Supporting Information

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SI Materials and Methods

Animals. The B10.Q founder mice were obtained originally from Jan Klein (Tübingen University, Tübingen, Germany) and have been maintained in our laboratory for more than 20 y. The BALB/c founder mice were obtained from Jackson Laboratories. (BALB/c × B10.Q) F1 mice, named QB, were bred in the Medical Inflammation Research animal house facility in Lund and Stockholm. The animals were kept in a specific pathogen-free animal facility with a climate-controlled environment having 12-h light/dark cycles in polystyrene cages containing wood shavings and were fed standard rodent chow and water for ad libitum consumption. Male mice 2–4 mo old were used in all of the experiments, with the experimental groups matched for age, mixed in cages and run blindly. Splenectomy or sham operation was done under isofluran anesthesia. Pups 2–3 d old were used for immunohistochemical studies. Regional ethics committees (Lund/Malmö and Stockholm regions, Sweden) approved the animal experiments.

Monoclonal Antibodies. The CII-specific hybridomas (M2139, M284, CIIC1, CIIC2, CB20, and UL1) were generated and characterized as described elsewhere (1–3). ACC4 antibody recognizes the citrullinated CII epitope, C1 (4). A mouse antitrinitrophenol (anti-TNP) antibody-producing hybridoma (Hy2.15) was a gift from Georges Köhler (Basel Institute for Immunology, Basel, Switzerland). A human HLA-DR α-chain–specific antibody clone (L243) was obtained from ATCC. M284 and CB20 mAbs bind CII epitopes J1 and C1, respectively. All hybridomas were cultured in ultra-low bovine IgG-containing DMEM Glutamax-I culture medium (Gibco BRL and Invitrogen AB) with 100 mg/L of kanamycin monosulfate (Sigma). MAbs were generated on a large scale as culture supernatant using Integra cell line 1000 (CL-1000) flasks (Integra Biosciences). Antibodies were purified using γ-bind plus affinity gel matrix (GE Healthcare) and the Äkta purification system (GE Healthcare) as described earlier (5). Briefly, culture supernatants were centrifuged at 26,786 × *g* for 30 min, filtered, and degassed before applying them to the gel matrix. The gel was washed extensively and the antibodies were eluted using acetic acid buffer at pH 3.0 and neutralized with 1 M Tris·HCl, pH 9.0. The peak fractions were pooled and dialyzed extensively against PBS, pH 7.0, with or without azide. The IgG content was determined by freeze drying. The antibody solutions were sterilized using 0.2-μm syringe filters (Dynagard; Spectrum Laboratories), aliquoted, and stored at −70 °C.

Collagen Antibody-Induced Arthritis. Endo-β-*N*-acetylglucosaminidase (EndoS)-hydrolyzed and unhydrolyzed, two or four arthritogenic mAb combinations were studied: M2139 (γ2b), CIIC1 (γ2a), CIIC2 (γ2b), and UL1 (γ2b) bind to triple helical J1 [MP*GERGAAGIAGPK; P* indicates hydroxyproline], $C1^I$ (GARGLTGRO) (6), D3 (RGAQGPOGATGF), and U1 (GLVGPRGERGF) CII epitopes, respectively. The mixture of the mAbs (9 or 4 mg per mouse) was prepared by mixing equal concentrations of each of the sterile filtered antibody solutions. Mice were injected i.v. with 250–500 μL of solution. Unless stated otherwise, all of the mice received i.p. LPS (25 μg per mouse) at day 5.

Clinical Evaluation of Arthritis. Mice were examined daily for development of arthritis. Scoring of the inflammation was done blindly using a scoring system based on the number of inflamed joints in each paw, inflammation being defined by swelling and redness. Scores were recorded for the phalangeal joints (maximum of 1 point per digit, 5 points per paw), the metacarpus or metatarsus (5 points), and the wrist and ankle joints (5 points). The maximum score was 15 per paw, and 60 for all four paws.

Histological Preparations. Paws were dissected from each group of mice (three or four mice per group), fixed in 4% (wt/vol) phosphate-buffered paraformaldehyde solution (pH 7.0) for 24 h, decalcified for 3–4 wk in a solution containing EDTA, polyvinylpyrrolidone, and Tris·HCl (pH 6.95), followed by dehydration and embedding in paraffin. Sections of 6 μm were stained with H&E to determine cellular infiltration and bone and cartilage morphology.

For immunohistochemistry, QB pups 2–3 d old (three or four mice per group) were injected with 1 mg each of a unhydrolyzed antibody mixture containing M2139 + CIIC2 + UL1 antibodies, EndoS-hydrolyzed IgG (M2139H + CIIC2H + UL1H), or a mixture of unhydrolyzed and EndoS-hydrolyzed IgG at 1:1 ratio. Twenty-four hours later, mice were killed and paw samples were snap-frozen in optimum cutting temperature compound using cold isopentane and dry ice. Sections of 6 μm were stained with biotinylated anti-kappa (clone: 187.1) or goat anti-mouse anti-C3c antibodies (Nordic Immunological Laboratories). Extravidin-peroxidase and diaminobenzidine were used for detection. CIIC1 mAb was excluded from this mixture because of its inability to activate complement after binding to CII. Histology scoring of joint sections after anti-C3c antibodies was done as follows: 0, no staining; 1, staining only at subchondral bone junction area; 2, weak; and 3, strong staining uniformly on the cartilage surface.

Complement Activation and Rheumatoid Factor-Like Activity of CII-Binding Antibodies. Microtiter plates were coated with 3 μg/mL of M2139 or CIIC1 alone or in combination with 3, 6, or 12 μg/mL of EndoS-hydrolyzed or unhydrolyzed Hy2.15, L243, M2139 and CIIC1 IgG in 75 mM sodium carbonate buffer (pH 9.6) overnight at 4 °C. Plates were washed between each step with 50 mM Tris·HCl, 150 mM NaCl, and 0.1% Tween-20, pH 8.0. Wells were blocked with 1% BSA in PBS for 2 h at room temperature to prevent unspecific binding. Serum was diluted to 0.5% in GVB^{++} [5 mM veronal buffer (pH 7.4), 144 mM NaCl, 1 mM $MgCl_2$, 0.15 mM $CaCl_2$, and 1% gelatin] and added to the plates, followed by 1 h of incubation at 37 °C. Deposited C3b was detected using a goat anti-C3 antibody (ICN Pharmaceuticals/Cappel) and a rabbit anti-goat HRP conjugate (Dako Denmark A/S). Deposited C1q was detected using a biotinylated mouse anti-C1q (clone JL-1) antibody (Hycult Biotech) and a streptavidin–HRP conjugate (ThermoFisher Scientific). The plates were developed using *o*-phenylenediamine substrate (Dako) and H_2O_2 and the absorbance at 490 nm was measured.

To study complement activation on CII-bound antibodies, microtiter plates were coated with 10 μg/mL of CII in 75 mM sodium carbonate buffer (pH 9.6) overnight at 4 °C. The wells were blocked using 3% (wt/vol) fish gelatin in 50 mM Tris·HCl, 150 mM NaCl, 0.1% Tween-20, pH 8.0 (blocking buffer). M2139 mAb alone at a concentration of 10 μg/mL or in combination with 10, 20, or 40 μg/mL of EndoS-hydrolyzed or unhydrolyzed Hy2.15, L243, or M2139 IgG diluted in blocking buffer was added and the plates were incubated at 4 °C overnight. Serum diluted to 4% (vol/vol) in $DGVB^{++}$ [2.5 mM veronal buffer (pH 7.35), 72 mM NaCl, 0.1% gelatin, 1 mM $MgCl_2$, 0.15 mM $CaCl_2$, and 2.5% (wt/vol) glucose] was added to the wells and complement activation was allowed to proceed for 1 h at 37 °C. Deposited C3b was detected as above.

Rheumatoid factor (RF)-like activity of CIIC1 antibodies was determined as described earlier (7). Biotin-labeled CIIC1 was detected by europium-conjugated streptavidin using the dissociation-enhanced lanthanide fluoroimmunoassay system.

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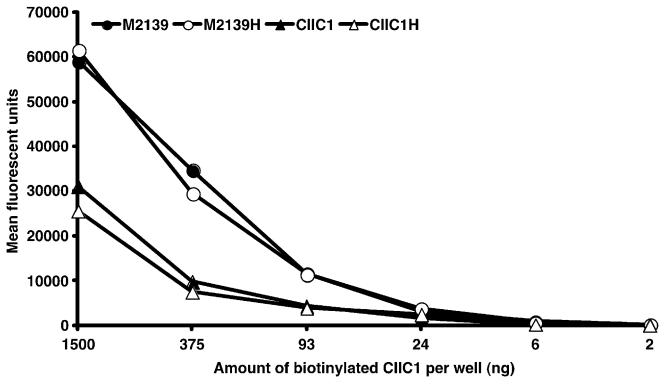

Fig. S1. Disturbance of immune complex formation by RF-like activity of CIIC1 mAb. There was no difference in biotinylated CIIC1 antibody binding to EndoS-hydrolyzed or unhydrolyzed M2139 and CIIC1 mAbs.

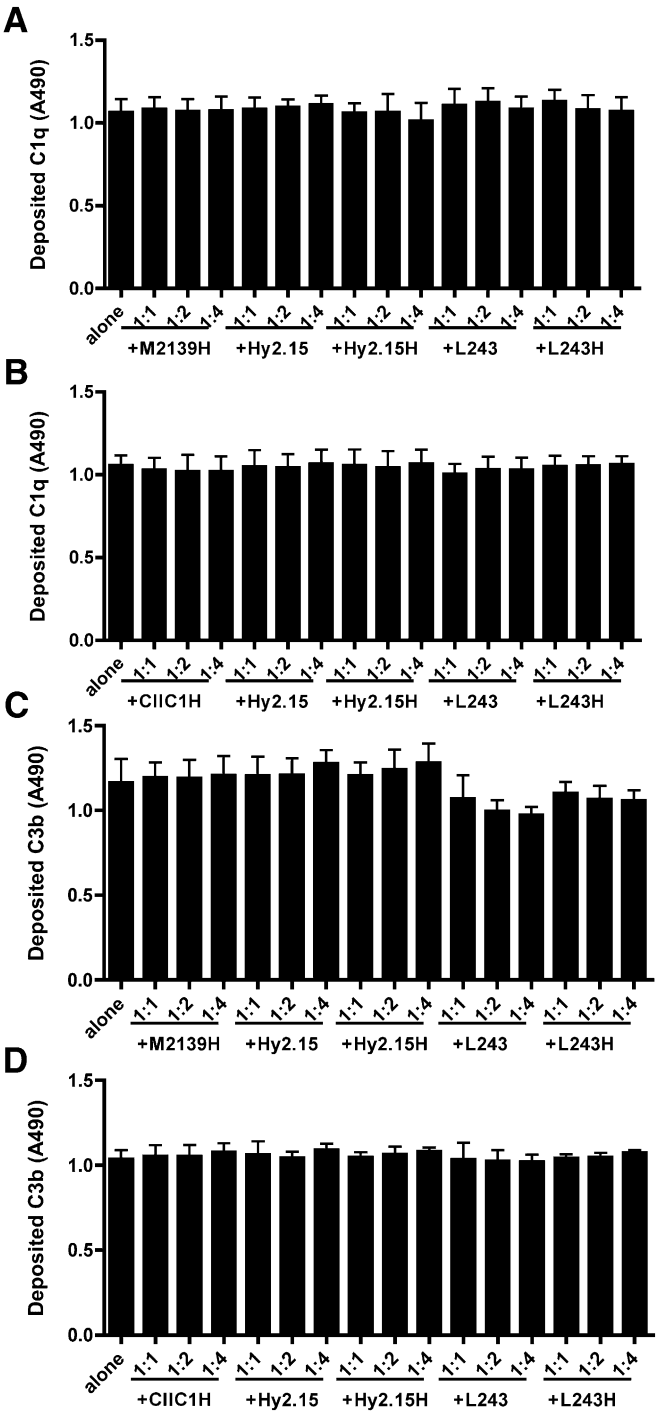

Fig. S2. In vitro complement activation. Activation of complement factors C1q (*A* and *B*) and C3b (*C* and *D*) on directly coated M2139 (*A* and *C*) or CIIC1 (*B* and *D*) anti-CII mAbs in the presence of EndoS-hydrolyzed or unhydrolyzed IgG. CII-specific (M2139 and CIIC1) and joint unrelated (Hy2.15 and L243) IgGs were used as shown in the figures. H denotes EndoS-hydrolyzed IgG. Each bar represents mean values from three experiments ± SD.

Table S1. Anti-CII IgG glycoforms before and after EndoS hydrolyzation

Glycan[†]	Antibody (sequence), %			
	M2139 (EDY**N**STIR)	M2139H (EDY**N**STIR)	CIIC1 (EDY**N**STLR)	CIIC1H (EDY**N**STLR)
HexNAc (1)	—	2.6	—	3.8
HexNAc(1)dHex (1)	—	97	—	95
HexNAc(4)Hex (3)	—	—	—	—
HexNAc(4)Hex (4)	—	—	—	—
HexNAc(3)Hex(3)dHex (1)	1.5	—	6.5	—
HexNAc(3)Hex(4)dHex (1)	0.4	—	1.3	—
HexNAc(4)Hex(3)dHex (1)	48	—	35	0.7
HexNAc(4)Hex(4)dHex (1)	43	—	42	0.5
HexNAc(4)Hex(5)dHex (1)	4.3	—	4.4	—
HexNAc(4)Hex(6)dHex (1)	0.3	—	0.2	—
HexNAc(3)Hex(4)dHex(1)NeuGc (1)	—	—	5.0	—
HexNAc(4)Hex(4)dHex(1)NeuGc (1)	0.5	—	3.2	—
HexNAc(4)Hex(5)dHex(1)NeuGc (1)	1.8	—	1.8	—
HexNAc(4)Hex(6)dHex(1)NeuGc (1)	—	—	0.3	—

Relative abundances (%) of the main Fc IgG glycoforms found in the antibodies and EndoS-treated antibodies, respectively. Glycopeptides were identified by their characteristic retention times and accurate monoisotopic masses (within <10 ppm from the theoretical values) of doubly and triply charged ions. Peptide sequences and glycan compositions are indicated. Glycans substituting EDY**N**STIR and EDY**N**STLR eluted at ~18–20 min and EEQF**N**STFR at ~21–23 min, respectively.
[†]dHex, deoxy-hexose; H, EndoS-hydrolyzed; Hex, hexose; HexNAc, *N*-acetylhexoseamine; NeuGc, *N*-glycolylneuraminic acid.

Table S2. Joint unrelated antigen(s)-specific IgG glycoforms before and after EndoS hydrolyzation

Glycan[†]	Antibody (sequence), %			
	L243 (EDY**N**STLR)	L243H (EDY**N**STLR)	Hy2.15 (EEQF**N**STFR)	Hy2.15H (EEQF**N**STFR)
HexNAc (1)	—	2.6	—	15
HexNAc(1)dHex (1)	—	97	—	84
HexNAc(4)Hex (3)	—	—	3.4	—
HexNAc(4)Hex (4)	—	—	1.3	—
HexNAc(3)Hex(3)dHex (1)	4.6	—	2.5	—
HexNAc(3)Hex(4)dHex (1)	3.3	—	0.7	—
HexNAc(4)Hex(3)dHex (1)	22	—	46	0.1
HexNAc(4)Hex(4)dHex (1)	50	—	27	0.5
HexNAc(4)Hex(5)dHex (1)	10	—	5.0	0.2
HexNAc(4)Hex(6)dHex (1)	4.6	—	0.1	—
HexNAc(3)Hex(4)dHex(1)NeuGc (1)	2.6	—	2.5	—
HexNAc(4)Hex(4)dHex(1)NeuGc (1)	0.3	—	5.9	—
HexNAc(4)Hex(5)dHex(1)NeuGc (1)	1.7	—	4.5	—
HexNAc(4)Hex(6)dHex(1)NeuGc (1)	0.7	—	1.5	—

Relative abundances (%) of the main Fc IgG glycoforms found in the antibodies and EndoS-treated antibodies, respectively. Glycopeptides were identified by their characteristic retention times and accurate monoisotopic masses (within <10 ppm from the theoretical values) of doubly and triply charged ions. Peptide sequences and glycan compositions are indicated. Glycans substituting EDY**N**STIR and EDY**N**STLR eluted at ~18–20 min and EEQF**N**STFR at ~21–23 min, respectively.
[†]dHex, deoxy-hexose; H, EndoS-hydrolyzed; Hex, hexose; HexNAc, *N*-acetylhexoseamine; NeuGc, *N*-glycolylneuraminic acid.