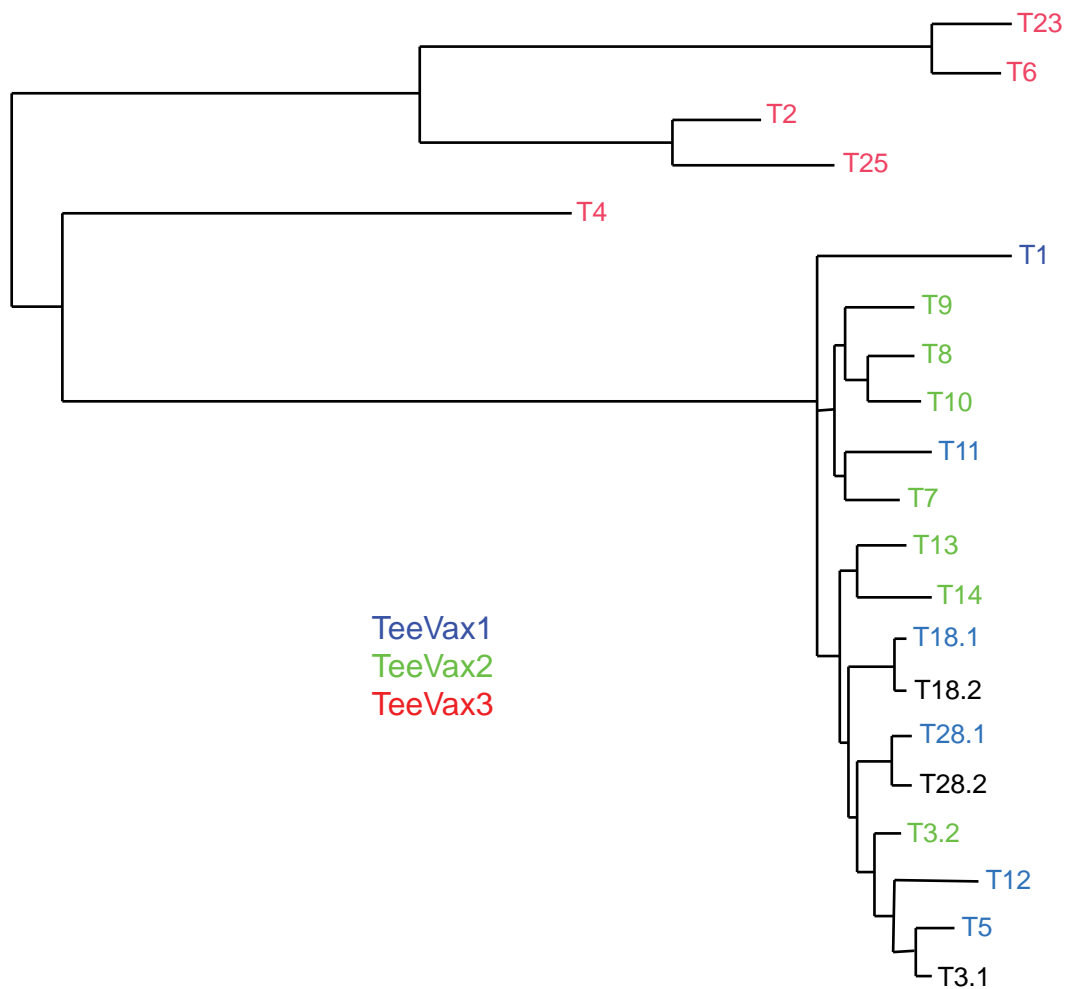


Fig. S2. Phylogenetic tree of T-antigen sequences.

The phylogenetic tree was generated by multiple sequence alignment of T-antigen amino acid sequences from strains listed in Table S1 using ClustalW. The tree was inferred using PhyML v20160115 at [<https://www.genome.jp/tools-bin/clustalw>]. T-antigens incorporated into TeeVax1 (blue), TeeVax2 (green), and TeeVax3 (red) are indicated.

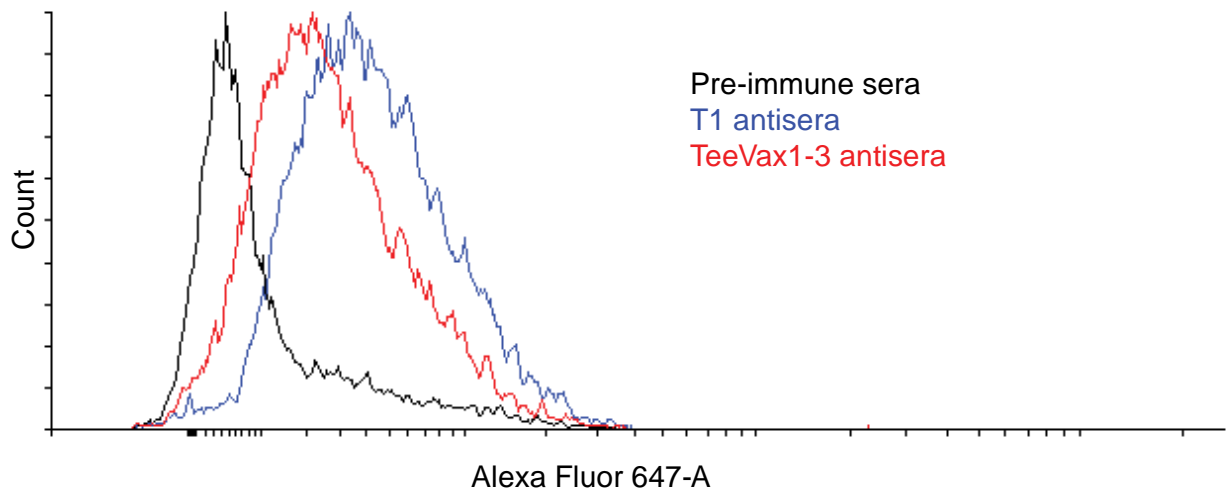


Fig. S3. Flow cytometry showing antibody detection of native pilus on GAS.

GAS SF370 (M1T1) grown to early exponential phase was incubated with rabbit sera and antibody-binding detected with anti-rabbit IgG- Alexa Fluor 674.

| Strain | <i>emm</i>-type | <i>tee</i>-type | Origin |
|---------------|------------------------|------------------------|--------------------------------|
| SF370 | 1 | 1 | ATCC |
| 5448 | 1 | 1 | Clinical isolate ⁵⁰ |
| MGAS10270 | 2 | 2 | ATCC |
| GAS13123 | 78 | 3.1 | ESR |
| GAS1142 | 65 | 3.2 | ESR |
| MGAS10750 | 4 | 4 | ATCC |
| 9893 | 82 | 5 | ESR |
| MGAS10394 | 6 | 6 | ATCC |
| GAS10143 | 114 | 7 | ESR |
| GAS13101 | 85 | 8 | ESR |
| GAS13345 | 74 | 9 | ESR |
| GAS10233 | 81 | 10 | ESR |
| 89/54 | 89 | 11 | ESR |
| 94/354 | 12 | 12 | ESR |
| 10028 | 41 | 13 | ESR |
| GAS09426 | 101 | 14 | ESR |
| MGAS8232 | 18 | 18.1 | ATCC |
| 57G | 49 | 18.2 | ESR |
| GAS11550 | 75 | 25 | ESR |
| MGAS6180 | 28 | 28.1 | ATCC |
| GAS11209 | 91 | 28.2 | ESR |

Supplementary Table 1. GAS strains used in this study.

Abbreviations: ATCC, American Type Culture Collection; ESR, Institute of Environmental Science and Research New Zealand.

Supplementary Table 2. Primers used in this study

| PCR primer pair | Template | Description of product amplified |
|--|---------------------|--|
| Spy0128.fw cgggatccgctacaacagttcacgg | gDNA from SF370 | T1-antigen |
| Spy0128.rv cggaattcttattcaagactttttatttg | | |
| Spy0128.fw cgggatccgctacaacagttcacgg | gDNA from MGAS10270 | N-term domain of T1-antigen included in TeeVax1 |
| M1_FctAN.rev gcgaattcaagatct agtagaatcaagctatttttg | | |
| spyM2_0109.fw ggatcc gaggacaccagagtgcttctc | gDNA from MGAS10270 | T2-antigen |
| spyM2_0109.rev aagctt tta accaccagtattaggattgaagg | | |
| LT2M.fw cgggatccggcagcggtagcggcaaaaatacaacagttaatgaacctaaag | gDNA from MGAS10270 | Linker + Middle domain of T2-antigen included in TeeVax3 |
| T2M.rv gcgaattcaagatcttctgtatgcacttcaggaacttc | | |
| T3.2m65.fw cgggatcc gagacggcaggagtgctc | gDNA from GAS13123 | T3.1-antigen |
| T5 M82R gcgaattcaagatct agtgcacgctattttgacaac | | |
| T3.2m65.fw cgggatcc gagacggcaggagtgctc | gDNA from GAS1142 | T3.2-antigen |
| m41m65_fctac_no sm.rev gcgaattcaagatct agtgcacgctatttttg | | |
| M65_FctAC.fw cgggatcc tcgttaaaagttaagaagaatg | gDNA from GAS1142 | C-term domain of T3.2-antigen included in TeeVax2 |
| m41m65_fctac_no sm.rev gcgaattcaagatct agtgcacgctatttttg | | |
| spyM4_0116.fw cgggatcc gaatcatcacataaaaccgatg | gDNA from MGAS10750 | T4-antigen |
| spyM4_0116.rev cggaattc tta accaccagtttgaggatgg | | |
| LT4N.fw cgggatccggcagcggtagcggcgaatcatcacataaaaccgatg | gDNA from MGAS10750 | Linker + N-term domain of T4-antigen included in TeeVax3 |
| LT4N.rev gcgtcgaacttaagatctctctgttggattcttagg | | |
| T5 M82F cgggatcc gagacggcaggagttgtaac | gDNA from 9893 | T5-antigen |
| T5 M82R gcgaattcaagatct agtgcacgctattttgacaac | | |
| M82_FctAC.fw cgggatcc tccttaaaaattgaaaaacaataac | gDNA from 9893 | C-term domain of T5-antigen included in TeeVax1 |
| M82_FctAC.rev gcgaattcaagatct agttggaacttgagtgctcac | | |
| spyM6_0160.fw ggatcc ttatcaaaagatgatactgcacaac | gDNA from MGAS10394 | T6-antigen |
| spyM6_0160.rev aagctt tta tcc accttcgaagtaattcacc | | |
| T6M.fw cgggatccccatctattacaagaagaatcacc | gDNA from MGAS10394 | Middle domain of T6-antigen included in TeeVax3 |
| T6M.rev gcgaattcaagatctataagataaacaattttagaatctctcttg | | |
| T7m114.fw cgggatcc gagacggcaggggtgattg | gDNA from GAS10143 | T7-antigen |
| M41M65_FctAC_no SM.rev gcgaattcaagatct agtgcacgctatttttg | | |
| T7m114C.fw cgggatccctattagaagttaaaaag | gDNA from GAS10143 | C-term domain of T7-antigen included in TeeVax2 |
| M41M65_FctAC_no SM.rev gcgaattcaagatct agtgcacgctatttttg | | |
| M85_FctAN.fw cgggatcc gagacggcaggggtgac | gDNA from GAS13101 | T8-antigen |
| M41M65_FctAC_no SM.rev gcgaattcaagatct agtgcacgctatttttg | | |
| M85_FctAN.fw cgggatcc gagacggcaggggtgac | gDNA from GAS13101 | N-term domain of T8-antigen included in TeeVax2 |
| M85_FctAN.rev gcgaattcaagatct tgggtatcaaaaagaattttcaata | | |
| M74_FctAN.fw cgggatcc gaaggtggagtgagtagtctgg | gDNA from GAS13345 | T9-antigen |
| M41M65_FctAC_no SM.rev gcgaattcaagatct agtgcacgctatttttg | | |
| M74_FctAN.fw cgggatcc gaaggtggagtgagtagtctgg | gDNA from GAS13345 | N-term domain of T9-antigen included in TeeVax2 |
| M74_FctAN.rev gcgaattcaagatct tgggttgaattcgttctg | | |
| T10m81.fw cgggatcc gagacggcaggggttgt | gDNA from GAS10233 | T10-antigen |
| M81_FctAC.rev gcgaattcaagatct agttggataggcaagtttctc | | |
| M81_FctAC.fw | gDNA from GAS10233 | C-term domain of T10-antigen included in TeeVax2 |

| | | |
|--|------------------------|---|
| cgggatcc tcttaactatcaaaaaagaagttac | | |
| M81_FctAC.rev gcgaattcaagatct agtgggataggaagttctc | | |
| T11 M89F cgggatcc gaagtaaattatgtaaatcaggag | gDNA from 89/54 | T11-antigen |
| T11 M89R gcgaattcaagatct agtgcacgcttattgtgactg | | |
| M89_FctAC.fw cgggatcc tctttagttgtaaaaaagagg | gDNA from 89/54 | C-term domain of T11-antigen included in TeeVax1 |
| M89_FctAC.rev gcgaattcaagatct agttggaacttgagtgcac | | |
| T12 M12F cgggatcc gagacggcaggggtttag | gDNA from 94/354 | T12-antigen |
| T12 M12R gcgaattcaagatct agtgcacggttattgtgacaac | | |
| M12_FctAC.fw cgggatcc tcattaaaaattgaaaggaag | gDNA from 94/354 | C-term domain of T12-antigen included in TeeVax1 |
| M12_FctAC.rev gcgaattcaagatct agttggaacttgagtgcac | | |
| M101_FctAN.fw cgggatcc gagacggcaggggttgaacag | gDNA from 10028 | T13-antigen, also included in TeeVax2 |
| T14m101_noSM.rev gcgaattcaagatct agtgcacggttattataactaag | | |
| M101_FctAN.fw cgggatcc gagacggcaggggttgaacag | gDNA from GAS09426 | T14-antigen |
| T14m101_noSM.rev gcgaattcaagatct agtgcacggttattataactaag | | |
| M101_FctAN.fw cgggatcc gagacggcaggggttgaacag | gDNA from GAS09426 | N-term domain of T14-antigen included in TeeVax2 |
| M101_FctAN.rev gcgaattcaagatct agttgtatcaaaaagaatttttaaaa | | |
| M18_0128.fw cgggatccgagacagcaggagtgattg | gDNA from MGAS8232 | T18.1-antigen |
| M18_0128.rev cggaattcttattcgtcagtagattg | | |
| M18_0128.fw cgggatccgagacagcaggagtgattg | gDNA from MGAS8232 | N-term domain of T18-antigen included in TeeVax1 |
| M18_FctAN.rev gcgaattcaagatct agttgtatcaaaaaatttttaaac | | |
| spyM49_fctA.fw ctaggatccgagacagcaggagtgattg | gDNA from 57G | T18.2-antigen |
| spyM28_0109.rev cggaattcttaaacaccagttggaacttgagtg | | |
| T23m23F cgggatccttatacaaaagatgataaggcggag | Synthetic DNA from IDT | T23-antigen |
| T23m23R gcgaattcaagatcttccactaattgtgttaggaattc | | |
| LT23M.fw cgggatccgagcagcggtagcggcccaacaattagcaagtcaattac | Synthetic DNA from IDT | Linker + Middle domain of T23-antigen included in TeeVax3 |
| LT23M.rv gcgaattcaagatctataggttagacggttttttatcc | | |
| TEE25FWD aaaggatccaataccaagtagtacggt aaggg | gDNA from GAS11550 | T25-antigen |
| TEE25REV aaagaattcttattcaggacgtttgt gtttctac | | |
| LT25M.fw cgggatccgagcagcggtagcggcccaaaaccggtaaggatg | gDNA from GAS11550 | Linker + Middle domain of T25-antigen included in TeeVax3 |
| T25M.rv gcgaattcaagatctttagtaactcaggattatctgaag | | |
| spyM28_0109.fw cgggatcc gagacggcagagtgctccgaaaatg | gDNA from MGAS6180 | T28.1-antigen |
| spyM28_0109.rev cggaattcttaaacaccagttggaacttgagtg | | |
| spyM28_0109.fw cgggatcc gagacggcagagtgctccgaaaatg | gDNA from MGAS6180 | N-term domain of T28.1-antigen included in TeeVax1 |
| M28_FctAN.rev gcgaattcaagatct agttgtctaaaggattttc | | |
| TEE28.1FWD aaaggatctgagacggcagggtaac taa | gDNA from GAS11209 | T28.2-antigen |
| TEE28.1REV aaagaattcttattcaggttattgt gacaacgat | | |