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Supplementary Materials for

Antibodies to the conserved region of the M protein and a streptococcal superantigen cooperatively resolve toxic shock-like syndrome in HLA-humanized mice

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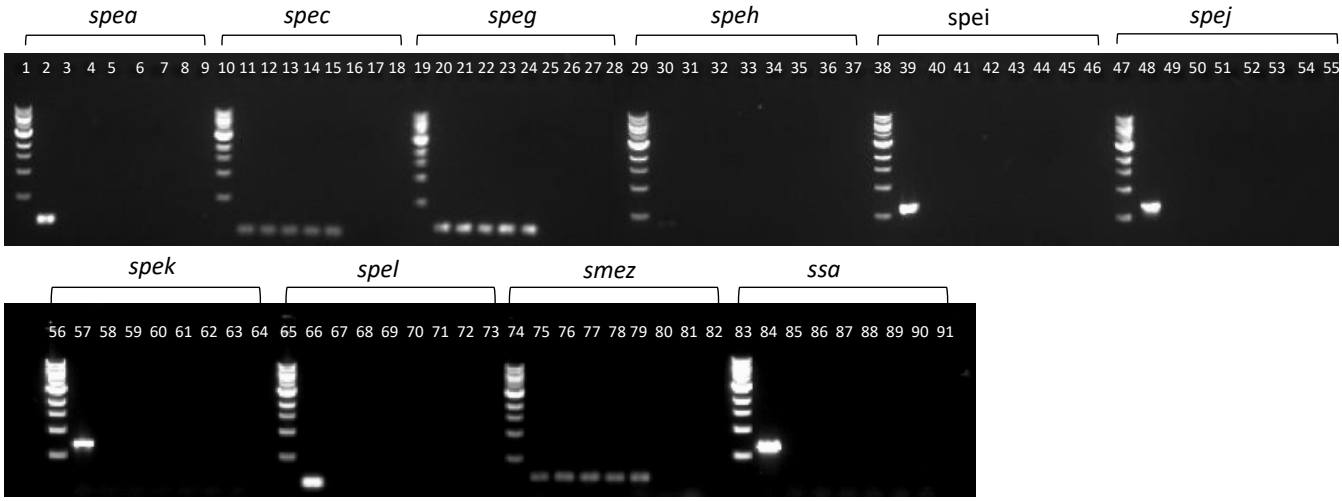
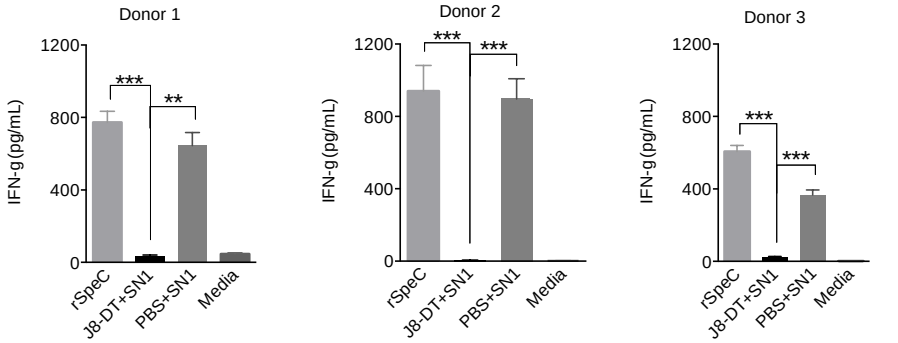
This PDF file includes:



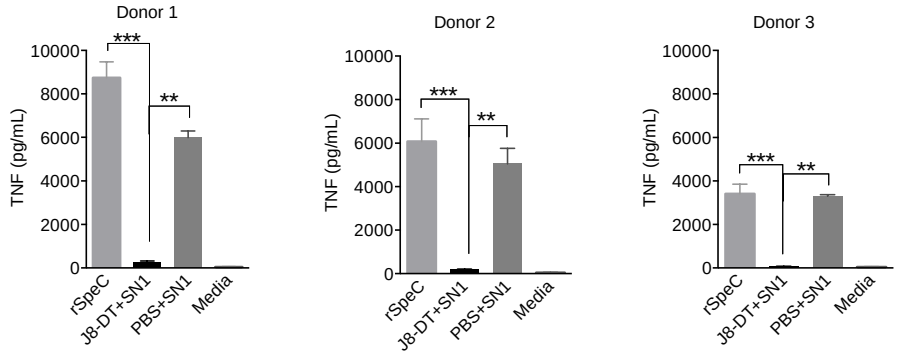
Spea	*spec*	*speg*	*speh*	*Spei*	*spej*	*spek*	*spel*	*smez*	*ssa*
1. 1 kb ladder	10. 1 kb ladder	19. 1 kb ladder	29. 1 kb ladder	38. 1 kb ladder	47. 1 kb ladder	56. 1 kb ladder	65. 1 kb ladder	74. 1 kb ladder	83. 1 kb ladder
2. Pos. control	11. Pos. control	20. Pos. control	30. Pos. control	39. Pos. control	48. Pos. control	57. Pos. control	66. Pos. control	75. Pos. control	84. Pos. control
3. SN1	12. SN1	21. SN1	31. SN1	40. SN1	49. SN1	58. SN1	67. SN1	76. SN1	85. SN1
4. SN2	13. SN2	22. SN2	32. SN2	41. SN2	50. SN2	59. SN2	68. SN2	77. SN2	86. SN2
5. SN3	14. SN3	23. SN3	33. SN3	42. SN3	51. SN3	60. SN3	69. SN3	78. SN3	87. SN3
6. SN4	15. SN4	24. SN4	34. SN4	43. SN4	52. SN4	61. SN4	70. SN4	79. SN4	88. SN4
7. NS33	16. NS33	25. NS33	35. NS33	44. NS33	53. NS33	62. NS33	71. NS33	80. NS33	89. NS33
8. H_2O	17. H_2O	26. H_2O	36. H_2O	45. H_2O	54. H_2O	63. H_2O	72. H_2O	81. H_2O	90. H_2O

Fig. S1. SAg profile of streptococcal isolates. The genomic DNA (gDNA) was extracted from overnight stationary phase cultures using the GenElute bacterial gDNA extraction kit from Sigma. The gDNA was qualified using the Nanodrop1000 and then 2 μg of gDNA used for amplification of the superantigens. Gels were then run as per the image legend. NS33 is a group C streptococcal isolate that was used as a 'superantigen negative' control.

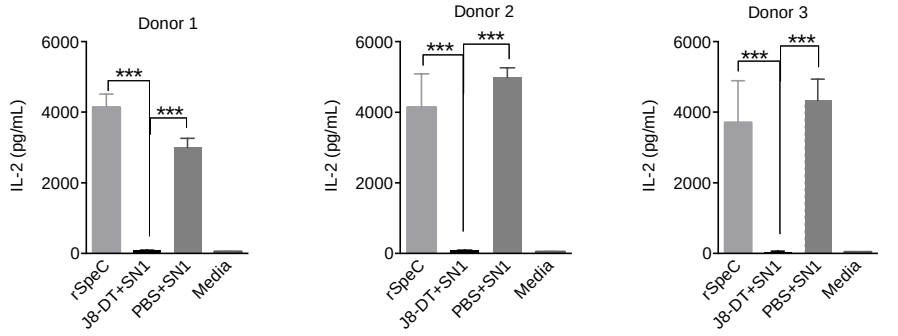
IFN-g



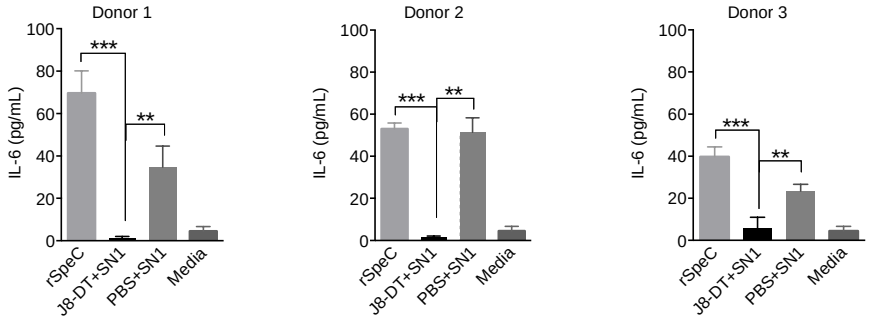
TNF



IL-2



IL-6



IL-17

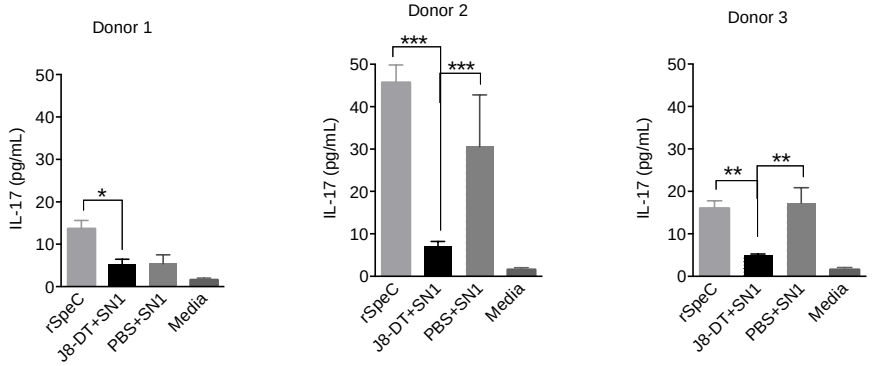


Fig. S2. Cytokine response of PBMCs following stimulation with vaccinated and control sera. PBMC from three different individuals were stimulated with 20 μL of serum from vaccinated-SN1 infected or control-SN1 infected mice. Optimal concentrations of rSpeC and PHA were used as a positive control for stimulation. The inhibitory effect of rSpeC antisera was assessed by adding a pre-optimized amount (20 μL) of rSpeC antisera to selected wells containing vaccinated-SN1 infected or control-SN1 infected sera or rSpeC. Media alone wells were used as negative controls. Cytokine responses were measured in the culture supernatants using CBA kit after 72 h of *in vitro* culture. Data are Mean± SEM of 3 replicates in each experiment with experiments repeated twice. Statistical analysis was performed using non-parametric, unpaired Mann-Whitney U-test to compare the two groups. *$p<0.05$, **$p<0.01$ and ***$p<0.001$.

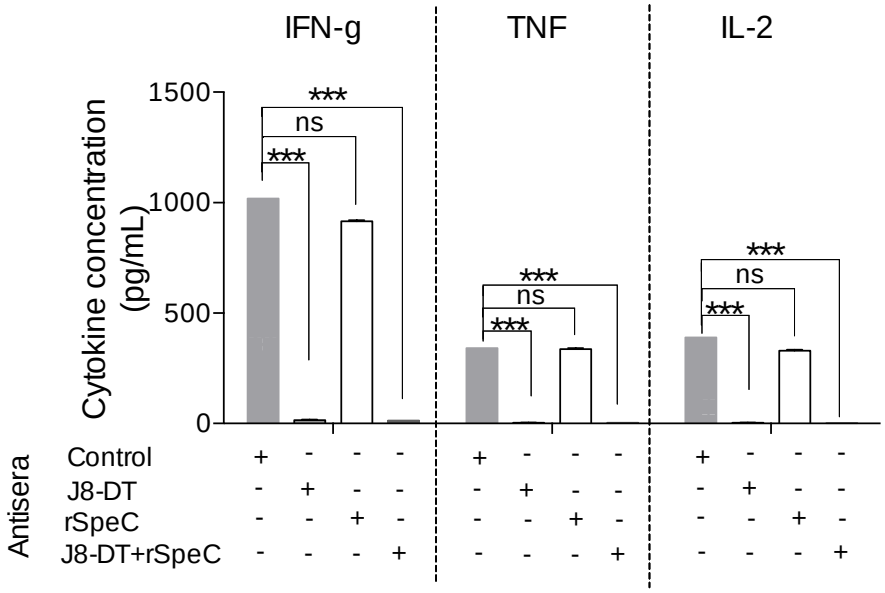


Fig. S3. Cytokine profile in HLA-B6 mice after stimulation with rM1. Splenocytes from HLA-B6 mice were stimulated with a pre-optimised concentration (5 μg/mL) of rM1. To assess inhibition of rM1 by antibodies to various antigens, 20 μL of J8-DT, rSpeC or J8-DT+rSpeC antisera were added to each well. Non-immune sera from PBS treated mice was used as control. The culture supernatants were collected at 72 h post stimulation and cytokine induction by splenocytes in the presence or absence of various antisera assessed using a CBA kit. Concentrations of IFN-γ, TNF and IL-2 in the presence or absence of various antisera are shown. One-way ANOVA with Tukey's post-hoc method was utilised to calculate significance between various groups. ***$p<0.001$ and ns $p>0.05$.

A

Peptide	Sequence
J14	**KQAEDKVKASREAKKQVEKALEQLEDKVK**
J14.1	**KQAEDKVKASREAKKKVEADLAQLEDKVK**

B

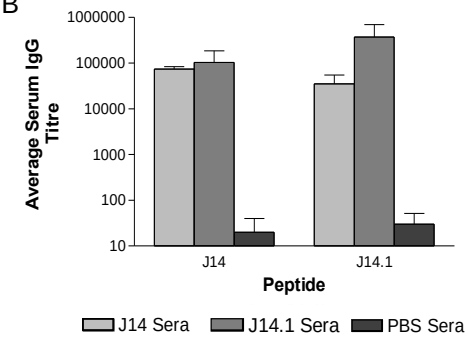


Fig. S4. Immunological cross-reactivity between J14 and J14.1. To assess immunological cross-reactivity between J8 and J8.1, we tested cross-reactivity between two slightly longer peptides, J14 and J14.1, which contain the J8 and J8.1 sequence, respectively. J14 and J14.1 ELISA assays were performed. (**A**) The peptide sequences are shown in the table. The sequence identity between the peptides is shown in bold and the underlined residues highlight the differences. (**B**) 96 well plates were coated with J14 or J14.1 peptide at 5 μg/mL concentration. J14 and J14.1 antisera raised in B10.BR mice were tested against both peptides. As a control, PBS serum was also included. Antigen specific titers are shown.

Table S1. Clinical isolates and medical history of patients.

Laboratory Identifier	Group A Streptococcus culture	Location	Year	Genotype	Clinical history
SN1	Blood	Queensland, Australia	2013	*emm89*; *speb* and *spec* positive	91-year old male long stay inpatient of rehabilitation unit; new onset fever, septic shock. Deceased
SN2	Blood	Queensland, Australia	2013	*emm89*; *speb* and *spec* positive	95-year old male in same unit developed acute sepsis with hypotension, septic shock. Deceased
SN3	Blood and Skin	Queensland, Australia	2013	*emm89*; *speb* and *spec* positive	87-year old female - necrotising fasciitis associated with fever and hypotension; Surgical debridement. Survived
SN4	Skin	Queensland, Australia	2014	*emm89*; *speb* and *spec* positive	68-year old male undergoing rehabilitation following brain tumour resection. 2 months after the first 3 cases developed fever, rash, pharyngitis and blistering cellulitis. Survived

Table S2. Primers used for SAg gene profiling.

Gene	Forward primer (5'to 3')	Reverse primer (5'to 3')	Annealing temp (ºC)	Reference
spea	TGATAGGCTTTGGATACCATCG	GAACCAAGAGATGGCAACTTTATT	60	[*26*]
spec	GATTTCTACTATTTCACC	AAATATCTGATCTAGTCCC	62	[*46*]
speg	ACCCCATGCGATTATGAAAA	GGGAGACCAAAAACATCGAC	60	[*26*]
speh	TTGGATCCAATTCTTATAATACAACC	CCACTTCCTGAGCGGTTACTTTCGG	60	[*26*]
spei	ATGAGTAGTGTGGGAGTTATTAA	ATGAAGTTGATCAGAATAAGCG	55	[*46*]
spej	ATCTTATTTAGTCCAAAGGTAAAT	GTGAACGAGGAGAGGTATGAA	55	[*46*]
spek	GCGGATCCGATACGTACAATACAAATG	GCGAATTCAATAGCATTCAACCA	55	[*46*]
spel	ATAAGTCAGCACCTTCCTCTTTC	AAATCTCCCGTTACCTTCCA	60	[*26*]
smez	TTTCTCGTCCTGTGATTGGA	AATGGGACGGAGAACATAGC	60	[*26*]
ssa	AGTCAGCCTGACCCTACTCCA	TAAGGTGAACCTCTATAGCTATAG	59.1	[*46*]

Table S3. Distribution of *spec* and *spea* genes in ISD isolates from Canada.

2017-2018 collection for whole genome sequencing			
***emm* type**	**Number of isolates**	***spec* positive**	***spea* positive**
emm1	11	1	11
emm101	9	4	0
emm11	8	4	0
emm4	7	5	0
emm41	7	4	0
emm59	8	0	0
emm76	8	0	0
emm81	8	0	0
emm82	5	0	0
emm87	1	1	0
emm89	5	5	0
Percent positive	77	**24/77 = 31.17%**	**11/77 = 14.29%**