Supplementary text

Detection of human anti-CarP antibodies

Carbamylated and non-modified proteins were coated at 10ug/ml (diluted in 0.1M carbonate-bicarbonate buffer with a pH of 9.6) and incubated overnight on Nunc Maxisorp plates (Thermo Scientific). Plates were blocked for at least 6 hours with PBS/1% BSA (Sigma). Sera were diluted 50x in PBS/1%BSA/0.05%Tween (PBT) and incubated overnight. As standard, serial dilutions of a pool of positive sera was used. Binding of human IgG was detected using in PBT-diluted rabbit anti-human IgG conjugated to HRP (horseradish perixodase, DAKO, P0214), which was visualized with ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)). Washing with PBS/0.05% Tween was carried out between steps. All incubations, but the ABTS detection took place at 4 °C. The reactivity to the non-modified protein was subtracted from the reactivity to the corresponding carbamylated protein. The cut-off for positivity was set as mean plus two times the standard deviation of healthy individuals.

Detection of mouse anti-CarP antibodies

Non-modified or modified proteins were coated overnight at a concentration of 10 µg/ml (diluted in pH 9.6 0.1 M carbonate-bicarbonate buffer) on Nunc Maxisorp plates (Thermo Scientific). The plates were washed with PBS/0.05% Tween (Sigma) and subsequently blocked for 6 hours at 4°C with 100 µl of PBS/1% BSA (Sigma). After washing, the wells were incubated with 50 µl serum 1/50 diluted in PBS/1% BSA/0.05% Tween. The ELISA plates were incubated overnight at 4 °C. Total Ig, IgG1, and IgG2a were detected using HRP-conjugated rabbit anti-mouse Ig antibody (Dako), HRP-conjugated goat anti-mouse IgG2a, HRP-conjugated goat anti-mouse IgG1 (all from Southern Biotec). HRP enzyme activity was

visualized using ABTS. As a standard, serial dilutions of a pooled serum sample from mice with CIA were used.

Sample preparation for mass spectrometric analysis.

Synovial tissue samples (20 mg) from RA and OA patients obtained from joint replacement surgery were washed with PBS to remove adherent body fluids such as synovial fluid and blood. Samples were incubated in ST lysis buffer (4% SDS in 0.1 M Tris-Cl pH 7.6) for 15 min at 70°C. Initially, SDS lysates were subjected to FASP II as described above, but yielding low numbers of carbamylated peptide hits. In contrast, subsequent treatment of the samples with trypsin yielded many more hits. Therefore, the synovial tissue samples (after their extraction with hot SDS to remove adherent and easily soluble protein) were digested with trypsin using the following procedure; samples were incubated in 100 µl 100 mM DTT in 25 mM NH_4HCO_3 for 20 minutes at 54°C. After centrifugation, the supernatant was saved and the pellet incubated in 150 µl 15 mM iodoacetamide in 25 mM NH_4HCO_3 for 30 minutes at room temperature. After centrifugation, the supernatant was saved and the pellet incubated in 200 µl 25 mM NH_4HCO_3 containing 10 µg trypsin for 4 hours at 37°C. The combined supernatants from DTT and iodoacetamide incubation were concentrated on a 30 kDa filter (Microcon, Millipore), washed 3 times with 100 µl 25 mM NH_4HCO_3 and also incubated with 1 µg trypsin for 4 hours at 37°C. Next, the supernatant containing digested protein from the pellet was added to the digest on the filter. The filter was washed once with 100 µl 0.5 M NaCl. Peptides were recovered from the filtrate and subjected to solid phase extraction on C18 cartridges (Oasis HLB Waters).

Proteome analysis and mass spectrometric identification of carbamylation.

Peptides were analyzed via on-line C18-nano-HPLC-MS with a system consisting of an Easy nLC 1000 gradient HPLC system (Thermo Scientific), and a Q-Exactive mass spectrometer (Thermo Scientific). Fractions were injected onto a homemade precolumn (100 μm × 15 mm; Reprosil-Pur C18-AQ 3 μm, Dr. Maisch) and eluted via a homemade analytical nano-HPLC column (15 cm × 50 μm; Reprosil-Pur C18-AQ 3 um). The gradient was run from 0% to 50% solvent B (100/0.1 water/formic acid (FA) v/v) in 120 minutes. The nano-HPLC column was drawn to a tip of 5 μm and acted as the electrospray needle of the MS source. The Q-Exactive mass spectrometer was operated in top10-mode. Parameters were resolution 70,000 at an AGC target value of 3 million maximum fill time of 100 ms (full scan), and resolution 17,500 at an AGC target value of 100,000/maximum fill time of 60 ms for MS/MS at an intensity threshold of 17,000. Apex trigger was set to 1 to 5 seconds, and allowed charges were 2-5. For peptide identification, MS/MS spectra were submitted to the uniprot Homo Sapiens database (UP000005640; Jan 2015; 67911 entries) using Mascot Version 2.2.04 (Matrix Science) with the following settings: 10 ppm and 20 millimass units deviation for precursor and fragment masses, respectively; trypsin was set as enzyme.The fixed modification was carbamidomethyl on Cys. Variable modifications were carbamylation on K and protein N-terminus, oxidation on M and acetylation on the protein N-terminus.

Peptide synthesis and confirmation of identity.

Peptides for the confirmation of the sequences identified with mass spectrometry were synthesized according to standard fluorenylmethoxycarbonyl (Fmoc) chemistry using a SyroII peptide synthesizer (MultiSynTech). The integrity of the peptides was confirmed using reverse-phase HPLC and MS. Synthetic peptides were submitted to MS2 on the same instrument and compared with MS2 spectrum from biological samples to confirm the initial identification.

Generation of the anti-CarP monoclonal antibody.

Spleen cells of Ca-OVA immunized mice were fused with SP2/0 myeloma cells [1] using PEG1500 (Roche). Hybridoma cells were seeded in ten 96-wells plates and supernatant was tested for anti-CarP positivity by ELISA. SP2/0 cell line was tested for mycoplasma contamination.

Antibody variable region cloning.

Antibody variable region genes were amplified from hybridoma cells by RT-PCR, using heavy and light chain variable region specific primers. A pool of degenerate 'forward' primers that anneal to sequences encoding mouse immunoglobulin leader peptides were used with a pool of 'reverse' primers that anneal to sequences spanning the framework 4–constant region junctions of the heavy and light chains. Alternatively, a pool of degenerate 'forward' primers that anneal to sequences encoding the start of mature mouse heavy and light chain variable regions were used. Restriction sites incorporated in the PCR primers allowed cloning of the amplified variable region genes into mouse IgG2a or mouse kappa mammalian expression vectors.

Cultivating CHOSXE cells.

Large scale transient transfections were carried out using UCB's proprietary CHOSXE cell line and electroporation expression platform**.** Cells were maintained in logarithmic growth phase in CDCHO media (LifeTech) supplemented with 2mM Glutamax and agitated at 140rpm in a shaker incubator (Kuhner AG) supplemented with 8% CO_2 at 37°C

Electroporation Transfection.

Prior to transfection, the CHOSXE cell numbers and viability were determined using CEDEX cell counter (Innovatis AG) and the required amount of cells ($2x10^8$ cells/ml) were centrifuged at 1400 rpm for 10 minutes. The pelleted cells were washed in Hyclone ^{R} MaxCyte ^{R} buffer (Thermo Scientific) and re-suspended for a further 10 minutes and the pellets were re-suspended at $2x10^8$ cells/ml in fresh buffer. Plasmid DNA, purified using QIAGEN Plasmid *Plus* Giga Kit® was then added at 400ug/ml

Following electroporation using a MaxCyte STX® flow electroporation instrument, the cells were transferred into ProCHO medium (Lonza) containing 2mM Glutamax and antibiotic antimitotic solution and cultured in a wave bag (Cell Bag ^{TM} GE Healthcare) placed on Bioreactor platform set at 37^0C and 5% CO_2 with wave motion induced by 25rpm rocking.

24hr post transfection, a bolus feed was added and the temperature was reduced to 32^0C and maintain for the duration of the culture period (12-14days). At day four, 3mM Sodium butyrate was added to the culture. At day14, the cultures were centrifugation for 30 minutes at 4000rpm and the retained supernatants were filtered through 0.22um SARTO BRAN- P (Millipore) followed by 0.22um Gamma gold filters. Final expression levels were determined by Protein G-HPLC.

Antibody purification.

The murine IgG_{2A} antibodies were purified as follows. Following expression a Protein A affinity capture step was performed followed by a preparative size exclusion 'polishing' step. Clarified cell culture supernatants were first 0.22µm sterile filtered and loaded at 4ml/min onto 2x 5ml stacked MabSelect SuRe HiTrap columns (GE Healthcare) equilibrated in PBS pH7.4 (Sigma Aldrich Chemicals). After loading the columns were washed with PBS pH7.4 and then eluted with 0.1M Sodium Citrate pH3.4. The elution was followed by absorbance at

280nm, the elution peak collected, then neutralised with 1/5th volume of 2M Tris/HCl pH8.5. The neutralized samples were concentrated using Amicon Ultra-15 concentrators with a 30kDa molecular weight cut off membrane and centrifugation at 4000xg in a swing out rotor. Concentrated samples were applied to an XK26/60 Superdex200 column (GE Healthcare) equilibrated in PBS, pH7.4. The column was developed with an isocratic gradient of PBS, pH7.4 at 2.6ml/min respectively. Fractions were collected and analyzed by size exclusion chromatography on a TSK gel G3000SWXL; 5µm, 7.8 X 300mm column developed with an isocratic gradient of 0.2M phosphate, pH7.0 at 1ml/min, with detection by absorbance at 280nm. Selected monomer fractions were pooled. Final samples were assayed; for concentration by A280 Scanning UV-visible spectrophotometer (Cary 50Bio); for % monomer by size exclusion chromatography on a TSK gel G3000SWXL; 5µm, 7.8 X 300mm column developed with an isocratic gradient of 0.2M phosphate, pH7.0 at 1ml/min, with detection by absorbance at 280nm; by reducing and non-reducing SDS-PAGE run on 4-20% Tris-Glycine 1.5mm gels (Novex) at 50mA (per gel) for 53minutes; and for endotoxin by Charles River's EndoSafe® Portable Test System with Limulus Amebocyte Lysate (LAL) test cartridges.