

Supplementary Information

Identifying protective *Streptococcus pyogenes* vaccine antigens recognized by both B and T cells in human adults and children

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Supplementary Table 1. Genomic sequences used for analysis of sequence conservation for the GAS antigens in this study.

No. in database	Strain name	Type
1	SF370	<i>emm1</i>
2	MGAS5005	<i>emm1</i>
3	A20	<i>emm1</i>
4	M1GAS476	<i>emm1</i>
5	MGAS10270	<i>emm2</i>
6	MGAS315	<i>emm3</i>
7	SSI-1	<i>emm3</i>
8	MGAS10750	<i>emm4</i>
9	Manfredo	<i>emm5</i>
10	ATCC 19615	<i>emm5/49</i>
11	MGAS10394	<i>emm6</i>
12	MGAS9429	<i>emm12</i>
13	MGAS2096	<i>emm12</i>
14	HKU QMH11M0907901	<i>emm12</i>
15	HKU360	<i>emm12</i>
16	HSC5	<i>emm14</i>
17	MGAS8232	<i>emm18</i>
18	M23ND	<i>emm23</i>
19	MGAS6180	<i>emm28</i>
20	STAB901	<i>emm44</i>
21	1E1	<i>emm44</i>
22	NZ131	<i>emm49</i>
23	Alab49	<i>emm53</i>
24	MGAS15252	<i>emm59</i>
25	MGAS1882	<i>emm59</i>
26	7F7	<i>emm83</i>

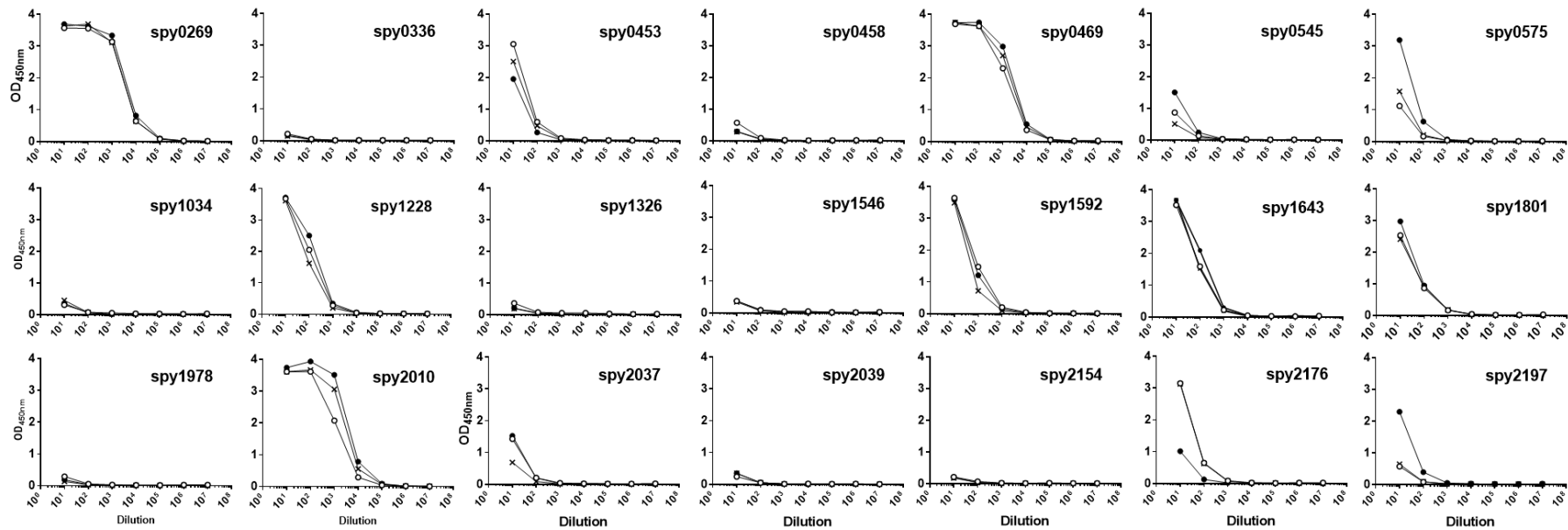
Supplementary Table 2. Cloning strategy for the selected antigens of the study

Locus name (M1 SF370)	MW (kDa)	Clonined fragment
spy0269	94.7	aa33-849
spy0336	57.9	aa40-190
spy0453	34.4	aa21-310
spy0458	89.3	aa180-801, Δ aa477-499
spy0469	41.1	aa24-389
spy0545	32.0	aa1-281, Δ aa185-203
spy0575	31.3	aa91-271, Δ 198-215
spy1034	29.6	aa1-263, Δ aa103-121
spy1228	36.4	aa27-350
spy1326	41.2	aa1-360
spy1546	16.0	aa1-120
spy1592	53.6	aa26-380
spy1643	53.0	aa1-489, Δ aa178-197
spy1801	54.5	aa25-503, Δ aa251-270
spy1978	32.5	aa1-282, Δ aa138-162, Δ aa189-209
spy2010	122.3	aa90-1039, Δ Asp130, Δ His193, Δ Ser512
spy2037	34.4	aa27-309
spy2039	40.3	aa146-398, Cys192 -> Ser, His340 -> Gln
spy2154	32.5	aa128-292, Δ aa155-173
spy2176	80.1	aa35-577
spy2197	37.9	aa1-341, Δ aa181-200

Δ :deletions (replaced by -(GGGS)₂-)

aa: amino acid number

->: amino acid substitution

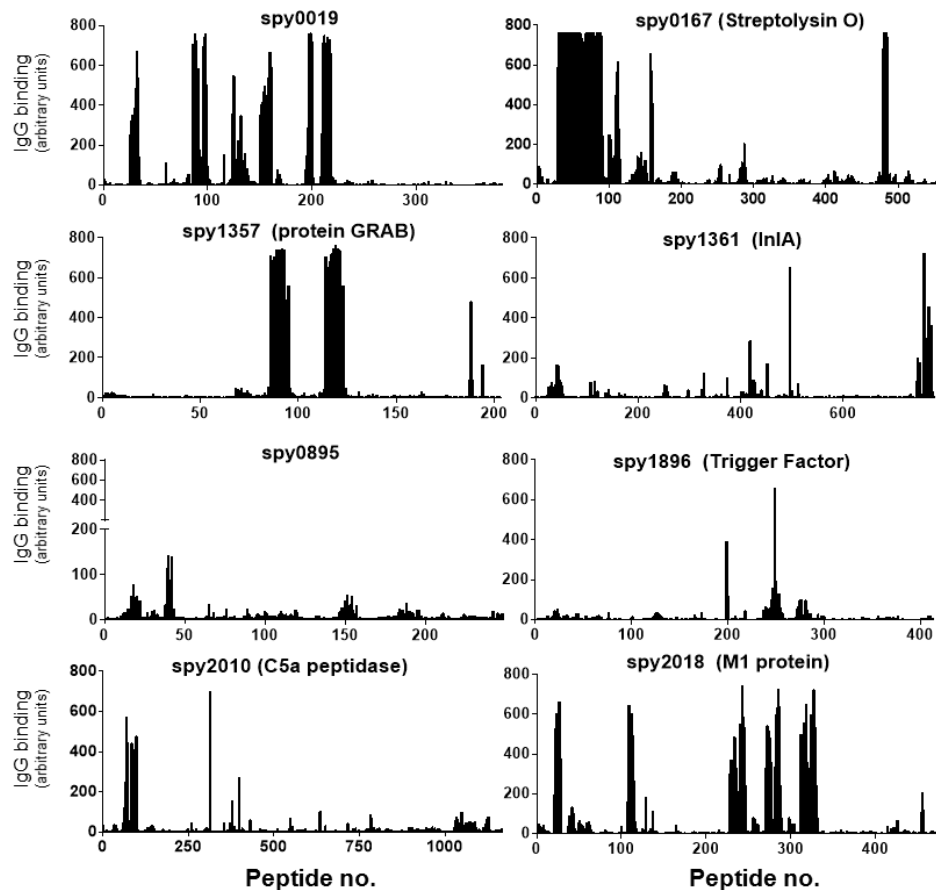


Supplementary Figure 1. Serum titration data for IgG reponses measured by ELISA

Antigen-specific IgG was determined by ELISA in three plasma pools from healthy adults (10-12 individuals in each pool). Symbols indicate individual data points for (x) pool I (●) pool II and (○) pool III

Supplementary Table 3. Immunogenic regions recognized by human IgG measured by a peptide array of 15-mers spanning the entire length of each antigen

Antigen	aa nr.	epitope sequence
spy0269	36-52	ADDRASGETKASNTHDD
	102-121	QDNEQKAL TSAQE IYTNTLA
	129-149	AQGAEHQRELTATE TELHNAQ
	185-216	QNI AKLNAMISNPDAITKAAQTANDNTKALSS
	743-765	TVPDLQVAPPLTG VKPLSYSKID
	758-782	PLSYSKIDTTPLVQEMVKETKQLLE
	856-873	VMLAAVGLTGFRFRKESK
spy0469	23-42	AQAQEWTPRSVTEIKSELVL
	140-161	ATPSDVPTTPFASAKPDSSVTA
	203-225	EPKTDISEAPTSANRPVPNESAS
spy1228	122-136	GVK FVI IDDI IEGKD
spy1643	167-184	SHHPAKQNTKKGWLIALF
	223-237	DSAKKATRLKAAAKA
	282-298	KAKVDSLKKAIAAITAV
spy1801	95-109	IGSSDNKAEAE NQVD
	106-120	NQVDDKSTDH SKSTD
	151-171	RTPIQSLSPYVSDLDLSEIDI
	329-356	DTTGSGKRRAQIMEDLDKWIDDHGGTPA



Supplementary Figure 2. Antibody epitopes of known antigens identified by peptide array technology.

Binding of human IgG on 15-mer peptides overlapping with 14 amino acids on a peptide array spanning every putative protein in the GAS M1 SF370 genome. Histograms are examples of known antigens. IgG binding was measured with a Cy3-conjugated goat anti-human antibody after incubation with plasma pooled from six adult donors. The black bars indicate fluorescence intensity for each of the peptides in arbitrary units that arise from summarizing over the R, G and B channels in the resulting image file. Peptides were randomly distributed on the array and data was mapped back in the chronological sequence of the antigen after the experiment. Spy0895 was included as an example of a known protective antigen, without an epitope >300AU.

Locus name (M1 SF370)	Description	References
spy0019	Putative secreted protein	[24,25]
spy0167	Streptolysin O	[25,42]
spy0895	Predicted histidine protein kinase	[24]
spy1357	Protein G-related alpha 2M-binding protein; GRAB	[27]
spy1361	Putative internalin A (InIA)	[25]
spy1896	Trigger Factor	[26]
spy2010	Streptococcal C5a peptidase; ScpA	[33]
spy2018	M protein type 1	[3,4]