Specific targeting of the IL-23 receptor, using a novel small peptide noncompetitive antagonist, decreases the inflammatory response

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Quiniou C, Domínguez-Punaro M, Cloutier F, Erfani A, Ennaciri J, Sivanesan D, Sanchez M, Chognard G, Hou X, Rivera JC, Beauchamp C, Charron G, Vilquin M, Kuchroo V, Michnick S, Rioux JD, Lesage S, Chemtob S. Specific targeting of the IL-23 receptor, using a novel small peptide noncompetitive antagonist, decreases the inflammatory response. *Am J Physiol Regul Integr Comp Physiol* 307: R1216–R1230, 2014. First published August 20, 2014; doi:10.1152/ajpregu.00540.2013.—IL-23 is part of the IL-12 family of cytokines and is composed of the p19 subunit specific to IL-23 and the p40 subunit shared with IL-12. IL-23 specifically contributes to the inflammatory process of multiple chronic inflammatory autoimmune disorders, including psoriasis, multiple sclerosis, inflammatory bowel disease, and rheumatoid arthritis. So far, one antibody targeting the shared p40 subunit of IL-12 and IL-23, Ustekinumab, is approved clinically to treat psoriasis. However, there are no treatments inhibiting specifically the IL-23 proinflammatory response. We have developed small IL-23R-specific antagonists by designing all D-peptides arising from flexible regions of IL-23R. Of these peptides, we selected 2305 (teeeqqly), since in addition to its soluble properties, it inhibited IL-23-induced STAT3 phosphorylation in spleen cells. Peptide 2305 specifically binds to IL-23R/IL-12Rβ1-expressing HEK-293 cells and not to cells devoid of the receptor. Peptide 2305 showed functional selectivity by modulating IL-23-induced gene expression in IL-23R/IL-12Rβ1-expressing cells and in Jurkat cells; 2305 does not inhibit IL-12-induced cytokine expression in IL-12Rβ-IL-12Rβ2-HEK-293 cells. Finally, compared with anti-p40 treatment, 2305 effectively and selectively inhibits IL-23-induced inflammation in three in vivo mouse models: IL-23-induced ear inflammation, anti-CD40-induced systemic inflammatory response, and collagen-induced arthritis. We, hereby, describe the discovery and characterization of a potent IL-23R small-peptide modulator, 2305 (teeeqqly), that is effective in vivo. 2305 may be more convenient, less cumbersome, less costly, and most importantly, more specific than current biologics for the treatment of inflammatory conditions, and conceivably complement the actual therapies for these chronic and debilitating inflammatory diseases.

peptides; cytokine receptors; interleukin-23R; inflammation; arthritis

IL-23, IDENTIFIED AS A HETERODIMERIC proinflammatory cytokine, is a member of the IL-12 superfamily composed of a p40 subunit (shared with the IL-12 cytokine) and a unique p19 subunit; IL-23 receptor complex is composed of IL-23R subunit and the IL-12Rβ1 subunit (shared with the IL-12 receptor complex). Over the past decade, IL-23 has been identified as a key cytokine in human chronic autoimmune inflammatory conditions and experimental mouse inflammatory disease models, such as psoriatic skin inflammation, inflammatory bowel disease (IBD), experimental autoimmune encephalomyelitis, and collagen-induced model of rheumatoid arthritis (2, 19, 23, 26, 52, 72). For instance, Yago et al. (72) demonstrated that the IL-23 pathway participates in joint destruction during the effector phase of rheumatoid arthritis (RA). Both IL-23 and IL-17 are found in the serum, synovial fluid, and synovial tissue of patients with RA, and appear to participate in inducing the production of proinflammatory cytokines that cause further damages to the joints (51, 53). Also, naturally occurring polymorphisms of the IL-23R gene have been linked to decreased susceptibility to Crohn's disease, ulcerative colitis, and psoriasis (9, 21).

IL-23 has been classified as a proinflammatory mediator mainly secreted by antigen-presenting cells (APC), such as monocytes, dendritic cells, and macrophages (70). The receptor for IL-23 is found on various T-cell subsets, some innate lymphoid cells, as well as a low number of B cells (5, 14). Activation of IL-23R induces JAK2 tyrosine kinase phosphorylation of STAT3, which prompts STAT3/STAT4 dimerization and transcription of inflammatory genes (30). In addition, IL-23 contributes to inflammation by maintaining Th17 cells (30) that produce IL-17, IL-21, IL-22, as well as several other proinflammatory cytokines and chemokines (1, 42).

IL-23R antagonists have been developed in recent years. Ustekinumab (Stelara-Centocor) was approved for the treatment of moderate-to-severe psoriasis (2009), for psoriatic arthritis (2013), and a phase III is ongoing for moderate-to-severe Crohn's disease; Briakinumab (Abbott) is under clinical evaluation at this time (23, 45). These two humanized antibodies target the IL-23/IL-12 p40 shared subunit. But IL-12 and IL-23 exert different effects. Because IL-12 promotes a Th1 response, while IL-23 favors Th17, a selective IL-23 receptor

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antagonist/modulator might provide clinical advantages without compromising the Th1 immune response to pathogens.

Hinge and flexible regions of receptors (and other proteins) are of great interest, as they affect appropriate conformation and/or interactions with other subunits and partners (10, 65), and peptides derived from the corresponding regions of VEGF and β-adrenergic receptors hinder dimerization (25, 68). A comparable approach using peptides derived from juxtamembranous regions has yielded efficacious peptides against prostaglandin $F_{2\alpha}$ receptor (FP) (24), vasopressin receptor (V_2R) (58), prostaglandin E_2 receptor (EP4) (44), and IL-1R/IL-1RAcP receptor (interleukin receptor 1) (57). In vitro and in vivo characterization of these peptides revealed functional selectivity and binding to a site distinct from that of the orthosteric (natural) ligand, a characteristic of allosteric modulators (43, 56). Allosterism can alter signaling modalities (56) and downstream responses without completely inhibiting receptor function (69), and, thus, confer greater selectivity. This has also been described for thiochrome, a selective allosteric enhancer of the M4 muscarinic receptor (43), for TNF-α-RI (50), and for adhesion molecules, such as the LFA-1 integrin of lymphocytes, where a peptide binds to an allosteric site and interferes with some (but not all) of its functions (3).

We, hereby, describe for the first time, a small octapeptide modulator of IL-23 receptor that displays specificity of binding and action, reveals allosteric properties (such as functional selectivity), and exhibits efficacy in IL-23-dependent animal models of inflammation.

MATERIALS AND METHODS

Animals and Cells

HEK-293 cells were purchased from American Type Culture Collection (ATCC) (CRL1573). CD-1 (male, 5 wk), DBA/IJ mice (male, 8 wk), and C57BL/6 mice (male 8–12 wk) were purchased from The Jackson Laboratories (Bar Harbor, ME). Jurkat cells and human rheumatoid arthritis synoviocytes were obtained from Dr. Florina Moldovan, CHU (Centre Hospitalier Universitaire)-Ste-Justine, Montréal, Canada. IL-23R-eGFP homozygous mice (referred to in the text as IL-23R$^{-/-}$) were maintained on a C57BL/6 background (5). CD-1 and DBA/IJ mice were maintained at CHU-Ste-Justine Hospital, while C57BL/6 and IL-23R$^{-/-}$ mice were maintained at the Maisonneuve-Rosemont Hospital. All animals were used according to the protocols by the Animal Care Committees of the CHU-Ste-Justine and Maisonneuve-Rosemont Hospitals along with the principles of the Canadian Council on Animal Care.

Peptide Design

The in silico model of the IL-23R subunit (Fig. 1A) was generated with the MT4 Server version 3.0 (22, 62) using the gp130 receptor structural data (PDB: 1I1R_A) (15) and the chain B of the human granulocyte colony stimulating factor-receptor signaling complex (PDB: 2D9Q_B) (67) as templates.

Flexible regions of the IL-23R subunit were identified on the basis of modeling data and were supported by hydrophobic and flexibility profiles, as well as homology domains using computational analysis [ProDom (6), PROSITE (61), Predict Protein (61), and ProtScale (41)]. Seven corresponding homologous peptides [all D-8-10 amino acids, (NH3-COOH)] were derived from primary sequences of extra-
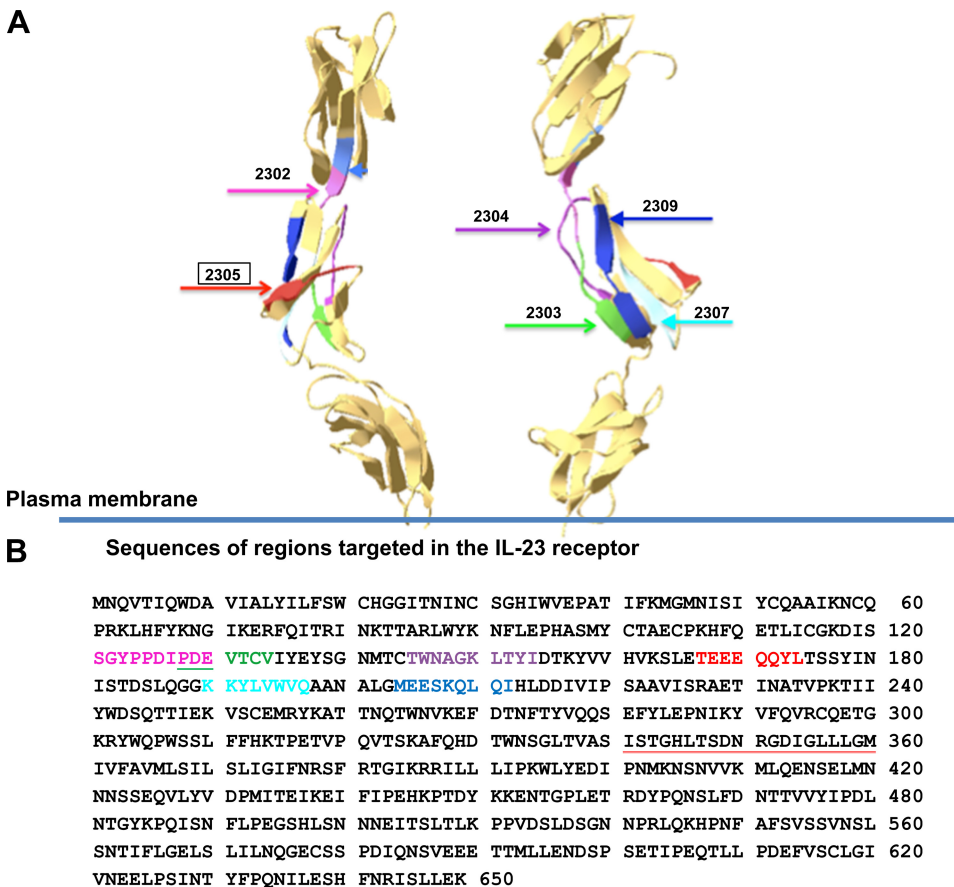

Fig. 1. Location of IL-23R regions targeted for peptide design. *A*: ribbon-like model of IL-23R chain (*right* and *left*) and identification of regions of derived effective peptides as identified with arrows. *B*: primary sequence of the human IL-23R. Colored sequences refer to corresponding loops indicated on *A*; in pink, 2302; in green and underlined in green, 2303; in magenta, 2304; in red, 2305 (surrounded with black box); in cyan, 2307; in dark blue, 2309; and in red and double underlined, juxtamembranous region.

A

2302 **2304** **2309**
2305 **2303** **2307**

Plasma membrane

B   **Sequences of regions targeted in the IL-23 receptor**

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MNQVTIQWDA VIALYILFSW CHGGITNINC SGHIWVEPAT IFKMGMNISI YCQAAIKNCQ   60
PRKLHFYKNG IKERFQITRI NKTTARLWYK NFLEPHASMY CTAECPKHFQ ETLICGKDIS  120
SGYPPDIPDE VTCVIYEYSG NMTCTWNAGK LTYIDTKYVV HVKSLETEEE QQYLTSSYIN  180
ISTDSLQGGK KYLVWVQAAN ALGMEESKQL QIHLDDIVIP SAAVISRAET INATVPKTII  240
YWDSQTTIEK VSCEMRYKAT TNQTWNVKEF DTNFTYVQQS EFYLEPNIKY VFQVRCQETG  300
KRYWQPWSSL FFHKTPETVP QVTSKAFQHD TWNSGLTVAS ISTGHLTSDN RGDIGLLLGM  360
IVFAVMLSIL SLIGIFNRSF RTGIKRRILL LIPKWLYEDI PNMKNSNVVK MLQENSELMN  420
NNSSEQVLYV DPMITEIKEI FIPEHKPTDY KKENTGPLET RDYPQNSLFD NTTVVYIPDL  480
NTGYKPQISN FLPEGSHLSN NNEITSLTLK PPVDSLDSGN NPRLQKHPNF AFSVSSVNSL  560
SNTIFLGELS LILNQGECSS PDIQNSVEEE TTMLLENDSP SETIPEQTLL PDEFVSCLGI  620
VNEELPSINT YFPQNILESH FNRISLLEK  650
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cellular regions (loops and interdomain regions) of the IL-23R sub-unit.

Gene Expression

Construction of plasmids for stable expression in mammalian cells. Complementary DNA of IL23R and IL12Rβ1 was amplified from pCDNA3.1IL23R and pCDNA3.1IL12Rβ1, respectively, and then further subcloned into pLpCMCS vector (kindly provided by the laboratory of Dr. Gerardo Ferbeyre) within the *Bam*HI/*Bsa*BI restriction sites to create the pLpCIL23R and *Pac*I/*Cla*I restriction sites to create pLpCIL12Rβ1. The construction of pLpCIL23R+Venus and pLpCIL12Rβ1+mPlum binary vectors were created by inserting PCR-amplified Venus and mPlum fragments between the *Pme*I and *Sal*I restrictions sites found in the multiple cloning sites following the IRES sequence. All of the plasmids constructs were verified by sequencing.

Creation of IL23R+IL12Rβ1 and IL12Rβ1 stable cell lines in HEK-293T. HEK-293T cells were cultured and kept in DMEM medium (Wisent) supplemented with 10% FBS. Stable cell lines of HEK-293T cells expressing IL23R and IL12Rβ1 were generated using retroviral gene transfer, as described elsewhere (64) using the pLpCIL12Rβ1+mPlum first, followed by a second round of trans-duction using the pLpCIL23R+Venus construct. The pLpC constructs carry the gene for the antibiotic resistance for puromycin, which was also used as a method of selection of stable population of HEK-293T cells that express the receptors.

Transient expression of IL12Rβ1 and IL12Rβ2 in HEK-293 cells. Transient expression in cells was performed by transfecting 5×10^4 cells with pcDNA3.1 plasmids containing either *IL12Rβ1* or *IL12Rβ2* genes (1 μg) using the polyethylenimine (1 μg/ml) as a transfecting agent. Expression of IL-12 receptor subunits was verified by performing SDS-PAGE electrophoresis followed with Western blot transfer. The following antibodies were used: anti-IL-23R (ab58367; Abcam, Cambridge UK), anti-IL-12Rβ1 (ab 96517; Abcam), and anti-IL-12Rβ2 (ab107204; Abcam).

Determination of IL-23- and/or IL-12-induced cytokine expression. HEK-293 expressing or not the human IL-23 receptor, Jurkat T lymphocytes, or Raw Blue mouse macrophage cells (Invivogen, San Diego, CA) were preincubated at 37°C with teeeqqyl (2305; Fig. 1*B*) at a concentration of 10^{-6} M for 30 min and then incubated for 4 h with human or mouse IL-23 (eBioscience) or hIL-12 (Peprotech, Rocky Hill, NJ). Raw Blue cells were pretreated with ionomycin (100 ng/ml) and PMA (phorbol 12-myristate 13-acetate 20 ng/ml) for 24 h prior to preincubation with 2305. After treatment with 2305 and IL-23, the cells were collected in TRIzol (Invitrogen, Burlington, Ontario, Canada), and total RNA was isolated. RNA tissue extraction from mouse ears was performed using the RNeasy extraction kit from Qiagen (Qiagen, Toronto, Canada), according to the manufacturer's instructions. 500 ng of RNA was combined to qScript cDNA Super-Mix (Quanta Bioscience, Gaithersburg, MD), and cDNA synthesis was performed following the manufacturer's protocol. Quantitative real-time PCR was performed on MxPro3000 (Stratagene Missis-sauga, Ontario, Canada) using iTaq SYBR Green SuperMix with ROX (Bio-Rad). Primers were synthesized by AlphaDNA (Mon-treal, Quebec, Canada), and sequences were hIL-1β-F (5′-AGCTGGAGAGTGTAGATCCCAA-3′); hIL-1β-R (5′-ACGGGCAT-GTTTTCTGCTTG-3′); hIL6-F (5′-TTCAATGAGGAGACTTGC-CTGG-3′); hIL6-R (5′-CTGGCATTTGTGGTTGGGTC-3′); hIL17A-F: (5′-CATTGGTGTCACTGCTACTGC-3′); hIL17A-R: (5′-AAGGTGAGGTGGATCGGTTG-3′); hIL22-F: (5′-CGGACT-CAGTATGAGTGAGCG-3′); hIL22-R: (5′-TGTGCTTAGCCTGT-TGCTGA-3′); hp35-F: (5′-TTTTATGATGGCCTGTGCCT-3′); hp35-R: (5′-TGGCACAGTCTCACTGTTGA-3′); hp40-F: (5′-GTGTCAAAAGCAGCAGAGGC-3′); hp40-R: (5′-GGTCAG-GTTTGATGATGTCCT-3′); mIL-6-F (5′-CAACGATGATGCACT-TGCAGA-3′); mIL-6-R (5′-TCTCTCTGAAGGACTCTGGCT-3′);

mIL-17-F (5′-TGAGTCCAGGGAGAGCTTCA-3′); mIL-17-R (5′-CATTGCGGTGGAGAGTCCAG-3′); IL-1β-F (5′-AGAT-GAAGGGCTGCTTCCAAA-3′); mIL-1β-R (5′-GGAAGGTC-CACGGGAAAGAC-3′); mIL-22-F (5′-TGCGATCTCTGATGGCT-GTC-3′); and mIL-22-R (5′-TATCTGCAAGGCTGGCCTCC-3′). mRNA expression levels were normalized against 18S rRNA endog-enous control levels in each sample and calculated relative to control vehicle-treated cells or control ears (Quantum RNA Universal primers 18S; Ambion; Life Technologies, Burlington, Ontario, Canada).

Isolation of Spleen Cells and Functional Assays

Briefly, mice were killed with CO_2, the spleen of each mouse was removed aseptically, cut, passed through a 26-gauge needle, and filtered through a 70-μm mesh nylon cell strainer. After centrifugation at 1,000 rpm, cells were exposed to water (1 ml) osmotic shock for 5 s, which was stopped with the addition of 10 ml of HBSS 2× buffer (HBSS 10× no phenol red, Life Technol-ogies, Burlington, Ontario, Canada). After centrifugation, cells were resuspended in RPMI supplemented with 10% FBS, and used immediately (40). Cells were counted, and cell viability was determined with the trypan blue exclusion assay. Cells were exclusively used when viability was >98%.

Cells resuspended in complete RPMI were used for subsequent anal-yses. For STAT3 phosphorylation dose-response curve, 10^5 cells were preincubated at 37°C for 30 min with different concentrations of 2305 and then incubated 15 min with 10 ng/ml of mIL-23 (eBioscience, San Diego, CA). Phosphorylated STAT3 was determined using the Alpha Screen Technology (Perkin-Elmer, Waltham, MA), according to man-ufacturer's instructions and normalized to total protein content and inhibition constant (IC_{50}), representing the concentration of 2305 that inhibits 50% of the total biological response was determined with the GraphPad Prism 6 (GraphPad Software, La Jolla, CA). IL-22, IL-6, and IL-17 production was determined using an ELISA kit (R&D Systems, Minneapolis, MN), according to manufacturer's instruc-tions. For STAT4 phosphorylation determination, 10^5 HL-60 (ATCC; CCL240) or mouse spleen cells were preincubated at 37°C with 2305 (10^{-6} M) 30 min and then incubated 15 min with 1 ng/ml of hIL-12 (Peprotech, Rocky Hills, NJ). Phosphorylated STAT4 was deter-mined by Western blot analysis using the anti-phosphorylated STAT4 and total STAT4 rabbit polyclonal antibodies (Cell Sig-naling, Boston, MA).

Radiolabeled Ligand Binding

Radiolabeled binding of [^{125}I]-2305 was performed on HEK-293 cells either expressing or not the IL-23 receptor complex. For maxi-mum specific binding, cells were preincubated with unlabeled peptide at 100 nM for 30 min in serum-free DMEM culture medium. [^{125}I]-2305 peptide was added at 75 pM, and the cells were incubated for another 2 h at room temperature with gentle shaking. The binding reaction was stopped by washing the cells four times with PBS 1× buffer and lysing with 0.1 N NaOH/0.1% Triton X-100. Displacement curves were obtained by preincubating HEK-IL-23R/IL-12Rβ1 or nontransfected HEK-293 cells with different concentrations of unla-beled 2305 for 30 min and subsequently incubating them with [^{125}I]-2305 at 75 pM for 2 h in the presence or absence of different concentrations of hIL-23. Bound radioactivity was measured on cell lysates with a Packard Cobrall autogamma counter. Displacement inhibition constants and logarithms [IC_{50} and $Log(IC_{50})$] of displace-ment inhibition constants were determined using the GraphPad Prism software version 5 (GraphPad Software).

IL-23-Induced Ear Inflammation Model

CD-1 mice were intradermally injected (exterior earlobe) daily for 5 days with 20 μl PBS or mIL-23 diluted in PBS at the indicated doses (eBioscience, San Diego CA) (26). 2305 was injected intraperitone-

ally twice daily (one injection in the morning at 5 mg/kg and the second injection 30 min before the IL-23 injection at 5 mg/kg). Some mice received 250 µg of the anti-p40 (eBioscience, San Diego, CA; clone C17.8) antibody on *day 1*. On *day 5*, ears were collected, weighed, and put in TRIzol for further RNA isolation using the Qiagen RNeasy universal plus kit. Quantitative PCR was performed on samples, as described above in the cytokine expression section.

Anti-CD40 Model of Systemic Inflammation

Eight- to twelve-week-old C57BL/6 (B6) and IL-23R$^{-/-}$ mice were used in this experimental model.

Treatment of mice with antibodies. Mice were injected intraperitoneally with 200 µg of anti-CD40 (CD40), IgG2a monoclonal antibody clone FGK45 (BioXCell, West Lebanon, NK; endotoxin level of <2.0 EU/mg) diluted in PBS. Control mice received the same volume of PBS. To evaluate the possible in vivo modulation of the IL-23R pathway, mice were injected intraperitoneally with 10 mg·kg^{-1}·day^{-1} of modulator peptide 2305 in PBS or with 200 µg of anti-p40 antibody, where the first dose was administered 6 h before the anti-CD40 antibody treatment and then every 24 h up to the end of the experiment. Mice were monitored daily and euthanized 7 days after anti-CD40 antibody injection. Animals exhibiting more than 25% weight loss were excluded from the study.

Evaluation of the systemic immune response. Serum was collected on *day 7*. Twenty-five microliters of undiluted mouse serum samples were used for the simultaneous detection of inflammatory molecules interleukin IL-2, IL-6, IL-12/23p40, IL-12p70, TNF-α, IFN-γ, CCL2, and CXCL1 by BD cytometric bead array (BD Biosciences, San Jose, CA), according to the manufacturer's instructions. Flow cytometric analysis was performed using a FACS Canto II flow cytometer (BD Biosciences). Data were acquired and analyzed using BD cytometric bead array software (v3.0).

Histopathology studies. For histopathological examination, liver samples were fixed in 10% buffered formalin. After paraffin embedding, 4-µm-wide tissue sections were stained with hematoxylin and eosin according to standard protocol and examined by light microscopy. All samples were examined in a blinded fashion. The severity of the inflammatory changes in liver sections was evaluated on a scale of 0 (no changes) to 4 (severe changes).

Collagen-Induced Model of Joint Inflammation

DBA/IJ male mice were obtained 1 wk prior the experiments.

Induction of collagen-induced arthritis model and assessment of arthritis. Bovine type-2 collagen (bCII, immunization grade) was purchased from Chondrex, (Redmond, WA). To induce collagen-induced arthritis (CIA), 8-wk-old mice were immunized with bCII (2 mg/ml), emulsified 1:1 with complete Freund's adjuvant (4 mg/ml) (Difco, Detroit, MI) intradermally at the base of the tail. The mice were boosted on *day 21* with a 50-µl injection intradermally at the base of the tail with a homogenate containing 1:1 CII (2 mg/ml) suspended in incomplete Freund's adjuvant. Mice were examined for the appearance of arthritis in the peripheral joints, and disease severity was graded on a scale (7). After the second immunization, all mice were examined daily. Mice that developed arthritis showed significant changes in redness and/or swelling in the digits or in other parts of the paws. The clinical severity of arthritis was graded in a double-blind manner on a scale of 0 to 4 for each paw, according to swelling and redness (7). Specifically, scoring was performed as follows: 0 = healthy; 1 = mild swelling and erythema; 2 = moderate swelling and erythema; 3 = more intense erythema, swelling, and redness affecting a greater proportion of the paw; 4 = severe erythema, swelling, and redness affecting the entire paw. A cumulative score ranging from 0 to 16, based on individual paw scores of 0 to 4, was assigned for each animal (7).

Experimental protocol. The study was divided into three groups of six animals: Control, 2305-treated, and anti-p40 treated. All treat-

ments started upon the first signs of arthritis that were visible between *days 5* and *14*. As soon as a mouse showed clinical signs of arthritis (score of 1–4, as described in the previous section) it was incorporated in a treatment group, and the next mouse was allocated to the next treatment. At the onset of disease and for 21 days, mice received 2305 twice daily (5 mg/kg ip), anti-p40 (250 µg) every 3 days, or saline twice daily.

Radiological examination of arthritic joints. Both fore- and hindpaws were radiographed with an X-ray Faxitron Imaging System (Faxitron X-Ray, Wheeling, IL). Forepaws and hindpaws were fixed in 10% buffered formalin. Data were digitized, and pictures of radiographs were prepared. Radiographs were examined for osteolysis, osteophyte presence, and disfigurement. Evaluators were blinded to treatment. For each animal, the overall score for the tarsal and carpal bones were given as follows: no damage = 0, minor damage = 1, mild damage = 2, and severe damage = 3. The tarsal-metatarsal joints, carpal-metacarpal joints, proximalis phalangeal joints, media phalangeal joints, and distalis phalangeal-digit joints were given a score 0 (not affected) or 1 (affected). Joints with lesions were counted, and a cumulative joint score was attained for each animal. The maximal score for each animal was 88.

Forepaws and hindpaws were fixed in 10% buffered formalin, decalcified, and embedded in paraffin. Joint sections (5–9 µm) were prepared and stained with hematoxylin and eosin. Microscopic evaluation of arthritic paws was done in a double-blind fashion. Arthritic changes in the ankle carpus tarsus, metacarpophalangeal or metatarsophalangeal, proximal interphalangeal joints, and digits were examined for inflammation, pannus formation, and joint damage. A lesion severity score of 0–3 was assigned to each joint. Thus, a cumulative score of 0–48 for each animal was possible. Scores were assigned as follows: 0 = normal, 1 = infiltration of inflammatory cells, 2 = synovial hyperplasia and pannus formation, 3 = bone erosion and destruction (7).

Statistical Analysis

Results are expressed as means ± SE. Two-tailed independent Student *t*-tests were used to analyze data. Comparisons between groups were made using one-way ANOVA followed by the post hoc Bonferroni's multiple-comparison test, or the Mann-Whitney *U*-test. Statistical significance was set at $P < 0.05$.

RESULTS

Screening of Peptides Derived From IL-23R Regions and Specificity of 2305 for IL-23R

Using the rationale presented above regarding the design of small peptides that interfere with actions of the protein of interest (12, 25, 55, 74), we identified flexible regions within the extracellular domain of IL-23R (Fig. 1A). On the basis of modeling data, which were supported by hydrophobic and flexibility profiles, as well as homology domains using computational analysis [ProDom (6), PROSITE (60), Predict Protein (61), ProtScale (41)], we designed D-8-10 amino acids homologous peptides (Fig. 1B). BLAST analysis confirmed that the chosen IL-23R regions were unique to IL-23R and exhibited interspecies homology for human, rat, and mouse, enabling us to investigate the properties of the peptides in systems of different species.

A first-round functional screening for efficacy of designed peptides was performed using IL-23-induced STAT3 phosphorylation in cultured mouse spleen cells. Peptides 2305 (teeeqqly) and 2309 (meeskqlqi) exhibited 100% efficacy (no statistical difference compared with control) in inhibiting IL-23-induced STAT3 phosphorylation (Fig. 2A). Peptide 2305 seemed to exhibit greater solubility (in normal saline) and was,

Fig. 2. Screening of peptides derived from IL-23R. *A*: primary screening of the designed peptides. STAT3 phosphorylation was determined on spleen cells preincubated with peptides (10^{-6} M) for 30 min and stimulated with mIL-23 (10 ng/ml) for 15 min. STAT3 phosphorylation was determined with Alpha Screen technology. *B*: dose-response effect of 2305 on STAT3 phosphorylation in spleen cells treated with mIL-23-induced (10 ng/ml). Values are expressed as means ± SE of three experiments each performed in triplicates. *A*: **$P < 0.01$ compared with control. #$P < 0.01$, ##$P < 0.001$, compared with IL-23-treated cells. *B*: *$P < 0.01$ compared with 10^{-11} M data (one-way ANOVA).
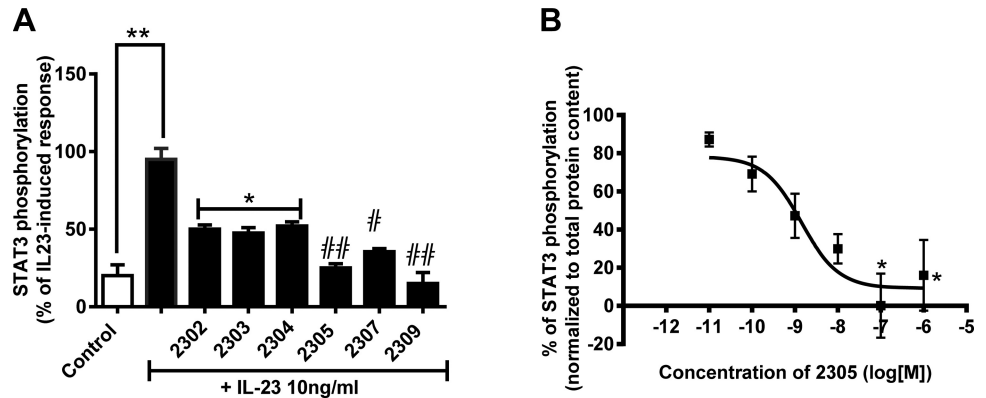
thus, selected for further in vitro and in vivo characterization; 2309 and 2307 were soluble in DMSO. Dose-response to 2305 revealed inhibition of STAT3 phosphorylation with a potency of 1.5 nM (Fig. 2*B*).

Binding Properties of 2305 on HEK-293 Cells Transfected with IL-23R/IL-12Rβ1 Complex

To ascertain the binding specificity of 2305 on IL-23R, binding studies using [^{125}I]-labeled 2305 were performed on HEK-293 cells transfected with pLpCIL-23R+Plum and pLp-CIL-12Rβ1+Venus plasmids to express both IL-23R and IL-12Rβ1 subunits composing the IL-23 receptor. [^{125}I]-2305 bound specifically to HEK-293 cells expressing the IL-23 receptor heterodimer with an estimated affinity of 3 nM (Fig. 3, *A* and *B*). [^{125}I]-2305 exhibited a much lower affinity (1.6 μM) on HEK-293 cells only expressing IL-12Rβ1 subunit, whereas it did not bind to nontransfected HEK-293 cells or to cells only expressing the IL-23R subunit (Fig. 3, *A* and *B*). These marked
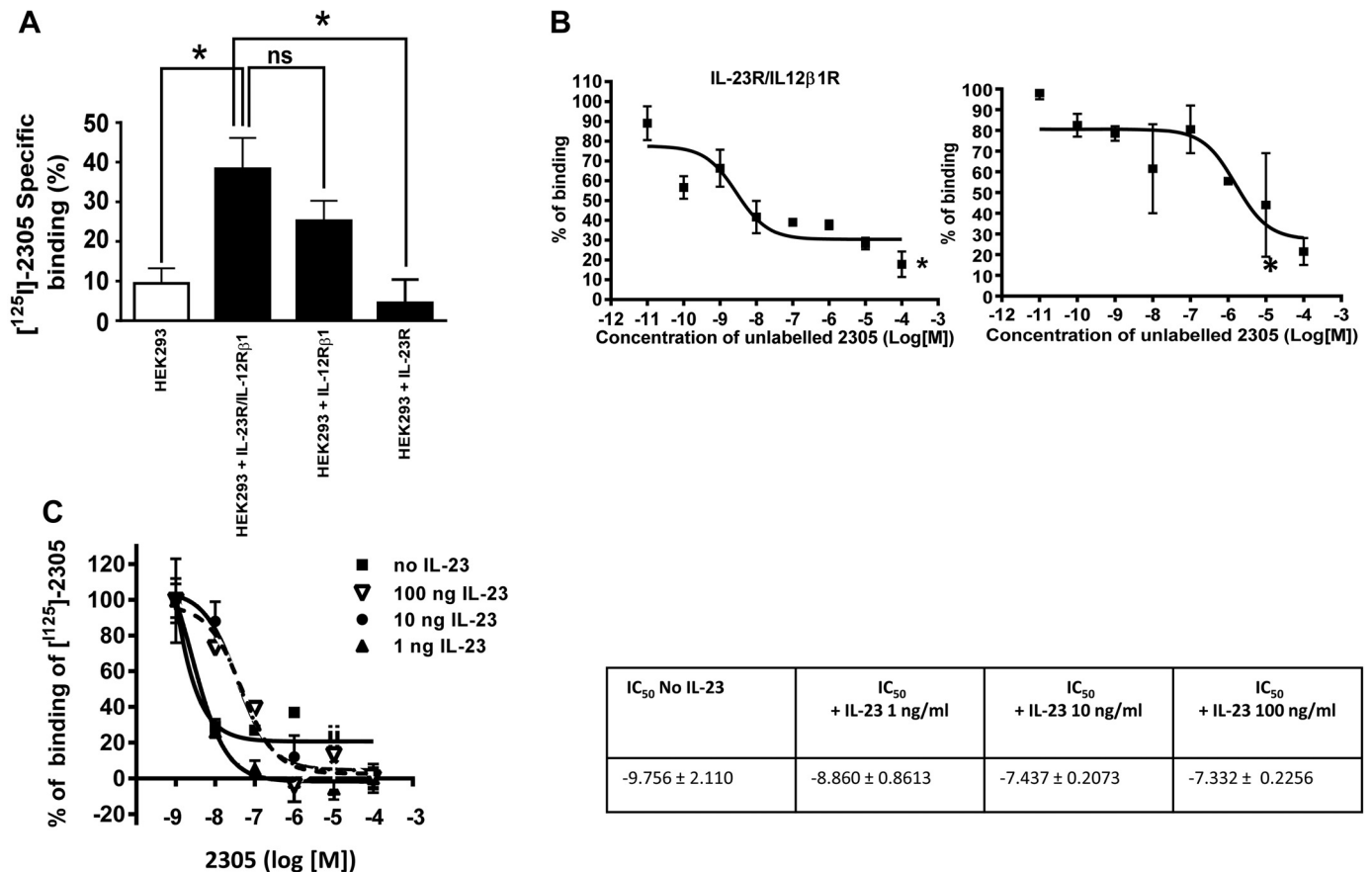
IC$_{50}$ No IL-23	IC$_{50}$ + IL-23 1 ng/ml	IC$_{50}$ + IL-23 10 ng/ml	IC$_{50}$ + IL-23 100 ng/ml
-9.756 ± 2.110	-8.860 ± 0.8613	-7.437 ± 0.2073	-7.332 ± 0.2256

Fig. 3. Binding properties of [^{125}I]-2305. *A*: total binding of [^{125}I]-2305 on recombinant HEK-293 cells expressing or not, IL-23R, IL-12Rβ1, or both subunits composing the IL-23 receptor. Cells were preincubated with unlabeled 2305 (75 nM, 1000× concentration compared with labeled compound) and incubated with [^{125}I]-2305 (75 pM) for 2 h. *B*: displacement of [^{125}I]-2305 binding (75 pM) to IL-23R/IL-12Rβ1R cells reveals much higher affinity (3 nM) compared with cells that only express the IL-12Rβ1 receptor subunit (affinity: 1.6 μM). *C*: displacement of [^{125}I]-2305 with unlabeled 2305 in the presence of different concentrations of IL-23. IL-23 induces modest changes in 2305 binding affinity on the receptor complex, consistent with an allosteric modulator (rather than an orthosteric competitive antagonist). Values are expressed as means ± SE of three experiments each performed in triplicate. *$P < 0.01$ compared with the corresponding value (one-way ANOVA).

differences in binding suggest that [^{125}I]-2305 requires both IL-23R and IL-12Rβ1 subunits for enhanced binding and suggest that 2305 interacts with IL-12Rβ1. Moreover, IL-23 induced a modest right shift (by ~100-fold) of the displacement curve of [^{125}I]-2305, which was saturable; higher concentrations of IL-23 could not further right-shift the displacement curves, as would be seen with competitive ligands; accordingly, IC$_{50}$ values for 2305 displacement of [^{125}I]-2305 did not significantly change in the presence of increasing concentrations of IL-23 [illustrated in Fig. 3C by the log(IC$_{50}$)]. This characteristic is consistent with a noncompetitive modulator binding to a site remote from the orthosteric site (the natural ligand binding site); on the contrary, a competitive relation between 2305 and IL-23 (if both were binding to the same site) would have yielded an infinite shift to the right, as IL-23 concentrations are increased. All in all, these results suggest that 2305 binds to the IL-12Rβ1 subunit, albeit with much greater affinity in presence of the whole IL-23R complex and behaves as a noncompetitive ligand.

In Vitro Functional Selectivity and Specificity of 2305

We assayed the specificity of action of 2305 on HEK-293 cells expressing or not the IL-23 receptor complex. As expected, IL-23 was able to induce the expression of inflammatory cytokines only in HEK-293 cells expressing the IL-23 receptor complex. 2305 also showed functional selectivity (modulation of selective signals), as it effectively reduced *IL-1*, *IL-6*, and *IL-22* gene expression in IL-23-stimulated HEK-293 cells expressing the IL-23 receptor to a similar extent as anti-p40 treatment (Fig. 4A). However, compared with anti-p40, 2305 did not significantly reduce IL-23-induced *IL17A*, *p35*, and *p40* (the latter two are subunits of IL-12 cytokine) mRNA levels (Fig. 4A). Efficacy of 2305 was also demonstrated on freshly isolated spleen cells from wild-type CD-1 mice, wherein it attenuated IL-23-induced IL-6 and IL-22, but not IL-17 protein levels (Fig. 4B). Likewise, 2305 significantly reduced the levels of IL-23-induced gene expression of *IL-1* and *IL-6* but not of *IL-17* in Jurkat cells (T-lymphocyte cell line) (Fig. 4C); the scrambled peptide was ineffective. Notably, 2305 did not interfere with IL-12-induced expression of *IL-22*, *p35*, and *p40* genes (Fig. 4D) in IL-12R$^+$HEK-293 cells and with IL-12-induced STAT4 phosphorylation in mouse spleen cells (Fig. 4E), further confirming specificity of 2305 to the IL-23 receptor. Interestingly, 2305 had a minor effect on *IL-1* and *IL-6* expression in Raw Blue mouse macrophages (Fig. 4F). Together, these data show that 2305 exhibits functional selectivity, as it modulates some, but not all, biological responses induced by IL-23.

Efficacy of 2305 in an IL-23-Induced Ear Inflammation Mouse Model

To further demonstrate in vivo specificity of action of 2305, we tested its efficacy in an IL-23-induced ear lobe inflammation model. Subcutaneous injection of IL-23 to the external earlobe generated a dose-dependent inflammatory edema (data not shown). We chose the higher dose to test efficacy of 2305. Systemic administration of 2305 at 10 mg·kg^{-1}·day^{-1} partially inhibited IL-23 (500 ng)-induced edema to a degree comparable to the anti-p40 antibody (Fig. 5A). In addition, levels of *IL-1*, *IL-6*, *IL-17*, and *IL-22* mRNA were measured in IL-23-injected external

earlobes and compared with controls. Peptide 2305 inhibited 50–75% of all gene expression, except for *IL-17*, which did not decrease; scrambled peptide was ineffective (Fig. 5B). Hence, 2305 specifically and effectively inhibits IL-23-induced inflammation in vivo.

Selectivity of 2305 in a Mouse Model of Systemic Inflammation

Activation of CD40 using a specific antibody (anti-CD40) is a p40-dependent model of systemic inflammation inducing the production of inflammatory cytokines (Table 1), as well as causing inflammation-derived cytotoxicity in several organs, notably in the liver (29, 36). We took advantage of this model to assess the selectivity of the 2305 peptide in an in vivo p40-dependent systemic inflammatory immune response. As expected, administration of anti-CD40 to wild-type mice caused an increase in serum levels of many inflammatory cytokines (Fig. 6). Concomitant treatment with 2305 significantly attenuated serum levels of most cytokines measured, with the exception of p40, TNF-α, and CCL2, which nonetheless decreased marginally ($P = 0.05$–0.1). In contrast to 2305, administration of anti-p40 antibody in anti-CD40-treated wild-type mice abrogated the rise of all serum cytokines measured.

To ascertain the role of IL-23R in this anti-CD40-induced inflammation model, we performed the same studies in IL-23R-null mice. Administration of anti-CD40 to IL-23R$^{-/-}$ mice induced an increase in serum levels of all cytokines measured with the exception of CCL2; albeit, this increase was attenuated compared with that seen in congener wild-type mice, as readily noted for IL-6, p40, p70, and TNF-α (Fig. 6), illustrating the difference in response due to the absence of the IL-23 receptor. Moreover, cytokine levels in anti-CD40-treated IL-23R-null mice did not differ statistically from those in anti-CD40/2305-treated wild-type mice, suggesting that the cytokine levels in response to 2305 in wild-type mice corresponds to the IL-23-independent cytokine expression in IL-23R-null mice. In addition, 2305 was also ineffective in anti-CD40-treated IL-23R-null mice; whereas in IL-23R-null mice anti-p40 reduced the levels of all cytokines that increased in response to anti-CD40, indicative of an IL-12-dependent response under these conditions (28). This observation was not limited to serum cytokine levels. Indeed, liver inflammation was comparable in C57BL/6 and IL-23R$^{-/-}$ mice treated with anti-CD40 (Fig. 6, B and C), underscoring this response as IL-23-independent. Altogether, these data suggest that part of the inflammatory response mediated by anti-CD40 treatment is IL-23-dependent, and the balance is IL-12-dependent; 2305 is ineffective in the absence of IL-23R, further supporting its specificity to IL-23R.

Efficacy of 2305 in CIA Model in Mouse

We determined the efficacy of 2305 in the pathologically relevant inflammatory model of arthritis, the CIA, reported to be at least partly IL-23-dependent (18, 73). 2305 inhibited 80% of the clinical signs of CIA, to the same extent as anti-p40 antibody (Fig. 7A); this is appreciated by daily scores from *day 14* to *21* of treatment and by the area under the clinical score
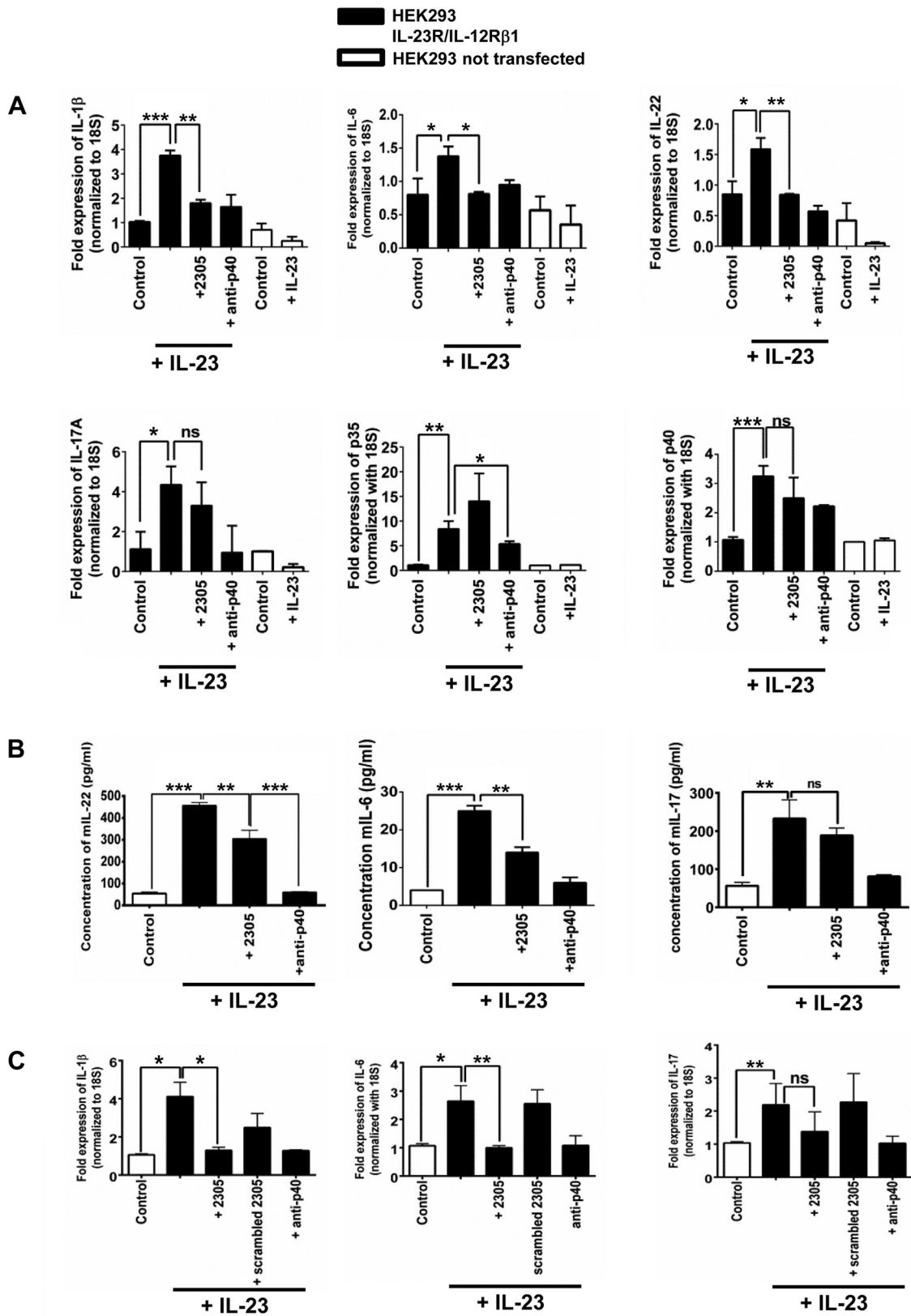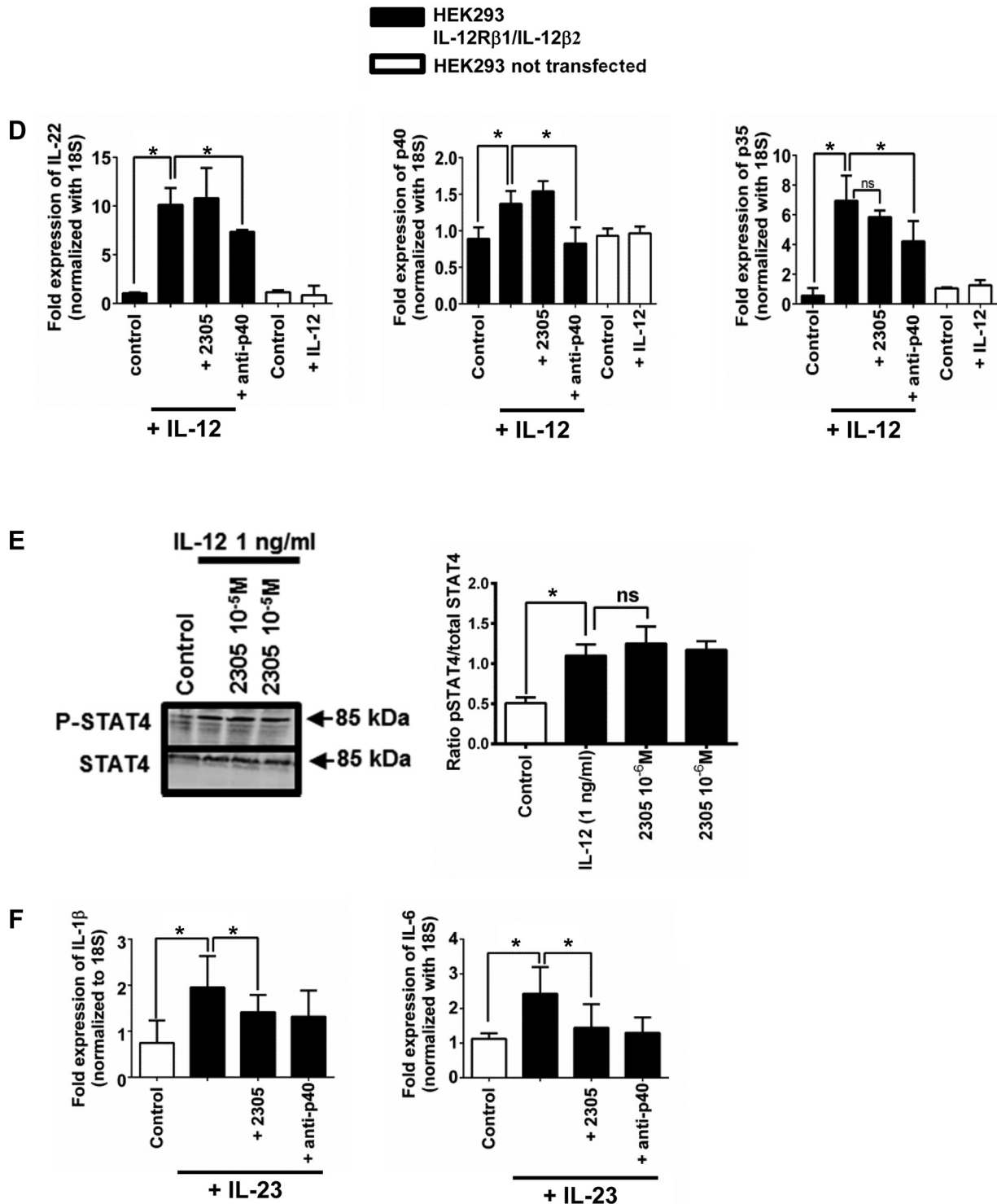
Fig. 4—*Continued*

Fig. 4. Functional selectivity of 2305 in HEK-293 cells, specifically expressing IL-23 receptor complex and in immune cells. A: peptide 2305 modulates cytokine gene expression in HEK-293 expressing the IL-23R complex. After preincubation with peptides (10^{-6} M), cells were stimulated for 4 h with hIL-23 at 50 ng/ml before real-time PCR was performed. Gene expression was normalized with ribosomal 18S production. B: efficacy of 2305 and scrambled peptide (10^{-6} M) on IL-23-induced (50 ng/ml) IL-6, IL-22, and IL-17 generation on mouse spleen cells. C: efficacy of 2305 and scrambled peptide (etqeqely 10^{-6} M) on IL-23-induced (50 ng/ml) *IL-1β*, *IL-6*, and *IL-17* mRNA in Jurkat cells. D: peptide 2305 has no inhibitory effect on IL-12-induced (1 ng/ml) expression of various cytokines in IL-12Rβ1/IL-12Rβ2[+]HEK-293 cells. E: 2305 has no inhibitory effect on IL-12 (1 ng/ml)-induced STAT4 phosphorylation in mouse spleen cells. Quantification of bands was performed with ImageJ software (National Institutes of Health). F: peptide 2305 has no inhibitory effect on IL-23-induced cytokine expression in RawBlue mouse macrophages. Values are expressed as means ± SE of three experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with corresponding value. ns, statistically not significant.
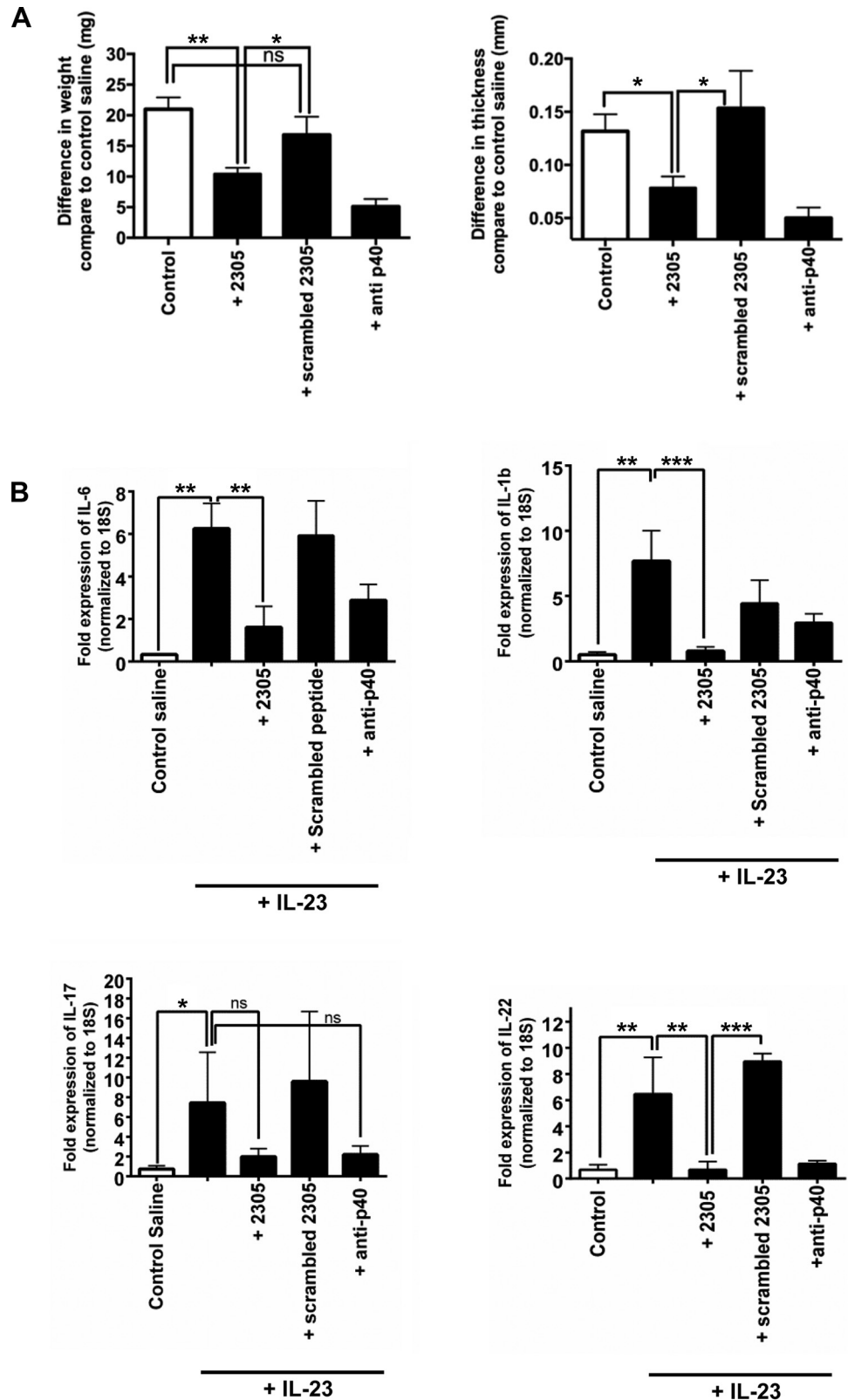
A

B

Fig. 5. Specificity of 2305 on IL-23-induced ear inflammation mouse model. *A*: effect of 2305 on mouse IL-23-induced ear inflammation. 2305 (10 mg·kg^{-1}·day^{-1} ip injections) inhibited >50% of edema induced with subcutaneous IL-23 injection (500 ng) to the external earlobe; the anti-p40 antibody (250 µg total) exhibited comparable efficacy; the scrambled peptide was ineffective. *B:* efficacy of 2305 and scrambled peptide on proinflammatory cytokine mRNA levels in IL-23-injected ear lobes compared with untreated (control) ear lobes. Values are expressed as means ± SE of three experiments (*n* = 6 per treatment); *P < 0.05, **P < 0.01, ***P < 0.001 compared with corresponding value (ANOVA); ns, statistically not significant.

curve (Fig. 7*A*, *inset*). X-rays of paws were consistent with clinical findings and revealed that animals treated with 2305 had diminished damage to joints (Fig. 7*B*). Histologic evaluation also revealed that 2305 markedly inhibited collagen-induced signs of inflammation (Fig. 7*B*). Accordingly, and in line with in vivo data, 2305 significantly inhibited IL-23-induced *IL-1* and *IL-6* cytokine gene expression but not *IL-17* in isolated human rheumatoid arthritis synoviocytes (Fig. 7*C*).

Table 1. *Statistical significance (P values) occuring between data samples obtained in the CD40 mouse model*

| Cytokines | Wild-Type C57BL/6 (B6) Treatments | | IL-23 Receptor Knockout Treatments | | |
	Saline vs. Anti-CD40	Anti-CD40 vs. Anti-CD40 + 2305	Saline vs. Anti-CD40	Anti-CD40 vs. Anti-CD40 + 2305	Anti-CD40 WT vs. Anti-CD40 KO
IL-2	0.02	0.04	0.02	0.62	0.35
IL-6	0.016	0.007	0.02	1	0.116
IL-12p40	0.018	0.134	0.043	0.886	0.2
IL-12p70	0.016	0.042	0.02	0.475	0.352
IFNγ	0.017	0.019	0.02	0.568	0.253
TNF-α	0.017	0.088	0.071	0.866	0.022
CCL2	0.05	0.255	0.599	0.769	0.085
CXCL1	0.017	0.042	0.02	0.866	0.775

Data were obtained using the Mann-Whitney *U*-test; see also Fig. 6.

Taken together, these data collectively suggest that CIA-induced inflammation is at least partly IL-23-dependent and that 2305 can efficiently be used to delay the progression of the disease.

DISCUSSION

On the basis of evidence that IL-23 receptor interacts with its IL-β1 subunit of the IL-23 receptor complex (54), we designed peptides that corresponded to the various flexible IL-23 receptor regions. Of these, one small octapeptide, termed 2305 (teeeqqly), was found to be particularly effective in inhibiting IL-23-dependent effects, both in vitro and in vivo. 2305 was a potent, selective, and reversible noncompetitive inhibitor of IL-23 receptor, exhibiting modulatory properties by not interacting with the ligand binding (orthosteric) site, albeit, slightly affected by IL-23 binding and by interfering variably with different in vitro responses to IL-23. Consistent with IL-23-induced in vitro effects, 2305 displayed effective anti-inflammatory capacity in inflammatory conditions involving IL-23. The findings describe a new small noncompetitive antagonist of IL-23 receptor with valuable and desirable modulatory pharmacologic properties, consistent with those of an allosteric negative modulator that exhibits functional selectivity (31, 32, 34, 69).

The peptides that we designed reproduced flexible regions of the IL-23R that possess high flexibility profiles to allow appropriate conformational changes to prompt the interaction with the IL-12β1 subunit. Primary sequence peptides reproducing specific protein regions have successfully been used to interfere with the effects of various receptors (12, 25, 55, 74), and the effects of such peptides coincide with those of corresponding mutations in the same region (48, 49). D-peptides [more active and stable than L-peptides (12)] derived from transmembrane and dimerization domains of β-adrenergic and VEGF receptors hinder dimerization (25, 68); similarly, a peptide fragment retaining a motif of the β-amyloid interferes with assembly of this protein into plaques (12). Because these regions of interest are often remote from the natural ligand-binding site (orthosteric site), these molecules are noncompetitive and can modulate ligand-binding affinity; these features are in line with characteristics of allosteric modulators (32, 33, 71), and on the basis of the data presented, apply to 2305, capable of binding specifically to the IL-23 receptor complex, but not to the IL-23 binding site. In addition, 2305 is a noncompetitive antagonist (43, 56) of IL-23-induced effects because it modulates (rather than fully interferes with) IL-23

binding to the IL-23 receptor complex. The labeled peptide shows specificity for binding to HEK-293 harboring the whole IL-23 receptor complex with an affinity of 3 nM; although $[I^{125}]$-2305 also binds to cells expressing exclusively the IL-12Rβ1 subunit, it does so with much lower affinity, indicative of the requirement of the full complex for efficient binding. The noncompetitive property of 2305 on IL-23-induced actions is supported by the inability of increasing concentrations of IL-23 to significantly overcome the displacement of $[I^{125}]$-2305.

Peptide 2305 reproduced a region of the extracellular portion of IL-23 receptor subunit. This subunit interacts specifically with the IL-12 receptor β1 subunit, and the complex will adopt a unique conformation enabling IL-23-induced signaling. Even though 2305 binds to the IL-12 receptor β1 subunit, it does so with a much lower affinity than to the IL-23R complex, wherein it appears to interfere with interactions between its two subunits, IL-23R and IL-12Rβ1. This is likely not the case for the interaction of IL-12Rβ1, with its signaling subunit IL-12Rβ2, consistent with inefficacy of 2305 in inhibiting signaling induced by IL-12.

Crystallographic analyses of protein complexes often fail to detect small conformational changes (≤ 2 Å), which may have profound effects on receptor function and are more readily appreciated by pharmacological binding and efficacy profiles (38). Altogether, one notes that IL-23 barely decreases binding affinity of 2305 for the receptor subunit, yet the latter markedly disrupts IL-23 functions; these observations contrast with those of orthosteric (competitive) inhibitors, in which changes in ligand binding somewhat correspond to those in function (17, 32).

Specific mutations (27, 48) or small molecules can affect some, but not all, functions evoked by a receptor (47, 59). This property is referred to as pharmacological permissivity or functional selectivity (31, 32, 69). This concept also appears to apply to 2305, which completely inhibited IL-23-induced expression of *IL-1*, *IL-6*, and *IL-22* in IL-23R/IL-12R HEK-293 cells. In addition, downstream signaling of STAT3 phosphorylation, conventionally activated by cytokine receptor activation, as well as IL-6 and IL-22 production in spleen cells were inhibited; 2305 was also effective in Jurkat T lymphocytes. Moreover, 2305 showed specificity of action and displayed no effect on IL-12-induced gene expression in IL-12R-expressing HEK-293 cells and on IL-12-induced STAT4 phosphorylation in HL-60 human monocytes and mouse spleen cells. However, treatment of 2305 had no effect on IL-23 induced *IL-17*, *p35*,
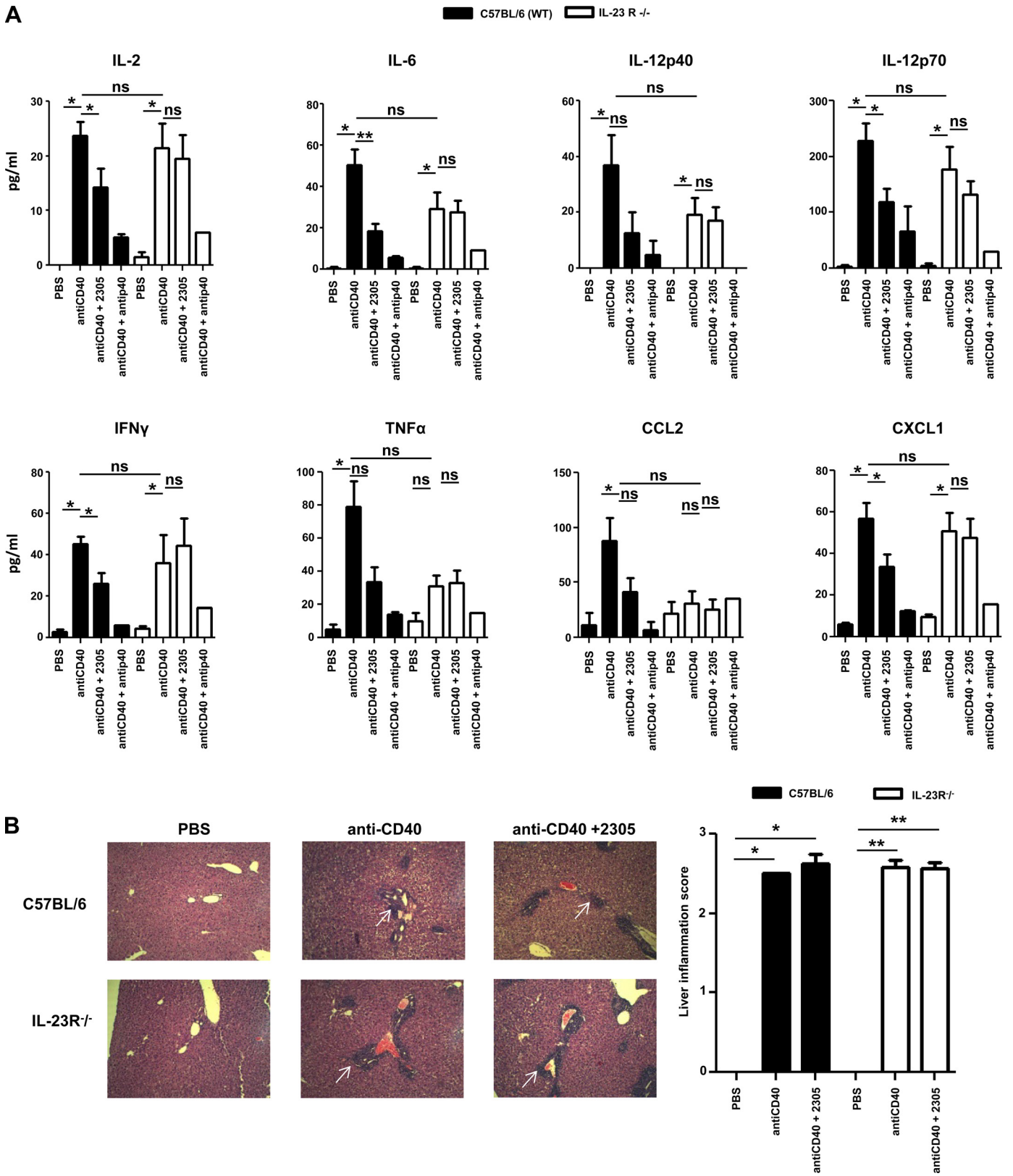
Fig. 6. Selectivity of 2305 in a model of systemic inflammation induced by anti-CD40. *A*: C57BL/6 and IL-23R$^{-/-}$ mice were treated as indicated. *Day 7* serum cytokine and chemokine levels are shown. *B*: 2305 does not affect the severity of the IL-23-independent liver inflammation induced by anti-CD40. C57BL/6 and IL-23R$^{-/-}$ mice were treated with PBS, anti-CD40, or anti-CD40 with 2305 as indicated. Liver histology is shown, and arrows point to anti-CD40-associated inflammation. Values are expressed as means ± SE; for saline, $n = 3$; for anti-CD40, $n = 5$; for anti-CD40 + 2305, $n = 5$; for anti-CD40 + anti-p40, $n = 2$. *$P < 0.05$, **$P < 0.01$ compared with corresponding value (ANOVA).
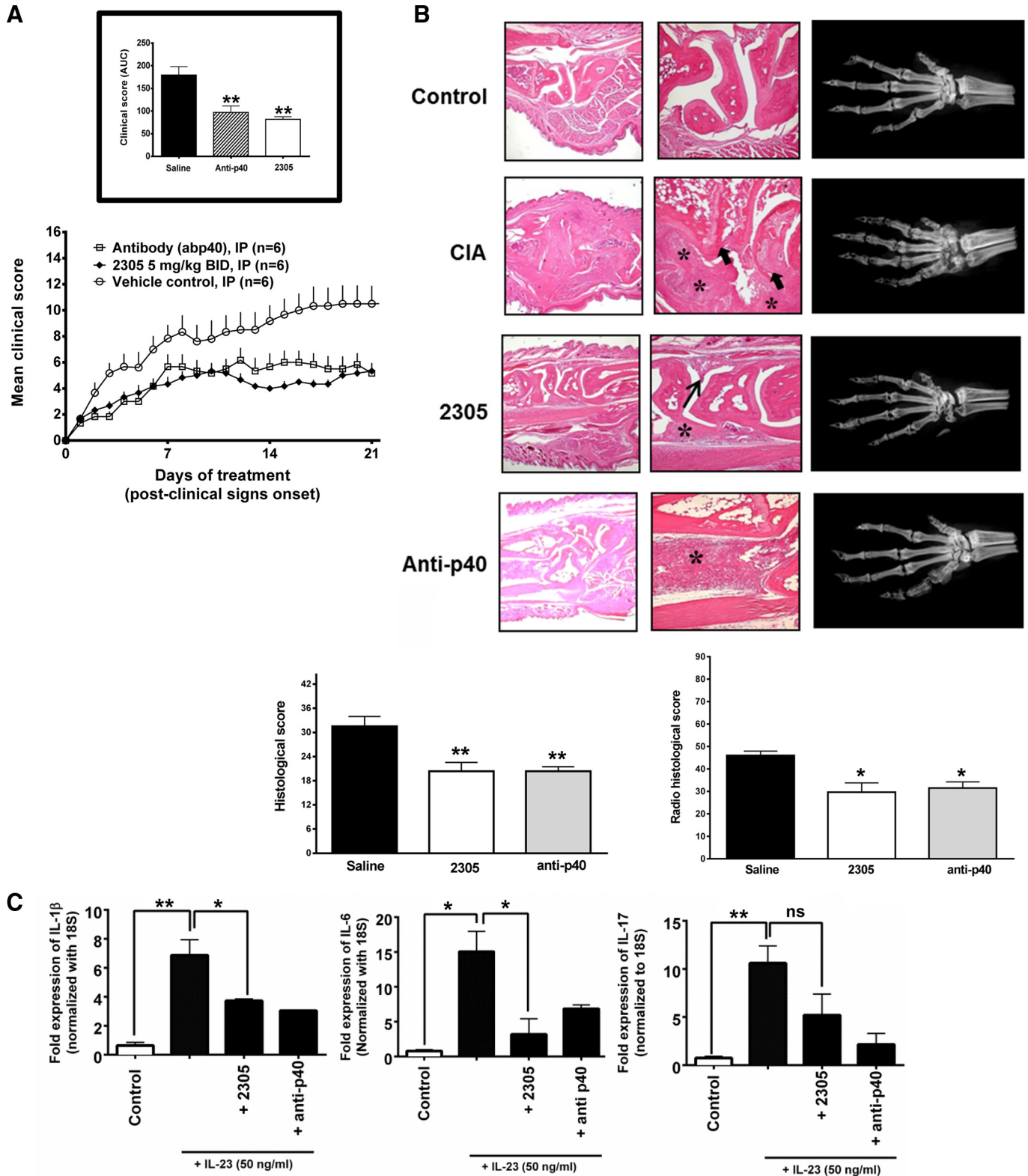
Fig. 7. 2305 inhibits joint inflammation in a collagen-induced arthritis (CIA) mouse model. *A, left*: graphic representation of collagen-induced inflammatory clinical signs. Intraperiteonal injections of 2305 (5 mg·kg^{-1}·day^{-1} twice a day) were given from *day 1* of appearance of clinical signs until *day 21* (for each animal). Clinical signs were evaluated every day according to the scoring method described in the MATERIALS AND METHODS. 2305 inhibited edema, redness, and ulcer formation as efficiently as the anti-p40 antibody (500 µg). *A, right:* area under the curve of the left panel graph. *B*: histology and X-rays representation of joints. Hematoxylin-and-eosin staining were performed on tissue sections. *Left* and *middle:* photographic representation of histology. Arrows point to bone erosion and asterisks within photomicrographs (*) represent neutrophil infiltration. *Lower:* scoring evaluation of tissue damages and cell infiltration. *Right:* representative X-rays of animal paws. *Lower:* scoring of X-rays reveal joint damages. *C:* efficacy of 2305 on IL-23-induced gene expression in human rheumatoid arthritis synoviocytes. Results are expressed as means ± SE of 6 mice. *$P < 0.05$ and **$P < 0.01$ compared with corresponding controls.

and *p40* gene expression. It has been suggested that IL-23 may activate a subset of macrophages and dendritic cells in an autocrine fashion, resulting in IL-6 production independently of IL-17 action (and T-cell activation) (4, 39, 66). IL-23 would, therefore, activate inflammation in two separate ways: *1*) activation of Th17 cells and *2*) induction of inflammatory mediators through innate immune cells (8, 13, 30), as presently shown herein on macrophages.

This functional selectivity is made possible by ligands, which bind in ways that affect the dynamic conformation of the receptor to interact with its natural ligand and associated proteins needed to activate normal signaling pathways (16, 35); hence, such ligands can alter signaling modalities (32, 56, 57), which may confer greater selectivity and reduce side effects (32) compared with orthosteric antagonists, which disable all functions triggered by the receptor. These features seem to apply to 2305 in line with recent work on other cytokine and noncytokine receptors (24, 44, 46, 57, 58).

In agreement with its specific anti-IL-23R actions in vitro, 2305 exerted corresponding effects in vivo by abolishing IL-23-induced acute ear inflammatory events comparably to the anti-p40 antibody. Remarkably, the peptide 2305 showed in vivo functional selectivity and, hence, acted as an allosteric modulator in a CD40 activation model of systemic inflammation. Binding of the anti-CD40 agonist antibody to B cells, macrophages, and nonimmune cells (fibroblasts, endothelial, and epithelial cells) leads to cell activation, systemic production of inflammatory cytokines, such as interleukin IL-23, IL-1, IL-6, IL-12, TNF-α, IFNγ, and CCL1 (11, 29), and to inflammation in different organs, mainly in the liver, but also to the gut (37). CD40 activation enhanced IL-23 secretion by activated cells and induced the expression of IL-23R, therefore, creating a positive feedback loop (20, 42). Along these lines, treatment of wild-type mice with 2305 attenuated the anti-CD40-induced release of some [but not all (consistent with functional selectivity)] proinflammatory mediators (IL-2, IL-6, IFNγ, and CXCL1) to values observed in the IL-23R-null mice, whereas anti-p40 antibody abrogated anti-CD40-induced rise in all cytokines. Findings are coherent with the fact that systemic cytokine production induced by CD40 activation is partially dependent on both the IL-12R and the IL-23R pathway.

Collagen-induced arthritis mouse model and human rheumatoid arthritis share many joint inflammation characteristics, such as neutrophil infiltrations in the synovium, osteoclast-mediated bone destruction, pannus formation, and the production of many proinflammatory cytokines (63) The collagen-induced arthritis model was reported to be IL-23-dependent (51, 72) Yago et al. (72) confirmed the role of IL-23 in the effector phase of CIA, and another study by Murphy et al. (51) demonstrated that p19$^{-/-}$ mice were completely resistant to joint damage in the CIA model. In line with the above in vivo results, 2305 abolished IL-23-induced inflammatory edema and joint damages in a collagen-induced model of autoimmune arthritis. The efficacy of peptide 2305 was comparable to that of the anti-p40 antibody.

Perspectives and Significance

In summary, we, hereby, report the discovery and pharmacological properties of a small stable (D-) octapeptide antago-nist of IL-23R, namely 2305, which is rationally derived from an extracellular loop region of IL-23R and exhibits properties consistent with those of an allosteric negative modulator. The clinically available molecule targeting IL-23 biological actions, namely, Ustekinumab, targets the IL-12- and IL-23-shared subunit p40. Drawbacks include higher risks of infections, allergic reactions, and tumor development, consistent with dual actions shared by IL-12 and IL-23. We describe in this report, the first IL-23 receptor-selective small molecule (peptide) antagonist, which seems to integrate allosteric modulatory properties; 2305 is a specific, potent, and effective in vitro and in vivo in IL-23-dependent models of inflammation following systemic application. Because 2305 appears to be as effective as the competitive antagonist to the p40 subunit in in vivo inflammatory conditions, 2305 (and small like-compounds) could offer therapeutic benefits, including those pertaining to simpler modes of administration and likely incurring lower costs. In addition, as the 2305 peptide shows increased specificity to the IL-23 pathway over the anti-p40 treatment, it may result in fewer side effects and complications (future studies will address this aspect).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: C.Q., M.C.D.-P., F.C., J.E., G. Chognard, X.H., J.D.R., S.L., and S.C. conception and design of research; C.Q., M.C.D.-P., F.C., A.E., J.E., D.S., G. Chognard, X.H., C.B., G. Charron, and M.V. performed experiments; C.Q., M.C.D.-P., F.C., J.E., M.S., G. Chognard, X.H., C.B., M.V., S.L., and S.C. analyzed data; C.Q., M.C.D.-P., F.C., G. Chognard, X.H., S.M., J.D.R., S.L., and S.C. interpreted results of experiments; C.Q., M.C.D.-P., F.C., A.E., J.E., G. Chognard, and S.L. prepared figures; C.Q. drafted manuscript; C.Q., M.C.D.-P., F.C., D.S., M.S., G. Chognard, J.C.R., C.B., G. Charron, M.V., V.K., S.M., J.D.R., S.L., and S.C. edited and revised manuscript; C.Q., M.C.D.-P., F.C., D.S., M.S., G. Chognard, V.K., S.M., J.D.R., S.L., and S.C. approved final version of manuscript.

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