Retraction and Correction

Retraction

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The authors wish to note the following: “Using studies of IgG hydrolyzed by the streptococcal glycan hydrolyzing enzyme EndoS, we found that treatment of mice with hydrolyzed IgG blocked antibody mediated arthritis. As an explanation for this observation, we suggested that EndoS-hydrolyzed IgG per se dominantly blocks local immune complex formation.

“With new data from our own follow up experiments, we have now found that this conclusion was incorrect.

“Our new data shows that injection of EndoS is much more potent in vivo than we could logically anticipate, as i.v. injection of doses containing less than 0.1 μg EndoS mixed with IgG suppressed arthritis using the same model as the one reported in the initial paper (collagen antibody-induced arthritis). We previously excluded the possibility that contaminating EndoS could play a role, as this contaminating amount was not detected using standard methods in the hydrolyzed IgG fraction we used in the experiments. Furthermore, much higher doses of EndoS injected in the same mouse strain as a control experiment did not affect collagen induced arthritis in earlier experiments. The correct interpretation of our collective data is that EndoS operates very potently in vivo on an immune complex-mediated disease, possibly by accumulating within immune complexes. Because this interpretation is different from our major conclusion of the published paper, the authors have unanimously decided to retract this paper to be able to publish the data connected with a correct interpretation. We sincerely apologize to readers of this paper, who might have been misled by our earlier interpretation.”

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Correction

MEDICAL SCIENCES

The authors note that the α-tubulin loading control blot in Fig. 4D appeared incorrectly. The corrected figure and its legend appear below.

Fig. 4. Increased PGC-1α levels in aging muscle prevent degradative processes. (A) Immunohistochemistry of biceps femoris using anti-active caspase 3 antibody to detect apoptosis. (B) Apoptotic index in skeletal muscle homogenates of wild-type and MCK-PGC-1α of different age-groups based on nucleosome fragmentation (n = 6 for each group). *, P < 0.05, **, P < 0.01, ***, P < 0.001. (C) Western blot of Bax and Bcl-2 in skeletal muscle homogenates. (D) Western blot of the 20S subunit of the proteasome and tubulin in skeletal muscle homogenates. (E) Western blot of LC3-I and LC3-II in skeletal muscle homogenates.
Dominant suppression of inflammation by glycan-hydrolyzed IgG

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A unique anti-inflammatory property of IgG, independent of antigen specificity, is described. IgG with modification of the heavy-chain glycan on asparagine 297 by the streptococcal enzyme endo-β-N-acetylglucosaminidase (EndoS) induced a dominant suppression of immune complex (IC)-mediated inflammation, such as arthritis, through destabilization of local ICs by fragment crystallizable (Fc-Fc) interactions. Small amounts (250 μg) of EndoS-hydrolyzed IgG were sufficient to inhibit arthritis in mice and most effective during the formation of ICs in the target tissue. The presence of EndoS-hydrolyzed IgG disrupted larger IC lattice formation both in vitro and in vivo, as visualized with anti-C3b staining. Neither complement binding in vitro nor antigen–antibody binding per se was affected.

Glycosylation is an important posttranslational modification affecting the structure and biological properties of glycoproteins. One of the most intensively studied is the asparagine 297 (Asn-297) glycan on the heavy chain (γ-chain) of IgG, which is sequenced within the internal space enclosed by the CH2 domains. Subtle changes in IgG N-glycome significantly fragment crystallizable (Fc) conformation with dramatic consequences for IgG effector functions (1). Extensive noncovalent interactions between the carbohydrate and the protein moiety in the IgG–Fc region result in reciprocal influences on conformation (2). NMR studies suggested a significant role for Fc–glycan dynamics in Fc receptor (FcR) interactions (3). The minimal oligosaccharide structure in IgG is a hexaasaccharide (GlcNAc2Man3GlcNAc) with variable sugar residues attached, resulting in the generation of many different glycoforms. Such altered IgG glycoforms lacking terminal steric acid and galactose residues were identified in rheumatoid arthritis (RA) patients (4). Recently, differential sialylation was reported to regulate the inflammatory property of IgG (5).

Endoglycosidases form a group (EC 3.2 subclass) of enzymes that hydrolyze nonterminal glycosidic bonds in oligosaccharides or polysaccharides. Endo-β-N-acetylglucosaminidase (EndoS) is a member of the GlcNAc polymer hydrolyzing glycosyl hydrolases of family 18 (FGH18) secreted by group A β-hemolytic Streptococcus pyogenes. It exclusively hydrolyzes the β-1, 4-di-N-acetylglucosamine moiety of the asparagine-linked complex-type glycan on Asn-297 of the γ-chains of IgG (6). EndoS has similarities to endo-β-N-acetylglucosaminidases, which cleave the β-1→4 linkages between N-acetylgalcosamines found in the core of the N-linked glycan of IgG.

Antibodies [anti-citrullinated protein antibodies (ACPA), rheumatoid factors (RF), and anti-type II collagen antibodies] and immune complexes (ICs) are prevalent in RA. ACPA and RF also precede disease development (7). IC-mediated pathology is evident in several autoimmune diseases. Importantly, the pathogenetic effect of circulating ICs was shown to be dependent on their size and composition (8). Both antigen-driven (soluble and target tissue-bound) and RF containing ICs present in RA patients are of intermediate (6S–19S) to large (22S–30S) size, and larger (>22S) ICs containing RF were implicated in extraarticular manifestations in RA (9). Furthermore, collagen type II (CII)-containing ICs from RA synovial fluid were shown to induce production of inflammatory cytokines (TNF-α, IL-1β, and IL-8) from peripheral blood mononuclear cells via FcyRIIA (10).

Antibodies from patients with RA upon passive transfer induced arthritis in mice (11). The effector phase of arthritis is optimally studied using the collagen antibody-induced arthritis model induced by anti-CII IgG mAbs (12). This model exhibits features of bone and cartilage erosions, major infiltrations of granulocytes, and deposition of IgG and complement factors on the cartilage surface, characteristic of RA, and is dependent on complement, FcyRs, TNF-α, IL-1β, and neutrophils and macrophages (12). Interestingly, removal of the N-linked glycan abrogated pathogenic potential of antibodies (13) and abolished all of the proinflammatory properties of IC from systemic lupus erythematosus patients (14). In addition, EndoS-hydrolyzed IgG ameliorated several antibody-mediated diseases in mice, including arthritis (15). Attenuation of inflammation was reported to be dependent on IgG1 and IgG2b subclasses, but the mechanisms were not clarified. Here we demonstrate suppression of inflammatory arthritis by EndoS-hydrolyzed IgG, which is dominant and mediated through disturbances in the formation of ICs within the target tissue. This dominant suppression of inflammation by EndoS-hydrolyzed IgG is a different and unique therapeutic effect of EndoS modification.

Results and Discussion

Dominant Inhibition of Inflammation by EndoS-Hydrolyzed IgG. EndoS treatment specifically cleaved the Asn-297 glycan on IgG (Fig. 1a), which removed almost all (99%) of the variable glycan chains attached to the first N-acetylgalcosamine (GlcNAc) residue of the Fc region (Fig. 1c and d and Tables S1 and S2). Upon anti-CII mAb (EndoS-unhydrolyzed IgG) transfer, arthritis developed in mice as early as 48 h with 100% incidence at day 10. Massive infiltration of immune cells, pannus formation, and distinct bone and cartilage erosions were observed in this group of mice (Fig. 1b). Interestingly, injection of EndoS-hydrolyzed IgG, irrespective of CII epitope specificity, potently inhibited such...
EndoS-hydrolyzed IgG dominantly inhibit inflammation. (A) SDS-PAGE and lectin blot analysis of mAbs incubated with (+) or without (−) EndoS hydrolysis and separated by 10% SDS-PAGE. The proteins were detected by PageBlue stain (Stain) or by blotting onto a PVDF membrane probed with *Lens culinaris* agglutinin (LCA). (B) Representative figures of H&E-stained ankle joints of mice (n = 3–4 per group) injected with anti-CII mAbs; unhydrolyzed (Left), EndoS-hydrolyzed (Center), or mixed IgG (Right). Magnification ×10. (C) Hy2-15 and (D) EndoS-treated Hy2.15. Showed spectra were acquired during the time period for which the majority of glycosylated peptides from EEQFNSTFR (21.5–23.0 min) elute. Doubly and triply charged ions as well as predicted glycan structures are shown. All numbers given are for the monoisotopic mass charge. In all of the animal experiments, male (BALB/c × B10.Q) F1 mice were used. Unless otherwise stated all of the mice received antibodies i.v. (d 0) and 25 μg of LPS i.p. (d 5). For arthritis induction in experiments shown in E, F, I, J, and L, 9 mg of two anti-CII mAb mixtures (M2139 + CIC1) were used, whereas for experiments in G and H 4 mg of four anti-CII mAb mixture (M2139 + CIC1 + CIC2 + UL1) was used. Antigen specificity is not required for inhibition. Mice (n = 42) were injected with 4 mg of EndoS-hydrolyzed IgG (E) M2139H + CIC1H or UL1H + CIC2H or (F) Hy2.15H + L243H followed by anti-CII mAb. Dose and subclass dependency. (G) Mice (n = 28) were injected with EndoS-hydrolyzed or unhydrolyzed IgG1 (Hy2.15) or IgG2a (L243) MAb binding to joint unrelated antigens at two different concentrations (1 mg and 0.25 mg), followed by anti-CII mAb. (H) Mice (n = 65) were injected with different subclasses of EndoS-hydrolyzed anti-CII (M2139H, M2139H + CIC2H, and UL1H) or anti-citrullinated CII peptide IgG (ACC4H) at two different concentrations (1 mg and 0.25 mg), followed by anti-CII mAb. (I) Mice (n = 25) were injected with a mixture of EndoS-hydrolyzed and/or unhydrolyzed anti-CII IgG at different combinations. In mixed IgG groups, group 1 received 4.5 mg of unhydrolyzed and 4.5 mg of EndoS-hydrolyzed IgG, group 2 had 6.75 mg of unhydrolyzed and 2.25 mg of EndoS-hydrolyzed IgG, and group 3 received 7.8775 mg of unhydrolyzed and 1.125 mg of EndoS-hydrolyzed IgG. (J) Mice (n = 25) were injected with different concentrations (50–4,000 μg) of EndoS-hydrolyzed single anti-CII IgG (M2139H), followed by anti-CII mAb. Three hours after the antibody transfer, LPS was injected. H denotes EndoS-hydrolyzed IgG. Hy2.15 and L243 represent mAbs binding to TNP hapten and human HLA-DR antigen, respectively. Error bars indicate ± SEM.

Inflammatory arthritis (Figs. 1 B and E–J and 2 A and B), resulting in normal joint architecture. Similarly, mice treated with a mixture of unhydrolyzed and EndoS-hydrolyzed IgG showed undisturbed joints. Surprisingly, mAbs to trinitrophenol (TNP) (Hy2.15) and to human HLA-DR (L243), which are commonly used as control antibodies in the mouse, also completely inhibited arthritis when EndoS-hydrolyzed (Fig. 1 F and G). Inhibition by EndoS-hydrolyzed antibodies occurred irrespective of the IgG subclass or the antigen specificity (Fig. 1 G and H).

When we analyzed the time and dose dependence of the arthritis suppressive effect, we found that 1 mg of EndoS-hydrolyzed IgG within the mixture of 9 mg of antibodies completely inhibited arthritis (Fig. 1 I) and 3 mg of anti-CII mAb was sufficient to induce arthritis (12), and when we titrated the EndoS-hydrolyzed IgG we showed the dose needed for complete inhibition of arthritis was as little as 250 μg (Fig. 1 J).

To find the effective therapeutic time point, EndoS-hydrolyzed IgG was administered to groups of mice at different time points and one group of mice was left untreated. Antibody mixture and then LPS were injected at 0 and 3 h, respectively. In the 0-h treatment group, EndoS-hydrolyzed IgG was injected initially, followed by an injection of mAb mixture. Unlike before or after 48h treatment, complete blocking of arthritis was observed when the treatment with EndoS-hydrolyzed IgG was done 3 h before or after the arthritogenic mixture injection (Fig. 2 A and B). Because antibodies are bound to cartilage within 30 min after injection (16), we concluded that EndoS-hydrolyzed IgG could block the disease most effectively if injected during the time when antibodies start binding to the cartilage surface.

EndoS Hydrolysis of IgG Does Not Affect Antigen Binding. Surface plasmon resonance (SPR) analysis of the antigen–antibody binding in the presence or absence of EndoS-hydrolyzed IgG clearly demonstrated that removal of carbohydrate moieties from mAbs did not affect their high-affinity binding to CII epitopes (Fig. 2 C).

We have earlier shown antibody-mediated damage in cartilage
explants cultured in vitro with the mAb to CII used in this study: This preinflammatory effect does not require living cells, is mediated by fragment antigen-binding (Fab), and is epitope-dependent (17). To test whether EndoS hydrolysis could change this effect, FTIR microspectroscopy (FTIRM) was used for chemical analysis of cartilage (17) cultured in the presence of 100 μg/mL EndoS-hydrolyzed or unhydrolyzed mAb (Fig. 2 D–G). The height and location of the amide 1 peak, which represents the total protein content of the tissue, in the region 1,600–1,700 cm$^{-1}$ (Fig. 2 D–G). The height and location of the amide 1 peak, representing protein, predominantly collagen, and the height of the proteoglycan peak at 1,076 cm$^{-1}$ were examined at the cartilage surface, where the mAb penetrates, and in the interior of cartilage explants. After 14 d, the amide 1 peak at the surface and in the interior of the control cartilage

Fig. 2. Inhibition of inflammation and SPR and FTIRM analysis. (A) Mice (n = 30) were injected with 1 mg of EndoS-hydrolyzed anti-CII IgG (M2139H + CIIC1H + CIIC2H + UL1H) at different time points (−48, −3, 0, +3, or +48 h). At 0 h and 3 h, anti-CII mAb (M2139 + CIIC + CIIC2 + UL1) and then LPS were injected. One group of mice received no treatment. (B) Effect of splenectomy. Mice (n = 21) were either splenectomized (Splx) or sham-operated (Sham). Three weeks later, they were injected with 4 mg of EndoS-hydrolyzed IgG (M2139H + CIICH) or left untreated, followed by anti-CII mAb (M2139 + CIIC1). H denotes EndoS-hydrolyzed IgG. Error bars indicate ±SEM. (C) SPR (Biacore) analysis of antibody binding capacity of EndoS-hydrolyzed and unhydrolyzed IgG was performed using CII immobilized on CMS sensor chip. MAbs were injected at different concentrations through flow cells at a flow rate of 30 μL/min. Antibodies were injected for 3 min and dissociation of bound molecules was observed for 7 min. There was no difference in antibody binding when EndoS-hydrolyzed or unhydrolyzed IgG was added at different ratios to anti-CII mAb mixture. (D and E) Changes in the chemical composition of the cartilage were assessed using FTIRM analysis. Representative mean spectra are shown from cartilage cultures without antibody (D), and from cartilage cultured for 14 d with 100 μg/mL of unhydrolyzed mAb M2139 (E). The results shown are the mean of 10 measurements taken from the central areas (red line) and near the surface of the tissue (black line). The mean spectra for surface and interior were calculated to assess the effects of antibody penetration on the peaks characteristic of CII and of proteoglycans. (F and G) The mean peaks from the surface cartilage were compared with those from antibody-exposed surface of cartilage exposed to the EndoS-hydrolyzed or unhydrolyzed IgG. Cartilage exposed to either EndoS-hydrolyzed or unhydrolyzed IgG (CIIC1, M2139, and UL1) showed similar changes. (F) The height and location of the amide 1 peak, which represents the total protein content of the tissue, in the region 1,600–1,700 cm$^{-1}$. (G) The height of the peak at 1,076 cm$^{-1}$ represents proteoglycans.
cultured without mAb was located at 1,659 cm$^{-1}$ (range 1,655–1,666 cm$^{-1}$), but there were striking changes in spectra from cartilage cultured in the presence of either EndoS-hydrolyzed or unhydrolyzed anti-CII IgG. Beyond the region of penetration by mAb, the spectra were generally similar to those of controls cultured without mAb, but spectra from the surface of the cartilage showed substantial changes (Fig. 2D). For both unhydrolyzed and EndoS-hydrolyzed anti-CII, there was a shift in the location of the amide I peak from 1,659 cm$^{-1}$ to as low as 1,643 cm$^{-1}$, indicative of denaturation of the CII, accompanied by substantial decreases in the height of the amide I peak, and the proteolytic peak at 1,076 cm$^{-1}$, indicating a total loss of matrix (Fig. 2E). These data confirm that the EndoS-hydrolyzed IgG retained its antibody reactivity and that the suppressive effect in fact resides in the Fc but not in the Fab part of the IgG molecules.

Spleen Is Not Required for Arthritis Inhibition. Recent studies demonstrated the anti-inflammatory property of terminal sialic acids present on IgG–Fc (5). C-type lectin receptor SIGN-R1 (CD209) expressed on macrophages in the splenic marginal zone is required for recognition of such sialic acids (18), which results in the production of IL-33 and expansion of IL-4-producing basophils promoting increased expression of the inhibitory FcγRIIB on effector macrophages leading to attenuation of inflammation (19). However, in the present study splenectomy did not alter the inhibitory capacity of EndoS-hydrolyzed IgG (Fig. 2B), suggesting involvement of mechanisms other than the SIGN-R1 pathway.

EndoS-Hydrolyzed IgG Disrupts Larger IC Formation. Specific IgG glycan hydrolysis alters both murine and human IgG–FcγR interactions (13, 15) and removal of outer-arm sugar residues affects the thermal stability and functionality of the CH2 domain of IgGs (20). However, the length and nature of residual carbohydrate structures could also affect Fc–Fc interactions and thereby IC formation, complement binding and FcγR binding.

Binding of the arthritogenic antibodies to the cartilage matrix and the subsequent formation of ICs on the joint surface is likely to be the major factor leading to the clinically apparent inflammation and arthritis. In the process of local IC formation, Fc–Fc interactions play an important role (21), and the specific glycans present in the CH2 domain of IgG might have a vital function in this process. Because the dominant suppression seems to be mediated through more acute effects during the binding of antibodies to the cartilage, we hypothesized this might be due to the instability of ICs formed within the target tissue, the articular joints. Because the Fab part did not influence the suppressive effect, the next step was therefore to analyze whether the EndoS-hydrolyzed antibodies can disrupt the growth of Fc-dependent ICs. For this we used the dynamic light scattering (DLS) technique and analyzed the formation of IC by CII and anti-CII mAb in the presence of EndoS-hydrolyzed or unhydrolyzed IgG. As shown in Fig. 3 A and B, larger IC formation was clearly disturbed by the presence of EndoS-hydrolyzed IgG. Interestingly, disturbance of large IC was more prominent in the presence of low-affinity (Fig. 3A) than high-affinity IgG (Fig. 3B). It has earlier been shown that the binding to low-affinity Fc receptors is decreased by EndoS modification (13). However, the binding is directly related to IC formation. Previously, we reported RF-like activity of one of the mAb (CIIC1) present in the arthritogenic mixture (22); however, we did not observe any difference in its binding activity to EndoS-hydrolyzed and unhydrolyzed IgG (Fig. S1). Different data have been reported regarding complement binding after EndoS hydrolysis (13, 15). To directly investigate this we analyzed complement binding using either immobilized EndoS-hydrolyzed or unhydrolyzed IgG, or CII–anti-CII ICs. The presence of EndoS-hydrolyzed IgG did not interfere with the ability of antibodies to stimulate complement deposition either when antibodies were immobilized directly on the plate (Fig. S2) or when bound to immobilized CII (Fig. 3C).

The presence of EndoS-hydrolyzed IgG did not affect complement activation but IC stability in vitro; hence, we further analyzed deposition of C3b, the activated product of complement factor C3, on the cartilage of mice as a measure of IC deposition and complement activation in vivo. Twenty-four hours after the injection of EndoS-hydrolyzed, unhydrolyzed, or mixed anti-CII IgG, mouse paws were analyzed for the binding of mAbs to cartilage using anti-kappa antibodies as well as for deposited C3b. The mAbs readily bound to the cartilage surface, but the pattern of C3b deposition between the groups was entirely different (Fig. 3 D and E). Minimal C3b staining was observed, only on the subchondral bone junction area of the joints from mice injected with EndoS-hydrolyzed IgG compared with a significant level of deposition throughout the cartilage surface in the unhydrolyzed IgG-injected group. In mice injected with mixed IgG, staining on the subchondral bone junction area was more intense and staining on the cartilage surface was weak. We conclude from these results that the presence of EndoS-hydrolyzed IgG decreased the formation of larger ICs in mice, most likely through the disturbance of Fc–Fc interactions, and this leads to decreased complement activation in the tissue. The significance of Fc–Fc interactions in precipitation (23) and formation of insoluble ICs (21) are early wisdoms. In addition, different FcγR-specific monoclonal antibodies (mAbs) binding to ICs may also inhibit and to solubilize IC between antigen and precipitating antibodies (24) that might involve Fc–Fc interactions. Later, structural evidence for such interactions involving the glycosylation loop of one Fc-fragment dimer binding to the CH2–CH3 interface of another Fc fragment has been demonstrated (25). Although oligosaccharides have been reported not to be involved in direct contacts with symmetry-related molecules (25), their interactions with the protein moiety in the IgG–Fc region could very well affect the reciprocal influences on conformation (2).

S. pyogenes secretes several enzymes and proteins that bind and modulate the functions of Igs as a part of its strategy for evading the immune system. Disruption of the development of larger IC lattices by EndoS-cleaved IgG could very well be one such strategy. Conversely, antibodies as a constituent of ICs play an important role in triggering various inflammatory processes leading to the development of a number of autoimmune diseases. Neutrophils play a vital part during this process, and sequential complement fixation generating C5a and direct engagement of FcγR receptors are needed to initiate and sustain such neutrophil recruitment in vivo and subsequent inflammation (26). Recent studies demonstrated bidirectional regulation of C5aR and FcγRs, which could significantly influence effector functions (27). Here we demonstrate that a specific modification of the N-linked glycan of IgG by EndoS leads to a profound anti-inflammatory effect and it does not require injection of a bacterial protein for protection. Disruption of larger IC formation at the target organs using host antibodies with a customized glycan profile (Fig. 3F) could be a unique therapeutic possibility for patients with IgG-mediated inflammatory diseases.

Materials and Methods

EndoS Hydrolysis of IgG. IgGs (CIIC1, M2139, M284, CIIC2, UL1, CB20, ACC4, Hy2.15, and L243) were hydrolyzed with recombinant EndoS fused to GST (GST–EndoS) as previously described (6). Five micrograms of GST–EndoS in PBS was added per milligram of mAb followed by incubation for 16 h at 37 °C. GST–EndoS was completely removed by three serial passages over Glutathione-Sepharose 4B columns with a 1,000-fold overcapacity of GST binding (GE Healthcare). SDS/PAGE and Lens culinaris agglutinin (LCA) lectin blotting were used to assess the purity and efficacy of EndoS cleavage. Briefly, 2 μg of EndoS-hydrolyzed and unhydrolyzed IgG were separated on 10% SDS/PAGE followed by staining with PageBlue protein stain (ThermoFisher Scientific), or blotted to PVDF using TransBlot Turbo transfer packs and apparatur (Bio-Rad). Membranes were blocked with 10 mM Heps (pH 7.5) with 0.15 M NaCl, 0.01 mM
MnCl$_2$, 0.1 mM CaCl$_2$, and 0.1% Tween-20 (HBST) and incubated with 1 μg/mL of biotinylated LCA lectin (Vector Laboratories). After washing in HBST, membranes were incubated with 50 ng/mL of peroxidase-labeled streptavidin (Vector Laboratories) and developed using Super Signal West Pico Chemiluminescent Substrate (ThermoFisher Scientific) and a ChemiDoc XRS imaging system (Bio-Rad).

**Glycopeptide Identification.** EndoS-hydrolyzed or unhydrolyzed antibody (15 μg) were trypsin-digested using Protease MAX Surfactant and trypsin enhancer (Promega). Samples were analyzed using a reversed-phase liquid chromatography system (Easy-nLC, Proxeon) connected to a Velos Orbitrap mass spectrometer (MS) (ThermoFisher Scientific). The MS was operated in positive mode and the survey MS scan in the range of m/z 300–2,000 was obtained at a resolution of 60,000. Following each MS scan, the top four most abundant precursor ions were selected for MS/MS using collision-induced dissociation and electron-transfer dissociation fragmentation. IgG Fc glycopeptides were identified in liquid chromatography-MS/MS datasets by their characteristic retention times and accurate monoisotopic masses (within <10 ppm).
ppm from the theoretical values) of doubly and triply charged ions from M2139: EDNYSITIR, CIIC1, and L243: EDNYSITLR as well as Hy2.15: EIQNYSITFR, respectively. Protein identity was confirmed using Mascot search engine (version 2.3.2) using International Protein Index mouse concatenated database. Search parameters were as follows: MS mass error tolerance at 10 ppm, MS/MS mass accuracy at 0.5 Da, tryptic digestion with a maximum of two missed cleavages, carbamidomethylation of cysteine as a fixed modification, asparagine and glutamine deamination, and methionine oxidation as well as N-glycosylation (HexNAc[β]Hex[Hex](Hex)) as variable modifications.

**Particle Size Measurement Using DLS.** DLS is a useful tool to detect and confirm the formation of a protein complex. Disturbance of stable IC formation by Endo-β-O-linked oligosaccharides was carried out using the DLS technique. Briefly, CII purified from rat chondrosarcoma dissolved in 0.1 M acetic acid at 5 mg/mL was diluted further in PBS (1 mg/mL) and anti-CII mAb (high-affinity M2139 or low-affinity CB20; 1 mg/mL) in PBS were mixed together at 1:1 ratio and incubated for 30 min at 37 °C, followed by addition of either unhydrolyzed (Hy2.15) or Endo-β-O-

**SPR Analysis.** SPR (Biacoore 2000; Biacore) analysis was performed using the following procedure (29). Briefly, CII immobilized on the surface of CM5 sensor chips. Endo-β-O-hydrolyzed and unhydrolyzed IgGs were injected at different concentrations through flow cells in the running buffer (10 mM Hepes (pH 7.4), 150 mM NaCl, 3.4 mM EDTA, and 0.05% surfactant P20) at a flow rate of 25 μl/min. All dissociation experiments were initiated by injection of 100% ethylene glycol followed by 2 M NaCl and 100 mM HCl. Background binding to control flow cells was subtracted automatically. The chips were regenerated using pulse injection of 100% ethylene glycol followed by 2 M NaCl and 100 mM HCl.

**Analysis of the in Vitro Effects of mAbs Using Bovine Cartilage Extracts.** Articular cartilage samples extracted from adult bovine metacarpophalangeal joints and cartilage shavings (5 x 5 x 1 mm) were cultured for up to 14 d in DMEM with 20% (vol/vol) FCS and 25 μg/mL ascorbic acid, with 100 μg/mL of mAb or in medium alone. Medium was changed every 2 d, and fresh ascorbic acid and mAb were added at each change. Cartilage samples were tested in duplicate, and all experiments were performed at least twice. On day 14, cartilage explants were fixed in 4% (wt/vol) paraformaldehyde and embedded in paraffin for FITRIM. Sections (5 μm) were placed onto MirrIR low-e microscope slides (Kevley Technologies), and adjacent sections were stained with toluidine blue. FITR images were recorded with a Sterngig Digital FT5 7000 series spectrometer coupled to a UMA 600 microscope equipped with a 64 x 4 focal plane detector. For each measurement, 16 scans were recorded at a resolution of 6 μm. The spectra were analyzed using CytoSpec imaging software (17). Raw chemical maps were generated from the integrated intensities of functional groups identified in the spectra, and 10 spectra from the surface of the explant and 10 from the interior were extracted from the raw chemical maps. The mean spectra for “surface” and “interior” were calculated to assess the effects of antibody penetration on the peaks characteristic of CII and proteoglycans. Analysis was performed on the location of the amide peak (1,640–1,670 cm⁻¹), which represents total protein, which for cartilage is primarily CII. For proteoglycans, analysis was based on the height of the peak at 1,076 cm⁻¹, within the region of 1,175–960 cm⁻¹ derived from carbohydrate moieties.

**Statistical Analyses.** All of the mice with arthritis were included for calculation of severity. The severity of arthritis was analyzed by Mann–Whitney U test and the incidence by chi square test using Statview (version 5.0.1). Significance was considered when P < 0.05, or for a 95% confidence interval.

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