

SUPPLEMENTAL MATERIAL

Nordenfelt et al., <http://www.jem.org/cgi/content/full/jem.20120325/DC1>

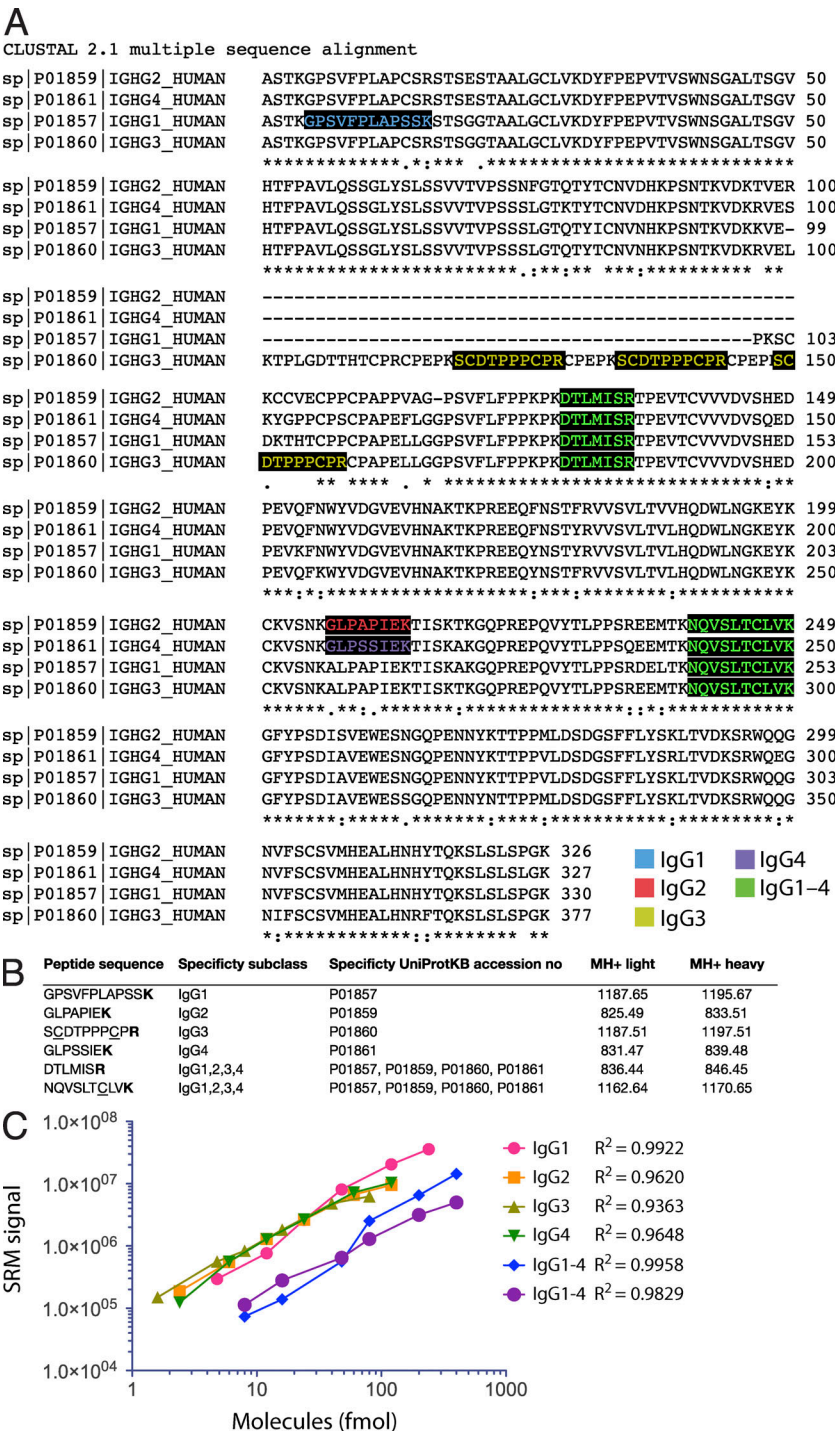


Figure S1. SRM analysis of IgG subclass distribution. (A) Sequence alignment comparing the different human IgG subclasses. Peptides that were selected for identification using SRM analysis are highlighted with the indicated colors. (B) Table of selected peptides for SRM analysis of IgG subclasses. Bold amino acids are isotopically labeled residues of heavy peptides. Cysteines (underlined) are carbamidomethylated. MH+ is the mass of precursor ions of either unlabeled (light) or labeled (heavy) peptides. (C) Signal to dose curve for the selected AQUA-IgG peptides with linear regression values listed in the legend. This information was used in all the SRM-IgG experiments to add the AQUA peptides at a concentration within their linear dynamic range..

| | plasma | | plasma | | plasma | | plasma | |
|---|---------|---------|---------|---------|---------|---------|---------|---------|
| | wt | wt | M- | M- | H- | H- | M- H- | M- H- |
| | AVERAGE | AVERAGE | AVERAGE | AVERAGE | AVERAGE | AVERAGE | AVERAGE | AVERAGE |
| Membrane cofactor protein | 2527 | 1001 | 2933 | 1522 | 4589 | 1265 | 2257 | 994 |
| Complement decay-accelerating factor | 724 | 2518 | 0 | 0 | 0 | 605 | 685 | 1492 |
| CD59 glycoprotein | 3359 | 5770 | 2817 | 2227 | 1797 | 3612 | 2715 | 4321 |
| C4b-binding protein alpha chain | 2236211 | 13551 | 3619122 | 976 | 22088 | 2178 | 31162 | 3826 |
| C4b-binding protein beta chain | 814793 | 3682 | 882229 | 722 | 6554 | 1951 | 8688 | 999 |
| Complement factor H (H factor 1) | 697000 | 97652 | 871810 | 26019 | 463907 | 24305 | 348603 | 20051 |
| Complement factor H-related protein 1 (FHR-1) | 15888 | 2800 | 7515 | 1381 | 40515 | 1597 | 34335 | 2517 |
| Complement factor H-related protein 2 (FHR-2) | 3637 | 2628 | 1048 | 1551 | 4470 | 2749 | 10573 | 0 |
| Complement factor H-related protein 3 (FHR-3) | 3787 | 533 | 7344 | 0 | 3797 | 0 | 4647 | 0 |
| Complement factor H-related protein 4 (FHR-4) | 5188 | 3066 | 27950 | 0 | 17255 | 0 | 14416 | 0 |
| Complement factor H-related protein 5 (FHR-5) | 19911 | 0 | 12336 | 0 | 40261 | 0 | 40464 | 1078 |
| Complement factor I | 2504 | 0 | 857 | 0 | 2148 | 756 | 2649 | 0 |
| Complement C1q subunit A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Complement C1q subunit B | 19716 | 0 | 147825 | 0 | 8005 | 4425 | 16183 | 618 |
| Complement C1q subunit C | 155887 | 1704 | 973750 | 0 | 101659 | 0 | 80529 | 473 |
| Complement C1r | 5018 | 0 | 7849 | 0 | 1900 | 0 | 4411 | 9 |
| Complement C1s | 113221 | 1114 | 111615 | 0 | 50120 | 531 | 41287 | 676 |
| Mannose-binding protein C | 0 | 1078 | 2775 | 1210 | 0 | 3005 | 0 | 547 |
| Mannan-binding lectin serine protease 1 | 0 | 670 | 809 | 0 | 0 | 3039 | 0 | 0 |
| Mannan-binding lectin serine protease 2 | 0 | 0 | 0 | 0 | 0 | 4870 | 0 | 0 |
| Ficolin-1 | 0 | 1494 | 0 | 0 | 0 | 2175 | 0 | 562 |
| Ficolin-3 | 0 | 0 | 0 | 0 | 0 | 1783 | 0 | 51 |
| Complement factor B | 25351 | 3010 | 16238 | 1122 | 100270 | 2268 | 91124 | 2140 |
| Complement factor D | 0 | 1871 | 0 | 1327 | 763 | 2043 | 327 | 1862 |
| Complement C3 | 568117 | 2129 | 301189 | 991 | 761947 | 3543 | 869422 | 4696 |
| Properdin | 1102256 | 2213 | 661483 | 589 | 3448146 | 709 | 3519376 | 937 |
| Complement C2 | 552 | 795 | 898 | 813 | 0 | 710 | 1928 | 3081 |
| Complement C4-A | 147533 | 4239 | 85388 | 0 | 91382 | 2300 | 81421 | 1492 |
| Complement C5 | 113786 | 565 | 57475 | 0 | 352535 | 1277 | 300604 | 1473 |
| Complement C6 | 58766 | 0 | 7998 | 0 | 68752 | 3917 | 77698 | 0 |
| Complement C7 | 124592 | 0 | 12820 | 0 | 119500 | 0 | 127520 | 0 |
| Complement C8 alpha chain | 22771 | 0 | 2205 | 0 | 21274 | 0 | 23694 | 0 |
| Complement C8 beta chain | 75380 | 910 | 8377 | 0 | 81529 | 580 | 77723 | 0 |
| Complement C8 gamma chain | 28166 | 0 | 3205 | 0 | 17468 | 0 | 14944 | 0 |
| Complement C9 | 719447 | 1498 | 116023 | 0 | 886405 | 1880 | 809255 | 1505 |
| C3a anaphylatoxin chemotactic receptor | 4945 | 886 | 1494 | 0 | 800 | 0 | 0 | 0 |
| Integrin beta-2/LFA-1/CR-3 | 0 | 0 | 0 | 0 | 0 | 1464 | 0 | 0 |
| Integrin alpha-M/CD11B | 0 | 0 | 0 | 0 | 0 | 1410 | 0 | 0 |
| Integrin alpha-X/CD11C | 0 | 0 | 676 | 0 | 0 | 2180 | 0 | 779 |
| C5a anaphylatoxin chemotactic receptor | 0 | 0 | 0 | 0 | 0 | 1496 | 0 | 0 |
| Complement receptor type 2/CD21 | 647 | 563 | 0 | 0 | 0 | 1870 | 0 | 0 |
| Complement receptor type 1/CD35 | 0 | 1689 | 0 | 765 | 0 | 1585 | 0 | 0 |

Figure S2. Heat map over bacteria-bound complement system molecules from SRM adsorption experiments. Data are represented as mean values from eight independent adsorption experiments. The heat map was constructed by comparing values between plasma and saliva conditions for each row. All values <500 was considered to be difficult to separate from background noise and were set to 0. Sample peptides were prepared as described in the Materials and methods.

Table S1. IgG levels in saliva and plasma

| | saliva ($\mu\text{g/ml}$) | Plasma ($\mu\text{g/ml}$) |
|-----------|--------------------------------|--------------------------------|
| IgG1 | 0.23 (0.05–0.72) | 4,470 (1,810–8,030) |
| IgG2 | 0.16 (0.03–0.42) | 3,130 (1,090–4,970) |
| IgG3 | 0.07 (0.02–0.23) | 1,770 (380–3,880) |
| IgG4 | 0.01 (0.01–0.03) | 280 (45–510) |
| total IgG | 0.47 (0.13–1.39) | 9.660 (3,790–14,310) |

SRM determination of IgG levels in paired saliva and plasma samples from five individuals. Data are represented as mean values including the range of all measurements. Sample peptides were prepared as described in the Materials and methods.

Supplemental text

Patient history. A 31-yr-old woman presented at the Department of Infectious Diseases, Skånes University Hospital, Lund, Sweden with a 36-h long history of high fever, chills, and profuse vomiting. She had seropositive rheumatoid arthritis and insulin-dependent diabetes mellitus and medicated with Prednisolone, Methotrexate, and Enbrel (TNF blocker). Her husband and two children had all suffered from milder upper respiratory tract infections in the preceding weeks, but the patient had had no such symptoms. The day before she fell ill she had been working in her garden, and afterward felt pain in her right thigh. At admission she was dehydrated, had a temperature of 39.5°C, pulse of 120 beats/minute, respiratory rate of 22 breaths per minute, and blood pressure of 120/70 mmHg. Routine physical examination was normal except for a tender, well-demarcated 7 × 5-cm erythema on the right ventral thigh. There were no surrounding wounds or hematoma. Strep A test was positive from a throat swab. Laboratory investigation revealed elevated levels of white blood cells ($21 \times 10^9/\text{L}$), C-reactive protein (CRP; 645 mg/liter), Prothrombin time (INR; 1.4), and Creatinine (157 $\mu\text{mol/liter}$). Liver function tests, aPTT (activated partial thromboplastin time), and platelet count were normal. After obtaining two aerobic and two anaerobic blood cultures (BacT/Alert; Biomérieux) as well as a throat and urinary culture, the patient received 2 g Cefotaxime (Pc-allergy) and 600 mg Clindamycin intravenously. 5 h later, a blood sample and a tissue scrap from the throat was taken for electron microscopy. The surgeons were contacted, the patient was operated on 12 h after admittance, and the operation showed necrotizing fasciitis in the right thigh. Blood and urine cultures turned out negative. *S. pyogenes* (Emm1) was isolated from the throat culture taken before antibiotics, and in perioperative wound fluid cultures from the necrotic tissue in the thigh collected after two doses of antibiotics. Samples for electron microscopy were also collected during the operation. The patient fully recovered and was released to her home after 9 d of hospital care.