# IL-4 controls activated neutrophil FcγR2b expression and migration into inflamed joints

Sudeepta Kumar Panda[a,b], Gustaf Wigerblad[a,c], Long Jiang[a], Yanek Jiménez-Andrade[a,d], Vaishnavi Srinivasan Iyer[a,e], Yunbing Shen[a], Sanjaykumar V. Boddul[a], André Ortlieb Guerreiro-Cacais[f], Bruno Raposo[a], Zsolt Kasza[a], and Fredrik Wermeling[a,1]

[a]Center for Molecular Medicine, Division of Rheumatology, Department of Medicine, Solna, Karolinska University Hospital, Karolinska Institutet, 17176 Stockholm, Sweden; [b]Structural Genomics Consortium, Department of Medicine, Karolinska University Hospital, Karolinska Institutet, 17176 Stockholm, Sweden; [c]Systemic Autoimmunity Branch, Intramural Research Program, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD 20892; [d]Cutaneous Biology Research Center, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA 02129; [e]School of Physical and Mathematical Sciences, Nanyang Technological University, Singapore 639798; and [f]Center for Molecular Medicine, Department of Clinical Neuroscience, Karolinska Institutet, 17176 Stockholm, Sweden

Edited by Tak W. Mak, University Health Network, Toronto, ON, Canada, and approved December 31, 2019 (received for review August 19, 2019)

**Neutrophils are the most abundant immune cells found in actively inflamed joints of patients with rheumatoid arthritis (RA), and most animal models for RA depend on neutrophils for the induction of joint inflammation. Exogenous IL-4 and IL-13 protect mice from antibody-mediated joint inflammation, although the mechanism is not understood. Neutrophils display a very strong basal expression of STAT6, which is responsible for signaling following exposure to IL-4 and IL-13. Still, the role of IL-4 and IL-13 in neutrophil biology has not been well studied. This can be explained by the low neutrophil surface expression of the IL-4 receptor α-chain (IL-4Rα), essential for IL-4– and IL-13–induced STAT6 signaling. Here we identify that colony stimulating factor 3 (CSF3), released during acute inflammation, mediates potent STAT3-dependent neutrophil IL-4Rα up-regulation during sterile inflammatory conditions. We further demonstrate that IL-4 limits neutrophil migration to inflamed joints, and that CSF3 combined with IL-4 or IL-13 results in a prominent neutrophil up-regulation of the inhibitory Fcγ receptor (FcγR2b). Taking these data together, we demonstrate that the IL-4 and CSF3 pathways are linked and play important roles in regulating proinflammatory neutrophil behavior.**

neutrophils | IL-4 | inflammation | arthritis | FcγR2b

Rheumatoid arthritis (RA) is a severe chronic autoimmune disease affecting 0.5 to 1% of the global population, thus ∼50 million people worldwide. RA is characterized by progressive inflammation of small joints in the hands and feet, but also often spreads to larger joints (1). Systemic effects are furthermore commonly seen, including symptoms affecting the lung, blood vessels, and heart (2, 3). A major proportion of RA patients are characterized by having disease-specific autoantibodies, and the presence of such autoantibodies is linked to an expected worsening of disease progression (4). Of note, these autoantibodies are associated with exposure to defined environmental factors, such as smoking, and genetic risk factors related to the immune system, such as specific HLA-DR alleles (5).

Studies with several animal models for RA have identified a central role for neutrophils in the induction and propagation of experimental autoimmune joint inflammation (6–10). The role of neutrophils in RA patients is harder to define, although neutrophils are typically making up the largest proportion of immune cells found in inflamed joints of patients with active disease (also seen in animal models for RA) (11–13). One hypothesis states that neutrophils are responsible for an inflammatory amplification loop. Here, an initial trigger results in neutrophil migration into the joint, local neutrophil activation, and release of proinflammatory effector molecules in the joint, in turn resulting in more inflammation and more migration of neutrophils into the joint, in a self-propagating inflammatory loop (10, 14). Of importance, activated neutrophils are known to release several modified proteins that are targets for autoantibodies found in RA patients, including citrullinated histones and vimentin (15, 16). In these patients, the release of such modified proteins locally in the joints is thought to result in the formation of antibody–antigen immune complexes (ICs), further propagating inflammation by interaction with activating Fcγ receptors (FcγRs) and by activating the complement system (17–19).

We and others have shown a protective role for IL-4 receptor (IL-4R) signaling in animal models for RA, induced by both IL-4 and IL-13 (20–26). However, it is not known how the IL-4R pathway is regulated during autoimmune inflammation. Combining gene-expression data and a targeted CRISPR screen, we here identify colony stimulating factor 3 (CSF3) as a potent positive regulator of neutrophil IL-4Rα expression. We further demonstrate that the neutrophil IL-4Rα up-regulation, seen during experimental joint inflammation (21), can be attributed to CSF3-induced STAT3 signaling. Importantly, we find that IL-4 and CSF3 synergize to up-regulate the inhibitory FcγR2b on

## Significance

**Joint inflammation is the defining characteristic of the autoimmune disease rheumatoid arthritis. Here we use experimental systems to show that the Th2 cytokine IL-4 suppresses the neutrophil potential of causing acute joint inflammation, including suppressing their migration to the inflamed joint, and by upregulating their expression of the inhibitory IgG Fc receptor. We also identify that the pathway is regulated by colony stimulating factor 3. Identifying strategies for early intervention in patients before they develop chronic, debilitating disease is a key focus of the current research in autoimmune diseases. Our study identifies mechanisms that could be exploited therapeutically in patient groups where IgG autoantibodies and neutrophils are thought to play an important role in the pathology.**

Author contributions: S.K.P., G.W., L.J., Z.K., and F.W. designed research; S.K.P., G.W., L.J., V.S.I., Z.K., and F.W. performed research; S.K.P., G.W., L.J., V.S.I., Z.K., and F.W. analyzed data; Y.J.A., V.S.I., Y.S., S.V.B., A.O.G.-C., and B.R. contributed to the research; S.K.P. and F.W. contributed new reagents/analytic tools; Z.K. and F.W. supervised the experiments; and S.K.P. and F.W. wrote the manuscript.

The authors declare no competing interest.

This article is a PNAS Direct Submission.



Data deposition: The data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, https://www.ncbi.nlm.nih.gov/geo (accession no. GSE134047 for Affymetrix expression data). Plasmids LentiGuide-Puro-P2A-EGFP and LentiGuide-Puro-P2A-EGFP_mRFPstuf have been deposited to AddGene (accession nos. 137729 and 137730, respectively).

[1]To whom correspondence may be addressed. Email: fredrik.wermeling@ki.se.

This article contains supporting information online at https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1914186117/-/DCSupplemental.

First published January 24, 2020.

neutrophils and that the interaction also affects neutrophil migration into inflamed joints. This is of importance since CSF3 is essential for joint inflammation in these models (27, 28). Taken together, our data identify that neutrophil IL-4Rα signaling suppresses several of the proinflammatory effects of CSF3 in the context of experimental joint inflammation, similar to what has been shown in models of infection (29). Neutrophil IL-4Rα thus seemingly behaves functionally as a paired inhibitory receptor to the CSF3 receptor, a concept commonly seen related to potent activating receptors expressed on immune cells, allowing for a fine-tuned activation and down-modulation of inflammation (30).

## Results

**IL-4 Protects Mice from Antibody-Mediated Joint Inflammation, and Neutrophil IL-4Rα Expression Is Potently Up-Regulated by a Serum Protein Related to Acute Inflammation.** A single injection of IL-4, formulated in a complex form, protects both C57BL/6 and BALB/c mice from joint inflammation in the K/BxN serum transfer model (Fig. 1 *A* and *B*) (20, 21). The acute joint inflammation triggered in this model is characterized by the infiltration of a large number of neutrophils into the joint, and the joint inflammation is related to FcγR-mediated activation (8, 10, 31). We have previously shown that these infiltrating neutrophils strongly up-regulate the IL-4Rα and that this is a general phenomenon for neutrophils triggered by a diverse range of acute inflammatory stimuli, including following the injection of LPS (21). Ex vivo stimulation of neutrophils with serum collected from mice injected with LPS ∼20 h earlier ("LPS serum") results in a strong up-regulation of the IL-4Rα chain at concentrations as low as 0.02% (Fig. 1 *C* and *D*) (21). Neutrophils incubated with serum from K/BxN mice similarly up-regulate the IL-4Rα, although several magnitudes lower compared to the LPS serum (Fig. 1*E*). Due to the strong activity of the LPS serum, we decided to initially use the LPS serum to study the characteristics of the IL-4Rα–regulating components. We ran LPS serum over several different protein-purification columns in a liquid chromatography system and found that the active component reproducibly segregates into defined fractions, behaving as expected from a protein or a stable protein complex. The active component showed, for example, strong binding to hydrophobic interaction columns (Butyl HIC), but no binding to Heparin columns (*SI Appendix*, Fig. S1). Taken together, these results suggest that a hydrophobic serum protein, or stable protein complex, released during acute sterile inflammation affects neutrophil IL-4Rα expression and that this protein could play a role controlling excessive inflammation.

**Identification of CSF3 as a Potent IL-4Rα Regulator in Neutrophils.** For more clues to the nature of the IL-4Rα regulating protein, we screened through supernatants of a panel of cell lines for their ability to up-regulate the IL-4Rα on neutrophils. The cell lines were stimulated overnight with or without LPS, and the cell-free conditioned supernatant was then transferred to ex vivo cultures with bone marrow cells, which contain a high proportion of neutrophils (Fig. 2*A*). Here, we usually used bone marrow cells from MyD88 knockout (KO) mice to eliminate the risk of residual LPS in the transferred conditioned supernatant interfering with the neutrophils through the Toll-like receptor 4 (TLR4)/MyD88 signaling pathway. Two cell lines were identified, from which the conditioned medium was able to potently up-regulate the IL-4Rα on neutrophils: 1) MH-S (32), a mouse alveolar macrophage cell line, and 2) HoxB8 Macpro (33), a mouse bone marrow-derived CSF2-dependent progenitor line that can be differentiated to macrophages (Fig. 2 *B* and *D* and *SI Appendix*, Fig. S2*A*). This is consistent with our previous observation showing that cells in the

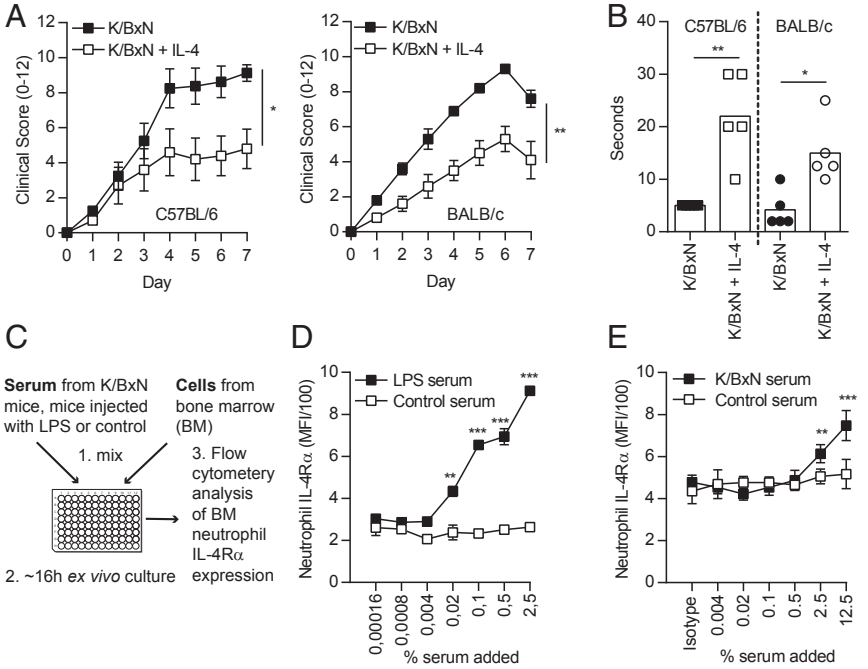


**Fig. 1.** IL-4 protects mice from antibody-mediated joint inflammation, and an inflammation-related serum factor regulates neutrophil IL-4Rα expression. (*A*) Joint inflammation in C57BL/6 and BALB/c mice injected intravenously with K/BxN serum (200 μL C57BL/6; 100 μL BALB/c) and IL-4 or control. (*B*) Functional test assessing the ability to hang on to a mesh at day 4. (*C*) Outline of experimental setup testing how serum from K/BxN mice or LPS injected mice affects neutrophil IL-4Rα expression. (*D* and *E*) Flow cytometry analysis of neutrophil IL-4Rα expression; cells isolated from bone marrow incubated overnight with different concentrations of serum collected from (*D*) LPS injected mice, and (*E*) K/BxN mice with active disease, as well as serum from naive control C57BL/6 mice. Data presented as mean and SEM in *A* ($n = 4$ to 5) and *D* and *E* ($n = 3$), and as mean and individual mice in *B* ($n = 4$ to 5). $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.005$ by Mann–Whitney *U* test (day 7 in *A*, and day 4 in *B*). Data are representative of at least two independent experiments. Differences in the basal IL-4Rα levels in *D* and *E* reflect the use of different flow cytometry machines with different sensitivities and settings.

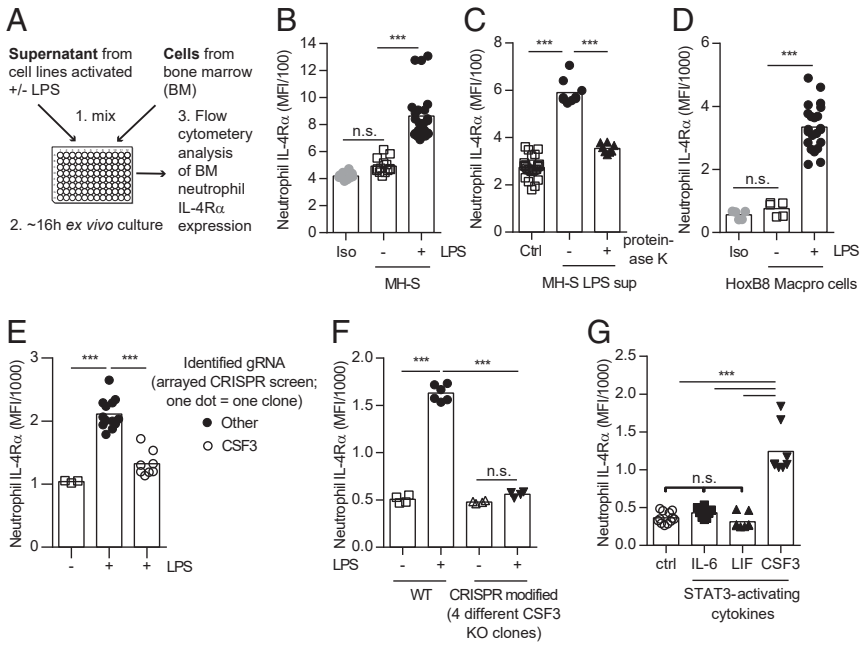


**Fig. 2.** CSF3 is a potent neutrophil IL-4Rα regulating protein. (*A*) Outline of experimental set-up testing how conditioned medium from different cell lines—activated by 0.2 μg/mL LPS or not—affects neutrophil IL-4Rα expression. (*B*) Flow cytometry analysis showing how 20% conditioned medium from the mouse alveolar macrophage cell line MH-S affects neutrophil IL-4Rα expression (cells from MyD88 KO bone marrow). (*C*) Supernatant from LPS-stimulated MH-S macrophages were incubated ± proteinase K beads and then incubated with bone marrow cells. The next day, neutrophil IL-4Rα expression was detected using flow cytometry. (*D*) Effect of 20% conditioned medium from HoxB8 Macpro cells, differentiated to macrophages, on neutrophil IL-4Rα expression, similar to experiment in *B*. (*E*) Summary of result from custom CRISPR screen targeting identified candidates for the IL-4Rα regulating protein. Single-cell colonies of CRISPR modified HoxB8 Macpro cells were expanded, differentiated to macrophages, activated by LPS, and tested for the ability of the conditioned medium to induce IL-4Rα expression on neutrophils. Cells with low ability to up-regulate the IL-4Rα were sequenced, and the CRISPR targeted gene identified. Each dot represents one single cell colony. (*F*) As a validation of the screen, four different CSF3 KO clones of the MH-S cell line was established by CRISPR (using a different CSF3 gRNA than the ones used in the screen). The cells were subsequently activated ±LPS and tested for the capacity of the conditioned medium to up-regulate the IL-4Rα on neutrophils. (*G*) Flow cytometer data of neutrophil IL-4Rα expression after overnight incubation with different STAT3-activating cytokines (20 ng/mL). Data presented as mean and individual samples in *B* ($n$ = 10 to 23), *C* ($n$ = 8 to 26), *D* ($n$ = 5 to 22), *E* (22 sequenced clones), *F* ($n$ = 4 to 6), and *G* ($n$ = 8 to 12) per group. ***$P < 0.005$, n.s. = not significant by one-way ANOVA with Tukey´s post hoc test. Data are representative of at least two independent experiments. (*B–D*) Represents pooled data from more than two independent experiments. Differences in the mean fluorescence intensity values comparing *B–G* is due to that different flow cytometry machines were used.

lung (and epididymal fat pads), isolated from mice injected with LPS, could readily release the IL-4Rα regulating protein/s (21). We could further confirm that the IL-4Rα regulatory activity of the conditioned medium was mediated by a protein, as the activity was abrogated by treatment with proteinase K (Fig. 2*C* and *SI Appendix*, Fig. S2*B*). We concluded that following activation of certain types of macrophages, a neutrophil IL-4Rα regulating protein is secreted.

We then generated single-cell colonies of the MH-S cell line and identified a set of clones that secreted higher amounts of the IL-4Rα regulating proteins ("high-activity clones") and a set of clones that secreted lower amounts ("low-activity clones"). Comparing these two groups revealed that the high-activity clones ($n$ = 3) produced at least four times more of the active IL-4Rα regulating proteins compared to the low clones ($n$ = 3) based on the ability to up-regulate neutrophil IL-4Rα at different dilutions (*SI Appendix*, Fig. S2*C*). Next, we performed a global gene-expression analysis comparing high- and low-activity clones stimulated with or without LPS. We had seen that the IL-4Rα regulating proteins accumulates in the supernatant of LPS-stimulated cell lines over several days. We, therefore, collected samples at a later time point (16 h) in order to limit picking up the burst of early response genes. At the assayed time point, 49 genes annotated as "extracellular" were significantly up-regulated more than twofold compared to unstimulated cells (*SI Appendix*, Fig. S2*D*). Of these, only seven genes (*Csf3*, *Il12b*, *Hp*, *Tnfsf15*, *Orm2*, *Lcn2*, and *Mmp9*) showed higher expression in the high-activity clones compared to the low-activity clones. Based on these assumptions, we performed a small custom CRISPR screen targeting only the differentially expressed genes and identified that CSF3, also known as G-CSF, was a plausible candidate for the IL-4Rα regulating protein (Fig. 2*E*). We subsequently generated individual CSF3 KO clones ($n$ = 4) of the MH-S cell line, using a guide RNA (gRNA) sequence that differed from the ones used in the screen, and validated that the cells lacked a functional *Csf3* gene (*SI Appendix*, Fig. S3 *A–C*). Stimulating the four different CSF3 KO clones with LPS and testing their supernatant for the ability to up-regulate IL-4Rα on neutrophils confirmed that the IL-4Rα regulating protein secreted by MH-S cells was CSF3 (Fig. 2*F*). Using recombinant proteins, we could further confirm that CSF3 has a potent ability to induce up-regulation of IL-4Rα on neutrophils as has been recently reported (29, 34), while no activity of the STAT3-activating cytokines IL-6 or LIF was seen (Fig. 2*G*). Notably, potent neutrophil IL-4Rα up-regulation can be observed at CSF3 levels as low as 1 ng/mL, indicating that this regulation is very sensitive (*SI Appendix*, Fig. S3*D*). In contrast, macrophages do not alter their IL-4Rα expression when incubated with CSF3, while IL-6 has a strong IL-4Rα up-regulating effect on macrophages (*SI Appendix*, Fig. S3 *E* and *F*) (35), hence highlighting differential regulatory properties of the IL-4Rα in myeloid cells. Finally, expanding and culturing bone marrow precursor cells, as described by Wang et al. (33), and then differentiating them in the presence of CSF3 further showed that the ability of CSF3 to up-regulate IL-4Rα is mainly confined to the final stage of differentiation when the cells express strong surface

levels of CXCR2 and the neutrophil-specific protein Ly6G (*SI Appendix*, Fig. S4*A*). We concluded that the cytokine CSF3 is released upon stimulation of certain macrophage cell lines and that CSF3 can potently up-regulate the IL-4Rα on neutrophils.

**CSF3 Is an Inflammation-Related Neutrophil IL-4Rα–Regulating Factor In Vivo and Has a Conserved Activity on Human Neutrophils.** To study how CSF3 levels are regulated in vivo, we measured CSF3 protein levels in sera from LPS-injected mice. We found that the cytokine level was significantly up-regulated 24 h after LPS injection (*SI Appendix*, Fig. S5*A*) and that its mRNA transcripts were kept elevated ∼10-fold in lung tissue at that same time point (*SI Appendix*, Fig. S5*B*). Nevertheless, this up-regulation was not lung-specific, as we could also observe CSF3 mRNA up-regulation in the spleen and liver (*SI Appendix*, Fig. S5*B*). To directly examine whether CSF3 in the LPS serum was responsible for the observed neutrophil IL-4Rα up-regulation, we incubated serum from LPS-injected mice with a neutralizing anti-CSF3 antibody before coculture with neutrophils. We found that the sera treated with neutralizing anti-CSF3 antibody lost its ability to up-regulate neutrophil IL-4Rα (Fig. 3*A*). In line with this, neutrophils from CSF3 receptor (CSF3R) KO mice did not up-regulate the IL-4Rα upon incubation with LPS serum (Fig. 3*A*). Furthermore, we found that the in vivo up-regulation of neutrophil IL-4Rα following injection of LPS could be abrogated by coinjecting a neutralizing anti-CSF3 antibody (Fig. 3*B*). Since CSF3 plays a central role in inducing inflammation in the K/BxN serum transfer model (27, 28), the administration of a neutralizing anti-CSF3 antibody in this model in order to test whether CSF3 is responsible for the observed IL-4Rα up-regulation on neutrophils is not a conclusive experiment. In this scenario, with low CSF3 levels, limited joint inflammation can be observed, and consequently, no activation of neutrophils can occur. Instead, we tested how the anti-CSF3 antibody affected neutrophil IL-4Rα up-regulation during ex vivo culture with serum from K/BxN mice and found that the up-regulation was entirely abolished by the anti-CSF3 antibody (Fig. 3*C*).

Performing another targeted CRISPR screen in the HoxB8 Macpro macrophage cells, targeting 22 genes linked to TLR signaling, we identified that clones lacking *Myd88*, *Irak4*, *Akt3*, *Rela*, and *Nfkb1/p50* did secrete low levels of CSF3 following stimulation with LPS (*SI Appendix*, Fig. S6 *A–C*). Although we did not further validate this, the observation indicates that the canonical NF-κB pathway (36) is likely responsible for the CSF3 secretion in the used experimental setup. Consistent with this, neutrophils in MyD88 KO mice did not up-regulate the IL-4Rα following LPS injection but still retain the ability to up-regulate the receptor upon CSF3 exposure (*SI Appendix*, Fig. S6*D*). Similar to mice, human neutrophils show a low basal expression of the IL-4Rα, while B cells show high expression (Fig. 3*D*). Human blood cells incubated with human CSF3, but not IL-6, resulted in clear neutrophil IL-4Rα up-regulation, although to a seemingly lower extent than observed in mice (Fig. 3*D*). Similarly to in mice, human CSF3 and IL-6 also do not affect IL-4Rα on human B cells (Fig. 3*E*).

STAT3 is the canonical signaling pathway downstream of CSF3R (37), and IL-6–induced STAT3 signaling has been linked to IL-4Rα up-regulation in macrophages (35). To test whether the CSF3-mediated up-regulation of neutrophil IL-4Rα can be attributed to STAT3 signaling, we used the STAT3 inhibitor Stattic (38) and found that Stattic inhibits the CSF3-mediated up-regulation of IL-4Rα on neutrophils in a dose-dependent manner (Fig. 3*F*). We also found that neutrophil survival is significantly decreased by Stattic (Fig. 3*F*). This emphasizes the

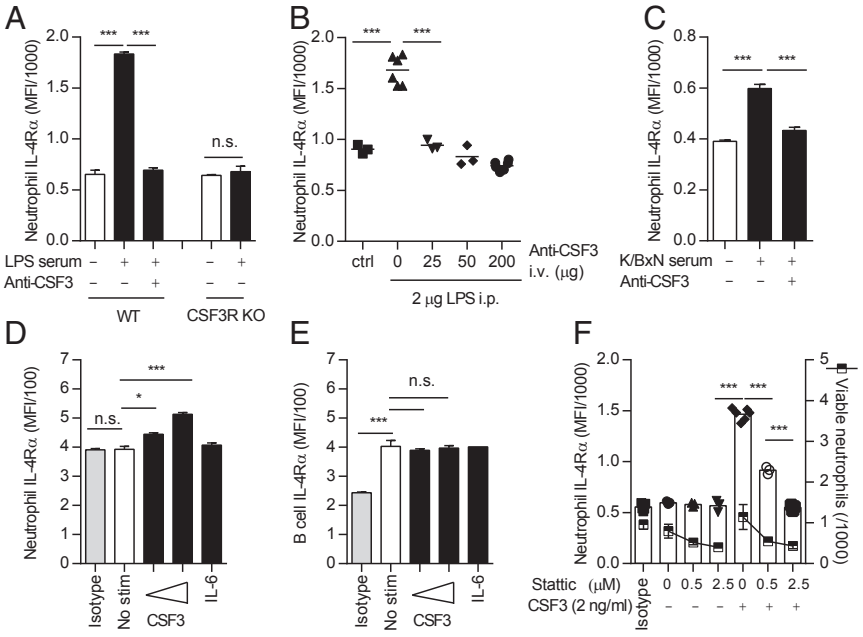


**Fig. 3.** CSF3 is an inflammation-related neutrophil IL-4Rα regulating protein in mice and humans. (*A*) Flow cytometry data of neutrophil IL-4Rα expression of WT and CSF3 receptor KO bone marrow cells after overnight incubation with 0.1% serum collected from LPS-injected mice, pretreated or not with a CSF3 neutralizing antibody (20 μg/mL). (*B*) Flow cytometry data of neutrophil IL-4Rα expression of blood neutrophils 24 h after injection of LPS and increasing doses of a neutralizing CSF3 antibody. (*C*) Neutrophil IL-4Rα expression after incubation with control or 15% K/BxN serum, pretreated or not with a CSF3 neutralizing antibody. (*D* and *E*) Human blood was incubated with CSF3 (10 or 25 ng/mL), or IL-6 (25 ng/mL) overnight, and IL-4Rα expression was detected on (*D*) neutrophils, and (*E*) B cells by flow cytometry. (*F*) Mouse bone marrow cells were stimulated with CSF3 overnight in the presence or absence of the STAT3 inhibitor Stattic at different concentrations, and neutrophil IL-4Rα expression and number of viable neutrophils were measured by flow cytometry. Data presented as mean and SEM in *A* ($n = 3$ to 6), *C* ($n = 3$), *D* and *E* ($n = 3$ to 4), and mean and individual samples in *B* ($n = 3$ to 6), and *F* ($n = 3$ to 6). $^*P < 0.05$, $^{***}P < 0.005$, n.s. = not significant by one-way ANOVA with Tukey's post hoc test. Data are representative of at least two independent experiments.

sensitivity of neutrophils, traditionally seen as very short-lived, and the central role for CSF3 and STAT3 in neutrophil biology (37, 39). Taken together, our data identify that CSF3 is the factor directly affecting the expression of IL-4Rα on neutrophils during acute inflammation and that this regulatory activity is conserved between mice and humans, as it been recently described (34). In this context, it is also notable that the ImmGen expression database (40) identifies that both mouse and human neutrophils have the highest expression of the IL-4R signaling adaptor *Stat6*/*STAT6* seen in any immune cell (*SI Appendix*, Fig. S7). This suggests that the neutrophils are hard-wired to respond to signals from the IL-4R and, thus, that CSF3-mediated IL-4Rα up-regulation can have profound effects on the cell behavior.

**CSF3 and IL-4 Synergize to Up-Regulate the Inhibitory FcγR2b on Neutrophils.** Inflammation triggered by autoantibodies depends on several processes, which are initiated by the formation of antibody-antigen ICs (41). In the K/BxN serum transfer model, IgG IC-mediated cross-linking of activating FcγRs on neutrophils is essential for the development of joint inflammation (8, 10, 42), which in turn can be suppressed by the inhibitory FcγR2b (43, 44). Mouse neutrophils have a clear basal expression of activating FcγR3 (CD16) and FcγR4 (CD16-2) (*SI Appendix*, Fig. S8 *A* and *B*), and no evident expression of the activating FcγR1 (CD64) (*SI Appendix*, Fig. S8*C*) or the inhibitory FcγR2b (CD32B) (Fig. 4 *A*–*C*). Taking into consideration that IL-4 is known to affect the expression of FcγR2b in certain myeloid cells (45, 46) and that CSF3 induces the up-regulation of IL-4Rα on neutrophils, we hypothesized that CSF3 and IL-4 could act synergistically to induce the expression of FcγR2b on neutrophils. Neutrophils isolated from naïve mice stimulated ex vivo with IL-4 overnight did not show any apparent up-regulation of FcγR2b. Intriguingly, neutrophils isolated from mice with inflammation, induced by LPS injection ∼20 h earlier, showed a strong up-regulation of FcγR2b following IL-4 stimulation (Fig. 4*A*). Based on our findings where CSF3 was identified as responsible for the neutrophil IL-4Rα up-regulation, we repeated these experiments in the presence of a neutralizing anti-CSF3 antibody. Under these conditions, anti-CSF3 could substantially block the IL-4–induced FcγR2b up-regulation in LPS injected mice, showing that the CSF3-mediated IL-4Rα up-regulation is central to the enhanced FcγR2b expression induced by IL-4 following LPS injection (Fig. 4*B*). A similar result was seen when first injecting mice with CSF3 and the next day injecting IL-4. In these conditions, a major synergistic activity on FcγR2b expression can be observed by CSF3 and IL-4, while only a minor effect was detected on other FcγRs (*SI Appendix*, Fig. S8 *A*–*C*). Importantly, exposure of neutrophils to CSF3 and IL-4 results in surface levels of FcγR2b that are higher than those observed on B cells, which are considered to be among the highest FcγR2b-expressing cells (Fig. 4*C*). Moreover, B cells display an opposite regulation of FcγR2b expression and instead down-regulate the receptor after IL-4 exposure (Fig. 4*C* and *SI Appendix*, Fig. S8*D*). The up-regulation of the FcγR2b protein induced by IL-4 could also be observed by qRT-PCR analysis in CSF3-stimulated neutrophils (*SI Appendix*, Fig. S4*B*), showing that the regulation is at least partly mediated at a transcriptional level.

Another known target locus induced by IL-4 is MHC-II (47). Expression of MHC-II by neutrophils and subsequent antigen presentation to $CD4^+$ T cells has been described in various contexts, including in the inflamed joints of RA patients (48, 49). Hence, we assessed whether the CSF3/IL-4 combination could



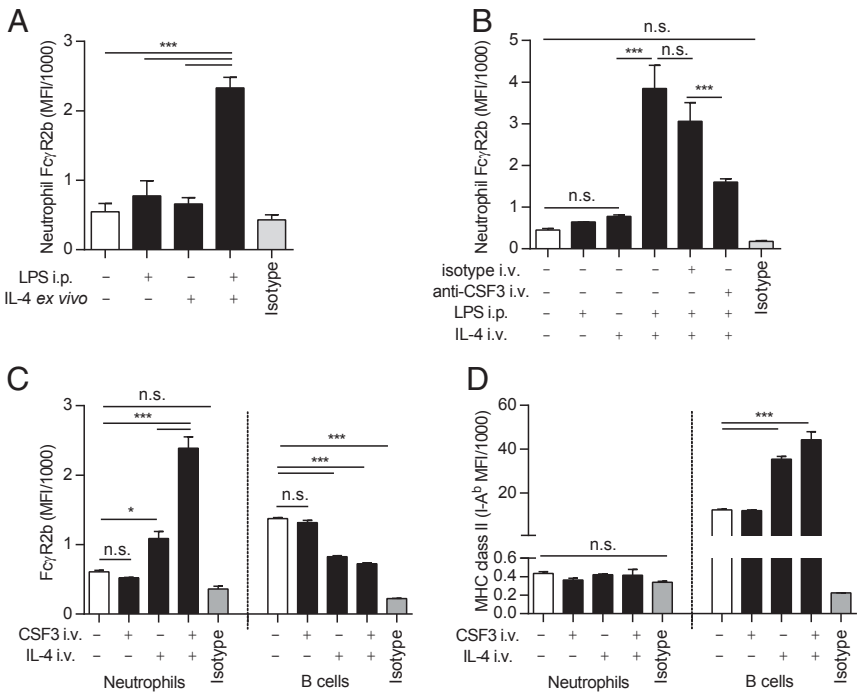


**Fig. 4.** *FcγR2b* is a target gene for IL-4–induced signaling in neutrophils. (*A*) Blood of mice injected with 10 μg LPS or control was collected ∼20 h postinjection and stimulated overnight with or without 20 ng/mL IL-4. Neutrophil FcγR2b expression was determined by flow cytometry the following day. (*B*) Mice were injected intravenously with 150 μg isotype control or anti-CSF3 and 30 min later intraperitoneally with 3.5 μg LPS (day 0), the next day mice were injected intravenously with IL-4 (day 1); the following day, blood neutrophil expression of FcγR2b was measured by flow cytometry (day 2). (*C*) Mice were injected intravenously with 2 μg CSF3 d 0, intravenously with IL-4 d 1, and cells in the blood analyzed by flow cytometry for neutrophil and B cell expression of FcγR2b day 2. (*D*) Neutrophil and B cell expression of MHC-II (I-Ab) after sequential injection of CSF3 and IL-4 as in *C*. Data presented as mean and SEM in *A* ($n = 4$), *B* ($n = 3$ to 4), and *C* and *D* ($n = 3$). $^*P < 0.05$, $^{***}P < 0.005$, n.s. = not significant by one-way ANOVA with Tukey´s post hoc test. Data are representative of at least two independent experiments.

also affect neutrophil MHC-II expression. Expectedly, IL-4 induced the up-regulation of MHC-II on B cells; however, no change in expression of the protein was observed on neutrophils (Fig. 4*D*). This observation shows that the up-regulation of MHC-II on neutrophils is insufficiently triggered by IL-4, even in a context where the neutrophils have up-regulated IL-4Rα by CSF3 exposure, possibly due to an inappropriate epigenetic state of the MHC-II locus. Overall, our data establish that CSF3 and IL-4 have a synergistic effect on the expression of the inhibitory FcγR2b on neutrophils, which is important for suppressing IC-mediated inflammation. Moreover, it shows that neutrophils can express levels of FcγR2b that surpass those seen on FcγR2b high-expressing B cells, although neutrophils have traditionally been described as not expressing FcγR2b.

**IL-4 Drives Neutrophil FcγR2b Expression Directly Downstream of the IL-4Rα and Dictates Neutrophil Migration to the Inflamed Joint.** To further explore the mechanisms involved in the FcγR2b regulation, we generated mixed bone marrow chimeric mice, where irradiated CD45.1$^+$ WT mice were grafted with CD45.1$^+$ WT and CD45.2$^+$ IL-4Rα KO bone marrow. The resulting mice thus contain immune cells with a WT expression of IL-4Rα as well as immune cells with absent IL-4Rα expression. Importantly, each of these cells can be easily distinguished by flow cytometry using CD45.1 and CD45.2 antibodies (*SI Appendix*, Fig. S9*A*). Injecting the chimeric mice with K/BxN serum and IL-4 or control showed that the binding of IL-4 to the IL-4Rα is directly responsible for the neutrophil FcγR2b up-regulation, as only IL-4Rα WT cells but not IL-4Rα KO cells up-regulated FcγR2b (Fig. 5*A*). Similar results were obtained in ex vivo cocultures of CD45.1 WT and CD45.2 IL-4Rα KO bone marrow cells, originated from mice previously injected with CSF3 or control the day before and then exposed to IL-4 or IL-13 in culture (Fig. 5*B* and *SI Appendix*, Fig. S9*B*). The bone marrow chimeric mice also allowed us to study how IL-4 affects neutrophil migration into different compartments by interacting with the IL-4Rα. Injection of K/BxN serum and IL-4 showed that neutrophil egress from the bone marrow and migration into the joint was significantly suppressed by the IL-4 injection. Migration was calculated as the frequency of WT neutrophils decreasing in competition with the IL-4Rα KO neutrophils in the K/BxN + IL-4 compared to the K/BxN + control group (Fig. 5*C*). Similarly, IL-4 could partly inhibit neutrophil migration toward CXCL2 and C5a chemoattractants in an in vitro transwell assay (*SI Appendix*, Fig. S4*C*). These data extend recent findings showing that IL-4 signaling in neutrophils inhibits their migration during inflammation and infection (29, 34), and at the same time illustrates this as a relevant mechanism also in the context of joint inflammation. We concluded that both IL-4 and IL-13 potently up-regulate the inhibitory FcγR2b in CSF3 primed neutrophils directly downstream of signaling through the IL-4Rα, and that IL-4 suppresses neutrophil egress from the bone marrow and migration into the joint in the context of K/BxN mediated joint inflammation.

CSF3 is known to be elevated in contexts of acute inflammation, including infection, sepsis, trauma, as well as in RA patients (50) and animal models for RA (28). To test whether

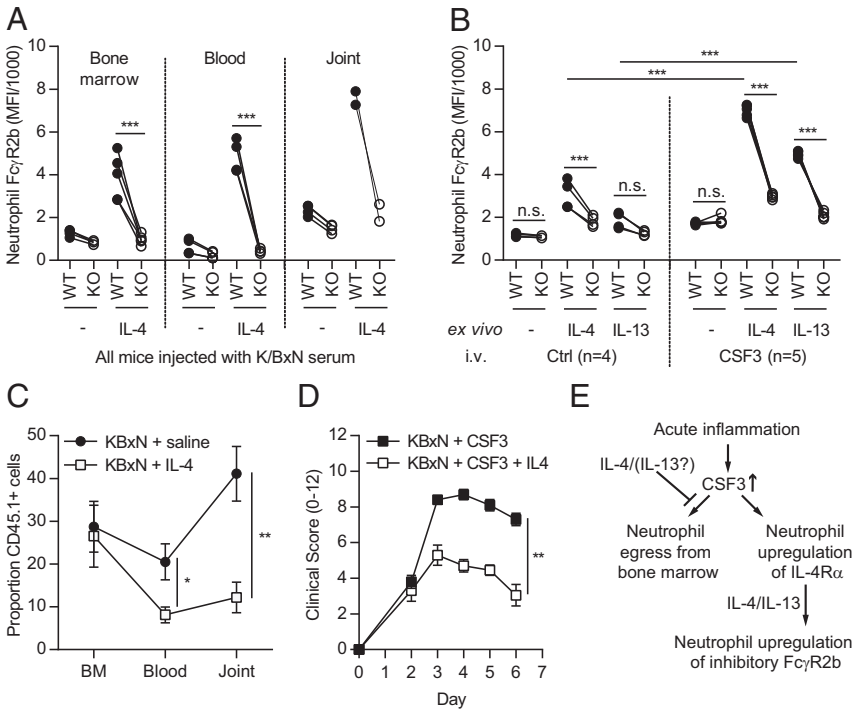


**Fig. 5.** IL-4 affects neutrophil FcγR2b expression directly downstream of the IL-4Rα and affects neutrophil migration to the inflamed joint. (*A*) Neutrophil FcγR2b expression in mixed bone marrow chimeric mice, CD45.1 WT, and CD45.2 IL-4Rα KO cells, injected with 200 μL K/BxN serum and IL-4 or control day 0, IL-4 or control day 2, and finally analyzed by flow cytometry day 3. Lines connect WT and IL-4Rα KO neutrophils in the same mouse. (*B*) Neutrophil FcγR2b expression from mixed ex vivo cultures of WT (CD45.1) and IL-4Rα KO (CD45.2) bone marrow from mice injected intravenously with 2 μg CSF3 or control the day before and incubated overnight with IL-4 or IL-13 (20 ng/mL). Lines connect WT and IL-4Rα KO neutrophils in the same sample. (*C*) The proportion of WT (CD45.1) and IL-4Rα KO (CD45.2) neutrophils in different compartments day 3 in mixed bone marrow chimeric mice injected day 0 with K/BxN and IL-4 or saline, and IL-4 or saline day 2. Data shows percent CD45.1 (WT) neutrophils based on flow cytometry analysis of CD45.1$^+$ and CD45.2$^+$ neutrophils. (*D*) Joint inflammation in C57BL/6 mice injected with a suboptimal dose of K/BxN serum (100 μL) with CSF3 and IL-4. (*E*) A model describing inhibitory effects of IL-4 on neutrophils during acute inflammation. Data presented as individual mice in *A* ($n = 2$ to 5), and *B* ($n = 4$ to 5), as well as mean and SEM in *C* ($n = 6$ to 7), and *D* ($n = 5$). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$, n.s. = not significant by one-way ANOVA with Tukey´s post hoc test (*A*–*B*) and by Mann–Whitney *U* test in *C* and *D* (at day 6). Data are representative of at least two independent experiments.

IL-4 can suppress joint inflammation in a context with increased CSF3 level, WT mice were injected with a suboptimal dose of K/BxN sera together with CSF3 and further treated with IL-4. Despite the suboptimal dose of K/BxN serum used, robust joint inflammation was observed in combination with CSF3, supporting the known role for CSF3 in this model (27). Nevertheless, the administration of IL-4 still suppressed the induced joint inflammation (Fig. 5*D*). Hence, we conclude that IL-4 can affect several proinflammatory features of CSF3 primed neutrophils with relevance for antibody-mediated joint inflammation, including in inflammatory conditions with high CSF3 levels (Fig. 5*E*).

## Discussion

IL-4 and its close relative IL-13 are central to T helper cell type 2 (TH2) responses, commonly associated with parasite infections and allergies (51, 52). Here we identify the IL-4/IL-4Rα signaling pathway as having several important effects related to proinflammatory neutrophil biology in the context of sterile inflammation. IL-4 has been widely studied in relation to its effect on lymphoid cells, centrally involved in T helper cell differentiation, B cell class switch (52, 53), as well as effects on nonimmune stromal cells (54). An emerging field related to IL-4R signaling in neutrophils is broadening the implications under conditions with increased IL-4 and IL-13 levels, such as allergic reactions and parasitic infections, but also related to therapeutic interventions affecting this pathway (55, 56). For example, an interesting connection between the increased susceptibility to skin infections in patients with the TH2 centric disease atopic dermatitis and the suppression of neutrophil migration by IL-4R signaling has been proposed (29, 34). Importantly, blockade of the IL-4R by the clinically approved antagonistic antibody Dupilumab decreases the risk of skin infections in atopic dermatitis patients, something that could be speculated to relate to better neutrophil activation in the absence of IL-4R–induced signaling, and as a consequence better control of the infection (57). In contrast, in a context where neutrophil activation is contributing to pathology, therapeutic activation of the IL-4R signaling pathway could be beneficial. Our data from mixed bone marrow chimeras extend the published observations of IL-4 suppressing neutrophil migration also in the context of joint inflammation.

The role of neutrophils in RA is debated. Neutrophils are typically making up the most abundant cell population in the synovial fluid from inflamed joints of patients with active disease, as well as in mouse models of acute joint inflammation. Furthermore, neutrophil deficiency in animal models for experimental joint inflammation results in significant suppression of the inflammation in most used models (6–10), suggesting that neutrophil localization to the joint in human RA patients could play an important role in the disease. Neutrophils are rapidly recruited to sites of inflammation and are activated in a rather nonspecific fashion compared to many other immune cells. During an infection, this behavior is often beneficial, helping to contain the invading infectious agent rapidly. In the context of sterile inflammation, such as during an autoreactive attack on the joint, neutrophil recruitment and activation is likely unfavorable, amplifying the unwanted inflammation. Limiting the migration of neutrophils into inflamed joints could thus be an interesting therapeutic approach in RA patients.

The IL-4R signaling pathway is fairly simple when compared to many other cytokine signaling pathways. It involves a type 1 IL-4 receptor made up of the IL-4Rα (CD124) and the common cytokine γ-chain CD132 that interacts with IL-4, and a type 2 IL-4 receptor made up of the IL-4Rα and IL-13Rα1 that interacts with both IL-4 and IL-13. Both type 1 and type 2 IL-4Rs activate STAT6 (58). Furthermore, a relative to the IL-13Rα1 called IL-13Rα2 (CD213A2) also exists, which only directly interacts with IL-13 (59). Importantly, the IL-4Rα protein is essential for all signaling pathways induced by IL-4. Neutrophils do not express high levels of the IL-4Rα at steady state compared to, for example, B cells. However, we and others have shown that IL-4Rα is highly up-regulated on several myeloid cell populations during different inflammatory conditions (21, 29, 35). Up-regulation of cytokine receptor α-chains on immune cells is well described where, for example, the up-regulation of IL-2Rα (CD25) on T cells is central to T cell activation and proliferation (60). In this example, the up-regulation of the receptor (IL-2Rα) results in an increased sensitivity to lower levels of the cytokine (IL-2), something that is important as many cytokines are found at low concentrations and are often rapidly consumed. The expression of the IL-4Rα is known to be regulated by several factors in B and T cells, including IL-2 and IL-4, and induced STAT5 and STAT6 signaling, respectively (61, 62). IL-2 and IL-4 do, however, not regulate the IL-4Rα on neutrophils (21).

Here we identify that activation of an alveolar macrophage cell line, as well as a CSF2/GM-CSF–dependent bone marrow-derived macrophage cell line, induces the releases a protein that in turn up-regulates the IL-4Rα on neutrophils. Combining gene-expression analysis and a targeted CRISPR screen, we identified this IL-4Rα–inducing factor as CSF3/G-CSF. Furthermore, we were able to show that also in vivo, CSF3 is the protein responsible for the up-regulation of IL-4Rα on neutrophils seen during sterile inflammation and that this is related to CSF3-induced STAT3 signaling. Previously, we identified that conditioned supernatant from lung and epididymal (visceral) fat pad organ cultures from mice injected with LPS were able to produce a factor that up-regulated the IL-4Rα on neutrophils (21). Interestingly, the Genotype-Tissue Expression (GTEx) project database (63) identifies that the lung has the highest basal CSF3 expression of 53 tested human organs, while visceral fat is among the top 5 organs in that same list. Following LPS injection into mice, we saw robust CSF3 levels in serum and broad *Csf3* mRNA up-regulation in all tested organs (liver, lung, and spleen).

The majority of RA patients display a spectrum of specific autoantibodies that characterize the patient as having seropositive RA. Notably, this subset of patients has an expected worsening disease progression (4, 5). An explanation for the accelerated disease progression of these patients has been speculated to relate to the fact of autoantibodies being involved in forming antibody–antigen ICs locally in the joints, which in turn further amplify the inflammation by interacting with activating FcγRs on different immune cells, such as neutrophils (8, 10). Animal studies have shown that activating FcγRs play a central role in antibody-mediated joint inflammation (10, 18, 19, 43) and that the inhibitory FcγR2b (CD32b) suppresses this interaction (20, 44). Significantly, here we identify that CSF3-activated neutrophils respond to IL-4 with strong up-regulation of the inhibitory FcγR2b while activating FcγRs are not majorly affected. FcγRs are expressed on most immune cells, and myeloid cells often express both activating and inhibitory FcγRs (41). The consequence of an interaction between an FcγR-expressing cell and an IC depends on the relative interaction of the IC with activating and inhibitory receptors on the specific cell. This is affected by multiple parameters, such as the subclass and glycoform of the IgG antibodies, but also by the expression levels of activating and inhibitory FcγRs on the cells (64, 65). We have previously shown that both IL-4 and IL-13 protect from antibody-mediated joint inflammation in WT mice, while this protection is not seen in mice lacking IL-4Rα, STAT6, or FcγR2b (20). Moreover, we have also previously shown that IL-4 does not protect mice where the IL-4Rα is deleted in LysM-Cre–expressing cells, such as macrophages and neutrophils (21). Therefore, the conclusion has been that IL-4R–induced STAT6 signaling results in the up-regulation of FcγR2b, which in turn is responsible for limiting the antibody-mediated joint inflammation. Due to the low basal expression of FcγR2b on neutrophils (66), we

have hypothesized that the IL-4–mediated protection could mainly be related to the up-regulation of FcγR2b on macrophages. However, the data presented here clearly show that also neutrophils can be induced to express very high levels of FcγR2b when primed by CSF3, a phenomenon commonly occurring during acute inflammation of various sorts.

Activated neutrophils are known to release targets for autoantibodies found in seropositive RA patients, such as citrullinated histones and vimentin, in a process referred to as netosis (15, 16). Limiting neutrophil recruitment, activation, and netosis in inflamed joints, could, therefore, be a therapeutic approach to suppress joint inflammation associated with local IC formation. Interestingly, it was recently shown that IL-4 could suppress neutrophil netosis (34), showing that IL-4–induced signaling in neutrophils results in several behavioral changes that could be expected to be beneficial in RA. Together, our data identify a regulatory loop where CSF3, known to activate neutrophils and to enhance neutrophil-mediated inflammation [including anecdotally resulting in flares of disease after injection into patients with RA (67)], also prime neutrophils for IL-4R signaling by up-regulating the IL-4Rα. In the presence of IL-4, CSF3-primed neutrophils up-regulate the inhibitory FcγR2b, and their egress from the bone marrow and migration into the inflammatory site is suppressed. Both these phenotypes are of significant relevance to antibody-mediated inflammation involving neutrophils. The suppressive activity of IL-4Rα signaling in neutrophils is thus directly linked to the activating signal induced by CSF3. Such paired activating/inhibitory pathways are commonly found in the immune system, exemplified by CD28/CTLA4 and activating/inhibitory FcγRs, important for fine-tuning the activation and down modulation of immune responses. Understanding these types of regulatory mechanisms could have important therapeutic implications, as shown by the many novel immune modulatory drugs targeting such pathways. We thus propose that the converging IL-4/CSF3 pathways could be explored therapeutically to both enhance and suppress immune responses involving neutrophils.

## Materials and Methods

**Animals.** Eight- to 12-wk-old, sex- and age-matched mice were used for experiments. All animal experiments have been approved by the local animal ethical board at the Karolinska Institute. Mice were housed under specific-pathogen-free conditions in a 12-h/12-h light–dark cycle and fed standard chow diet ad libitum. WT (C57BL/6J, BALB/cJ) or modified mice on C57BL/6 background from the Jackson Laboratory were used; MyD88 KO (stock no. 009088); CSF3 KO (stock no. 002398), Cas9 (stock no. 026179), CD45.1 (stock no. 002014). Germline IL-4Rα KO mice were created by breeding IL-4Rα floxed/floxed mice on a C57BL/6 background [generated by Frank Brombacher, University of Cape Town, Cape Town, South Africa (68), backcrossed to C57BL/6 and provided by Judith E. Allen, University of Manchester, Manchester, United Kingdom] with B6.Rosa26-Cre mice. The Rosa26-Cre allele was removed by breeding before the experiments were conducted. K/BxN mice were generated, and serum collected as described previously (21).

Bone marrow chimeric mice were generated by intravenous injection of ~$10^6$ bone marrow cells (~1:1 ratio of different bone marrows) 4 to 8 h after 900 rad γ-irradiation. Subsequent experiments were conducted >8 wk after the irradiation and bone marrow transplantation to allow for the establishment of the new immune system.

**Injections.** K/BxN serum transfer experiments were performed by intravenous injection of 100 or 200 μL serum as indicated in the figure legends. Clinical score of joint swelling and functional test was performed as described previously (21). IL-4 complexes with prolonged half-life were generated by incubating mouse IL-4 (Peprotech) with the anti–IL-4 clone 11B11 (BioXcell) as described previously (20). IL-4 complexes (5 μg mouse IL-4 + 25 μg 11B11) and/or mouse CSF3 (2 μg, Peprotech) in sterile PBS were intravenously injected 30 min before K/BxN serum or 2 d after K/BxN, as indicated in the figure legends.

TLR4-specific LPS [*Salmonella* Minnesota R595 (Re); Enzo Life Sciences] diluted in sterile PBS was injected intraperitoneally (2, 3.5, or 10 μg) as indicated in the figure legends. Anti-CSF3 (9B4CSF, functional grade, eBioscience) or isotype control (eBR2a, functional grade, eBioscience) was diluted in sterile PBS and injected intravenously, at doses indicated in the figure legends, 30 min before LPS injection. Mouse CSF3 (2 μg, Peprotech) diluted in sterile PBS was injected intravenously. When IL-4 complexes (5 μg mouse IL-4 + 25 μg 11B11) were injected in the same experiment as LPS or CSF3, the IL-4 was intravenously injected 20 to 24 h after the LPS/CSF3 injection. The effect of the injections was measured by flow cytometry 20 to 24 h after the IL-4 injection.

Thioglycolate-elicited macrophages were generated as described previously (21) with the modification that 0.5 mL 4% thioglycolate solution was injected intraperitoneally.

**Cell Lines and Ex Vivo Stimulations.** A549 (human lung epithelial carcinoma), Jurkat (human T cell leukemia), and MH-S (mouse alveolar macrophage cell line) were cultured in RPMI-1640 (Sigma-Aldrich) with 10% heat-inactivated bovine serum, 1% penicillin-streptomycin-glutamine, and 0.1% β-mercaptoethanol (Gibco; referred below to as cRPMI). The ER-HoxB8 Macpro cell line (mouse bone marrow macrophage precursor, a kind gift from Mark P. Kamps, University of California, San Diego) was cultured in cRPMI with 1 μM β-estradiol (Sigma-Aldrich) and 10% conditioned medium from a CSF2/GM-CSF expressing B16 cell line, as described previously (33). Cells were seeded at 250,000 cells per well in 6-well plates in 1.5 to 3 mL medium, and 50,000 cells per well in 96-well plates in 0.2 mL medium and split every 2 to 3 d. To differentiate the HoxB8 Macpro cells to mature macrophages, the cells were washed three times to remove the β-estradiol, and cells cultured in cRPMI with CSF2/GM-CSF but without β-estradiol for >5 d before being stimulated with LPS. Cell lines at 50 to 80% confluency cultured in six-well plates were stimulated with 0.2 μg/mL LPS [*Salmonella* Minnesota R595 (Re); Enzo Life Sciences] overnight. Conditioned supernatant was collected and separated by centrifugation (500 × *g*, 5 min). Single-cell colonies of the Macpro and MH-S cell lines were generated by limiting dilution. High- and low-producing MH-S clones were identified by assaying the conditioned media for the ability to up-regulate the IL-4Rα on neutrophils after LPS stimulation. For the generation of Cas9-expressing Macpro cells, the cells were spin-infected with Lenti-Cas9 Blast viral particles, described below, and selected after ~72 h with 10 μg/mL blasticidin for 10 d. All cell lines were tested negative for mycoplasma contamination.

Bone marrow cells from WT or MyD88 KO mice were collected by flushing femur/tibia with a syringe and a 25-G needle with sterile PBS. Blood cells were collected in EDTA tubes (Sarstedt) through tail vein bleeding or by terminal bleeding by cardiac puncture. Red blood cells (RBCs) were lysed using 1× RBC Lysis buffer (eBioscience), and cells stimulated overnight in 96-well plates (1:100 if cells from two femur were used per well in a 96-well plate). In Fig. 5*B*, bone marrow cells were mixed from WT ($CD45.1^+$) and IL-4Rα KO (CD45.2), injected or not with CSF3 as indicated in the figure legend. Stimulations include dilutions of serum from K/BxN mice (from mice with active disease), and mice injected with LPS ("LPS serum," collected 20 to 24 h after intraperitoneal injection of 10 μg LPS into WT C57BL/6 mice), 20% conditioned medium from cell lines stimulated overnight ±0.2 μg/mL LPS, and cytokines (mouse: IL-4, IL-6, CSF3, LIF at 20 ng/mL; and human: CSF3 [10 and 25 ng/mL], and IL-6 [25 ng/mL]; all from Peprotech). To degrade proteins in conditioned medium, the sample was incubated with proteinase K-agarose from Tritirachium album (P9290-10UN, Sigma-Aldrich). Briefly, the lyophilized beads were diluted in 4 mL mQ water and stored in 4 °C until being used. Next, 400 μL of the beads were collected with a cut pipette tip, added to a spin column, and washed three times with sterile PBS. Then, 400 μL conditioned medium was added to the washed beads and incubated for 90 min at 37 °C with slight movement. The treated samples were collected by centrifugation and assayed for its activity to up-regulate the IL-4Rα on neutrophils. To neutralize cytokines, conditioned medium or serum were preincubated for 45 min with 20 μg/mL anti-CSF3 (9B4CSF, functional grade, eBioscience). To inhibit STAT3, Stattic (Tocris) dissolved in sterile DMSO was added to cells at doses indicated in the figure legends. When conditioned supernatant containing LPS was used, cells from MyD88 KO mice were typically used in the culture testing the activity of the conditioned medium (this is not necessary but increases the survival of the neutrophils, and lowers the background staining).

Fresh human blood from donors was bought from the local blood center and used in experiments in line with national regulations. Following RBCs lysis using 1× RBC Lysis buffer (eBioscience), human blood cells were cultured in cRPMI and stimulated overnight with human cytokines from Peprotech; CSF3 (10 or 25 ng/mL) and IL-6 (25 ng/mL).

**Flow Cytometry, Sorting, and ELISA.** Single-cell preparation of mouse organs was generated as described previously (21), and RBCs were lysed using 1× RBC Lysis buffer (eBioscience). Cells isolated from joints of mice with K/BxN-induced inflammation were collected from mice perfused with cold PBS to

remove blood from tissues. Cells were stained with Fc-blocker (clone 2.4G2, importantly not included if cells were stained for FcγRs), and labeled with the following antibodies: CD11b (M1/70), Ly-6G (1A8), CD45.1 (A20), IL-4Rα/CD124 (mIL-4R-M1), I-Ab (AF6-120.1) from BD Bioscience; CD45.2 (104-2) from Miltenyi Biotec; CD45R/B220 (RA3-6B2), CD19 (6D5), FcγR2b/CD32b (AT130-2) from eBioscience; FcγR1/CD64 (X54-5/7.1), FcγR4/C16.2 (9E9), F4/80 (BM8), CXCR2 (SA044G4), rat IgG2a isotype control (RTK2758) from Biolegend; and FcγR3/CD16 (275003) from R&D Systems. 7-aminoactinomycin D (BD Bioscience) or LIVE/DEAD Fixable Aqua Dead Cell Stain (Invitrogen) was used to exclude dead cells. Human cells were stained with IL-4Rα/CD124 (hIL-4R-M57), CD19 (HIB19), CD16 (3G8), mouse IgG1 isotype control (MOPC-21) from BD Bioscience, and CD15 (W6D3) from Biolegend. BD Accuri C6, BD Verse, and Beckman Coulter Gallios cytometers were used for flow cytometry analysis. Acquired data were analyzed using the CSampler Analysis software (BD Bioscience) or FlowJo (FlowJo).

Cell-free supernatant was tested for IL-6 and CSF3/G-CSF levels using the protocols suggested by the manufacturer: ELISA MAX Standard Set mouse IL-6 (Biolegend), and Murine G-CSF ABTS ELISA Development Kit (Peprotech).

**Gene Expression.** TRIzol (Thermo Fisher) and Direct-zol RNA MiniPrep Kit (Zymo Research) were used to isolate RNA from cells, and genomic DNA removed with RNase-free DNase (Zymo Research). For global gene-expression analysis, frozen RNA samples were provided to the Bioinformatics and Expression Analysis (BEA) core facility, Karolinska Institutet, and samples were analyzed on Affymetrix Mouse Gene ST 2.1 Array. For qRT-PCR applications, cDNA was generated with the High Capacity RNA-to-cDNA kit (ThermoFisher Scientific), and mouse gene expression quantified with a CFX 384 Real-Time PCR machine (Bio-Rad) using TaqMan gene expression FAM assays for *Csf3* (Mm00438334), and *Fcgr2b* (Mm00438875) with the TaqMan Gene Expression Master Mix as suggested by the manufacturer (ThermoFisher Scientific). Expression was normalized by TaqMan gene expression VIC assays for *β-actin* (Mm00607939) in all qRT-PCR experiments. Gene expression was quantified using the ddCT method. Stat6/*STAT6* expression data from sorted mouse and human cells were extracted from the ImmGen webpage (www.immgen.org) as specified in the figure legend, and *CSF3* expression in whole human organs was extracted from the GTEx Portal (https://gtexportal.org/home/).

**gRNA Design and Cloning.** gRNAs with matching overhangs for the respective plasmids were extracted from the Green Listed software (http://greenlisted.cmm.ki.se/), described in Panda et al. (69), using the Brie reference library (70). gRNA oligomers for pSpCas9(BB)-2A-Puro (PX459) V2.0 (Addgene plasmid #62988; kindly provided by Feng Zhang, Broad Institute of MIT and Harvard, Cambridge, MA) experiments were ordered as sense and antisense 80-mers (*SI Appendix*, Table S2) and as sense 70- or 100-mers for the lentiGuide-Puro-P2A-EGFP_mRFPstuf experiments (*SI Appendix*, Tables S3 and S4). Ten non-targeting controls were included in screening experiments, as indicated in *SI Appendix*, Table S5 (oligos were ordered from Sigma-Aldrich or CustomArray).

For the introducing gRNAs into pX459 V2.0, paired sense and antisense gRNA oligomers for the CSF3 gene (*SI Appendix*, Table S2) were mixed at 10 μM each in 10 mM Tris-EDTA (TE) buffer in a total volume of 100 μL. Oligo hybridization was performed by incubating the mixture at 95 °C for 5 min at thermocycler and then cooled to room temperature. The duplex oligonucleotides were then cloned into the Bbs1 site of pX459 plasmid by the NEBuilder Hifi DNA assembly master mix (New England Biolabs). Chemical transformation was subsequently performed with 0.5 μL of the ligated product and 50 μL of One Shot TOP10 Chemically Competent *Escherichia coli* (Thermo Fischer Scientific) per reaction, according to the manufacturer's instruction. Sequence confirmed colonies were expanded, and the plasmid DNA was purified with the EndoFree Plasmid Maxi kit (Qiagen). Sanger sequencing was done by Eurofins genomics or KI Gene.

For introducing gRNA libraries into lentiGuide-Puro-P2A-EGFP_mRFPstuf, the concentration of individual oligomers (when ordered separately) were measured with Qubit ssDNA Assay Kit (Thermo Fischer Scientific) and an equal amount of each oligo mixed to have an oligo pool with equal representation. The single-stranded oligo pool was converted to double-stranded DNA and expanded by PCR using Phusion High-Fidelity PCR 2X Master Mix (New England Biolabs), as indicated in *SI Appendix*, Table S6. The PCR products were run on 2% agarose gel, and the 70 bp (or 100 bp when applicable) fragment was excised from the gel, and DNA eluted with the MinElute Gel Extraction Kit (Qiagen). Cloning was performed using 50 ng of Bsmb1-digested lentiGuide-Puro-P2A-EGFP_mRFPstuf vector with 3 ng of the 70 bp or 20 ng of the 100-bp fragments using the NEBuilder Hifi DNA assembly master mix (New England Biolabs) at 50 °C for 1 h. Three ligation reactions were combined and precipitated using Isopropanol/NaCl and dissolved in 5 μL of TE buffer. Four electroporations were carried out using 0.75 μL of the purified ligation product and 25 μL of Endura competent cells (Lucigen) per reaction according to the manufacturer's instruction. The electroporated cells were combined and plated on thirty 100-mm LB agar plates with 100 μg/mL carbenicillin and grown at 32 °C for 14 h. Plasmid DNA was purified with the EndoFree Plasmid Maxi kit (Qiagen).

**Production of Nonreplicating Viruses.** The production of lentiGuide-Puro-P2A-EGFP_mRFPstuf and lenti-Cas9 Blast (a kind gift from Feng Zhang) was initiated by seeding $2 \times 10^6$ cells HEK293T cells in 10 mL DMEM with 10% serum and 1% glutamine in 100-mm plates. After ∼24-h culture, the cells were at 60 to 70% confluency, and the medium was replaced by 6 mL fresh, prewarmed medium. Transfer plasmids were combined with either pCgpV, pRSV-Rev, and pCMV-Eco (all from Cell Biolabs) at a 3:1:1:1 ratio, or with psPAX2 (Addgene #12260), and pMD2.G (Addgene #12259) (both kind gifts from Didier Trono, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland) at a 2:2:1 ratio (the first number indicates the ratio of the transfer plasmid), and transfected into the HEK293T cells using the TransIT-LT1 Transfection Reagent (Mirus) according to the manufacturer´s protocol. Approximately 12 h later, the medium was removed and replaced by 5 mL of fresh DMEM with 30% serum and 1% glutamine. After another ∼36 h, the supernatant containing the virus was collected, briefly centrifuged to remove cellular debris, and used for spin-infection of bone marrow cells.

For CRISPR screen experiments, the multiplicity of infection (MOI) of the gRNA library cloned LentiGuide-Puro-P2A-EGFP viral particles were tested by infection with serial dilutions of virus particles, to find a dilution resulting in a low MOI suitable for a screen. Fifty-thousand cells per well in 12-well plates were spin-infected (1,200 × *g*, 90 min) in the presence of 8 μg/mL polybrene (Sigma-Aldrich). The Macpro cell line is difficult to infect, and an infection efficiency of ∼25% was used for the screens, as measured by the percentage of $GFP^+$ cells after 96 h.

**CRISPR Experiments.** To establish CSF3 KO versions of the MH-S cell line, the cells were electroporated with the pX459 V2.0 plasmid containing CSF3 gRNAs (*SI Appendix*, Tables S2 and S5), using the Neon Transfection System (Thermo Fischer Scientific). The following parameters were used: 1,350-pulse voltage, 30-pulse width, 1-pulse number. After 24 to 72 h, puromycin (1.5 μg/mL) selection was started and continued for 2 to 3 d. Limited dilution was performed to establish single-cell colonies. To test the genotype of the clonal lines, genomic DNA was extracted using DNeasy Blood & Tissue Kits (Qiagen), and the gRNA targeted sites were sequenced using CSF3 genotyping primers (*SI Appendix*, Table S6). The sequences were analyzed using the software tool at https://ice.synthego.com/.

For CRISPR screens, 50,000 Cas9-blast expressing Macpro cells, cultured in the presence of β-estradiol and GM-CSF/CSF2, were infected with the custom gRNA lentiviral library at an expected MOI of ∼0.25. Three independent infections were performed, thus producing three independent Cas9-HoxB8-ER macrophage libraries. After 24 h of transduction, media with puromycin (5 μg/mL; for selection of the lentiGuide-puro-EGFP) and blasticidin (10 μg/mL; for selection of the LentiCas9-Blast) were added every third day and continued for four passages to remove uninfected cells. Clonal dilution was performed at 0.5 cell per well to get single puromycin and blasticidin resistant colonies in 10 96-well plates with media containing GM-CSF and β-estradiol (as specified under "cell lines and ex vivo stimulations"). As clones expanded, part of the cells was matured to macrophages, by the withdrawal of β-estradiol for 6 d, and then stimulated with LPS. Twenty-four hours later conditioned medium from stimulated clones was collected and tested for the ability to up-regulate the IL-4Rα on bone marrow neutrophils in the first screen (Fig. 2*E*) or for the production of CSF3 by ELISA in the second screen (*SI Appendix*, Fig. S6*B*). The gRNA of the different single-cell clones was identified by Sanger sequencing, and the genotype correlated to the studied phenotype. Colonies having more than one gRNA were excluded from the analysis. Details of the PCR conditions and primers were listed in *SI Appendix*, Table S6.

**Materials and Data Availability.** The Affymetrix expression data of MH-S clones stimulated ±LPS have been deposited in the National Center for Biotechnology Information's Gene Expression Omnibus and are accessible through GEO series accession number GSE134047. The plasmids lentiGuide-Puro-P2A-EGFP and lentiGuide-Puro-P2A-EGFP_mRFPstuf have been deposited to the nonprofit plasmid repository AddGene.

**Statistics.** GraphPad Prism 6 was used for statistical analysis, as described in the figure legends.

**ACKNOWLEDGMENTS.** We are very grateful to Drs. Mikael C. I. Karlsson, and Robert M. Anthony for critically reading the manuscript; Drs. Jeffrey V. Ravetch, and Patrick Smith for providing $KRN^+$ mice, K/BxN serum, and cell

lines; Drs. Diane Mathis and Christophe Benoist for allowing us to use the KRN$^+$ mice strain; Dr. Judith E. Allen for providing the IL-4Rα floxed mice on a C57BL/6 background; Dr. Frank Brombacher for allowing us to use the IL-4Rα floxed mice strain; Dr. Robert Harris for sharing CSF3R receptor knockout mice; Dr. Mark P. Kamps and the University of California, San Diego for providing the ER-HoxB8 construct, the Macpro cell line, and a GM-CSF–producing B16 cell line; Dr. Erik Holmgren for experimental suggestions as well as the lacZ mRFP sequence; Drs. Manish Ponda and Pontus Almer Boström for experimental input; Annika van Vollenhoven for cell sorting; Dr. Eduardo Villablanca for formatting suggestions; and the staff working at the Karolinska Institute Department of Comparative Medicine and Preclinical Laboratory animal facilities for the help provided. LentiGuide-Puro (Addgene plasmid 52963), pSpCas9(BB)-2A-Puro (PX459) V2.0 (Addgene plasmid 62988), and LentiCas9-Blast (Addgene plasmid 52962) were kind gifts from Dr. Feng Zhang. psPAX2 (Addgene plasmid 12260) and pMD2.G (Addgene plasmid 12259) were kind gifts from Dr. Didier Trono. This work benefitted from data assembled by the ImmGen consortium and the Genotype-Tissue Expression (GTEx) project, for which we are grateful. This research was partly funded by grants from the Swedish Research Council, Karolinska Institutets Foundation Grants for Rheumatology, The Swedish Rheumatism Association, King Gustaf V's 80-y fund, Åke Wiberg Foundation, Stiftelsen Professor Nanna Svartz Fond, The Jeansson Foundations, Alex and Eva Wallström Foundation for scientific research and education (to F.W.), The Wenner-Gren Foundations (F.W., S.K.P., and S.V.B.), the Börje Dahlin fund, Karolinska Institutets Foundation Grants for Rheumatology (S.K.P.), the China Scholarship Council (L.J. and Y.S.), and the Nanyang Technological University–Karolinska Institutet Joint PhD Programme (V.S.I.). This work has received support from the European Union/European Federation of Pharmaceutical Industries and Associations (EU/EFPIA) Innovative Medicines Initiative Joint Undertaking (ULTRA-DD Grant 115766) (to S.K.P. and Z.K.) and from the EU/EFPIA Innovative Medicines Initiative 2 Joint Undertaking (RTCure Grant 777357) (to F.W.).

1. J. S. Smolen *et al*., Rheumatoid arthritis. *Nat. Rev. Dis. Primers* **4**, 18001 (2018).
2. A. I. Catrina, A. J. Ytterberg, G. Reynisdottir, V. Malmström, L. Klareskog, Lungs, joints and immunity against citrullinated proteins in rheumatoid arthritis. *Nat. Rev. Rheumatol.* **10**, 645–653 (2014).
3. C. Turesson, W. M. O'Fallon, C. S. Crowson, S. E. Gabriel, E. L. Matteson, Extra-articular disease manifestations in rheumatoid arthritis: Incidence trends and risk factors over 46 years. *Ann. Rheum. Dis.* **62**, 722–727 (2003).
4. A. Willemze, L. A. Trouw, R. E. Toes, T. W. Huizinga, The influence of ACPA status and characteristics on the course of RA. *Nat. Rev. Rheumatol.* **8**, 144–152 (2012).
5. L. Klareskog *et al*., A new model for an etiology of rheumatoid arthritis: Smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination. *Arthritis Rheum.* **54**, 38–46 (2006).
6. B. T. Wipke, P. M. Allen, Essential role of neutrophils in the initiation and progression of a murine model of rheumatoid arthritis. *J. Immunol.* **167**, 1601–1608 (2001).
7. D. Schrier, R. B. Gilbertsen, M. Lesch, J. Fantone, The role of neutrophils in type II collagen-induced arthritis in rats. *Am. J. Pathol.* **117**, 26–29 (1984).
8. P. A. Monach *et al*., Neutrophils in a mouse model of autoantibody-mediated arthritis: Critical producers of Fc receptor gamma, the receptor for C5a, and lymphocyte function-associated antigen 1. *Arthritis Rheum.* **62**, 753–764 (2010).
9. T. Németh, A. Mócsai, The role of neutrophils in autoimmune diseases. *Immunol. Lett.* **143**, 9–19 (2012).
10. C. D. Sadik, N. D. Kim, Y. Iwakura, A. D. Luster, Neutrophils orchestrate their own recruitment in murine arthritis through C5aR and FcγR signaling. *Proc. Natl. Acad. Sci. U.S.A.* **109**, E3177–E3185 (2012).
11. G. Weissmann, H. Korchak, Rheumatoid arthritis. The role of neutrophil activation. *Inflammation* **8** (suppl.), S3–S14 (1984).
12. S. W. Edwards, M. B. Hallett, Seeing the wood for the trees: The forgotten role of neutrophils in rheumatoid arthritis. *Immunol. Today* **18**, 320–324 (1997).
13. L. J. O'Neil, M. J. Kaplan, Neutrophils in rheumatoid arthritis: Breaking immune tolerance and fueling disease. *Trends Mol. Med.* **25**, 215–227 (2019).
14. T. Németh, A. Mócsai, Feedback amplification of neutrophil function. *Trends Immunol.* **37**, 412–424 (2016).
15. R. Khandpur *et al*., NETs are a source of citrullinated autoantigens and stimulate inflammatory responses in rheumatoid arthritis. *Sci. Transl. Med.* **5**, 178ra40 (2013).
16. K. Van Steendam *et al*., Citrullinated vimentin as an important antigen in immune complexes from synovial fluid of rheumatoid arthritis patients with antibodies against citrullinated proteins. *Arthritis Res. Ther.* **12**, R132 (2010).
17. L. Mathsson, J. Lampa, M. Mullazehi, J. Rönnelid, Immune complexes from rheumatoid arthritis synovial fluid induce FcgammaRIIa dependent and rheumatoid factor correlated production of tumour necrosis factor-alpha by peripheral blood mononuclear cells. *Arthritis Res. Ther.* **8**, R64 (2006).
18. H. Ji *et al*., Arthritis critically dependent on innate immune system players. *Immunity* **16**, 157–168 (2002).
19. S. Kleinau, P. Martinsson, B. Heyman, Induction and suppression of collagen-induced arthritis is dependent on distinct fcgamma receptors. *J. Exp. Med.* **191**, 1611–1616 (2000).
20. R. M. Anthony, T. Kobayashi, F. Wermeling, J. V. Ravetch, Intravenous gammaglobulin suppresses inflammation through a novel T(H)2 pathway. *Nature* **475**, 110–113 (2011).
21. F. Wermeling, R. M. Anthony, F. Brombacher, J. V. Ravetch, Acute inflammation primes myeloid effector cells for anti-inflammatory STAT6 signaling. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 13487–13491 (2013).
22. Y. Cao, F. Brombacher, M. Tunyogi-Csapo, T. T. Glant, A. Finnegan, Interleukin-4 regulates proteoglycan-induced arthritis by specifically suppressing the innate immune response. *Arthritis Rheum.* **56**, 861–870 (2007).
23. Z. Chen *et al*., Th2 and eosinophil responses suppress inflammatory arthritis. *Nat. Commun.* **7**, 11596 (2016).
24. A. C. Horsfall *et al*., Suppression of collagen-induced arthritis by continuous administration of IL-4. *J. Immunol.* **159**, 5687–5696 (1997).
25. D. L. Boyle *et al*., Intra-articular IL-4 gene therapy in arthritis: Anti-inflammatory effect and enhanced th2activity. *Gene Ther.* **6**, 1911–1918 (1999).
26. T. Hemmerle, F. Doll, D. Neri, Antibody-based delivery of IL4 to the neovasculature cures mice with arthritis. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 12008–12012 (2014).
27. I. K. Campbell *et al*., Therapeutic targeting of the G-CSF receptor reduces neutrophil trafficking and joint inflammation in antibody-mediated inflammatory arthritis. *J. Immunol.* **197**, 4392–4402 (2016).
28. J. L. Eyles *et al*., A key role for G-CSF-induced neutrophil production and trafficking during inflammatory arthritis. *Blood* **112**, 5193–5201 (2008).
29. J. Woytschak *et al*., Type 2 interleukin-4 receptor signaling in neutrophils antagonizes their expansion and migration during infection and inflammation. *Immunity* **45**, 172–184 (2016).
30. J. V. Ravetch, L. L. Lanier, Immune inhibitory receptors. *Science* **290**, 84–89 (2000).
31. J. P. Jacobs *et al*., Deficiency of CXCR2, but not other chemokine receptors, attenuates autoantibody-mediated arthritis in a murine model. *Arthritis Rheum.* **62**, 1921–1932 (2010).
32. I. N. Mbawuike, H. B. Herscowitz, MH-S, a murine alveolar macrophage cell line: Morphological, cytochemical, and functional characteristics. *J. Leukoc. Biol.* **46**, 119–127 (1989).
33. G. G. Wang *et al*., Quantitative production of macrophages or neutrophils ex vivo using conditional Hoxb8. *Nat. Methods* **3**, 287–293 (2006).
34. D. Impellizzieri *et al*., IL-4 receptor engagement in human neutrophils impairs their migration and extracellular trap formation. *J. Allergy. Clin. Immunol.* **144**, 267–279.e4 (2019).
35. J. Mauer *et al*., Signaling by IL-6 promotes alternative activation of macrophages to limit endotoxemia and obesity-associated resistance to insulin. *Nat. Immunol.* **15**, 423–430 (2014).
36. T. D. Gilmore, Introduction to NF-kappaB: Players, pathways, perspectives. *Oncogene* **25**, 6680–6684 (2006).
37. S. S. Tian, P. Lamb, H. M. Seidel, R. B. Stein, J. Rosen, Rapid activation of the STAT3 transcription factor by granulocyte colony-stimulating factor. *Blood* **84**, 1760–1764 (1994).
38. J. Schust, B. Sperl, A. Hollis, T. U. Mayer, T. Berg, Stattic: A small-molecule inhibitor of STAT3 activation and dimerization. *Chem. Biol.* **13**, 1235–1242 (2006).
39. F. Liu, H. Y. Wu, R. Wesselschmidt, T. Kornaga, D. C. Link, Impaired production and increased apoptosis of neutrophils in granulocyte colony-stimulating factor receptor-deficient mice. *Immunity* **5**, 491–501 (1996).
40. T. S. Heng, M. W. Painter; Immunological Genome Project Consortium, The Immunological Genome Project: Networks of gene expression in immune cells. *Nat. Immunol.* **9**, 1091–1094 (2008).
41. F. Nimmerjahn, J. V. Ravetch, Fcgamma receptors as regulators of immune responses. *Nat. Rev. Immunol.* **8**, 34–47 (2008).
42. C. M. Gillis *et al*., In vivo effector functions of high-affinity mouse IgG receptor FcγRI in disease and therapy models. *J. Autoimmun.* **80**, 95–102 (2017).
43. M. Corr, B. Crain, The role of FcgammaR signaling in the K/B x N serum transfer model of arthritis. *J. Immunol.* **169**, 6604–6609 (2002).
44. F. Li, P. Smith, J. V. Ravetch, Inhibitory Fcγ receptor is required for the maintenance of tolerance through distinct mechanisms. *J. Immunol.* **192**, 3021–3028 (2014).
45. S. Tridandapani *et al*., Regulated expression and inhibitory function of Fcgamma RIIb in human monocytic cells. *J. Biol. Chem.* **277**, 5082–5089 (2002).
46. A. A. te Velde, R. de Waal Malefijt, R. J. Huijbens, J. E. de Vries, C. G. Figdor, IL-10 stimulates monocyte Fc gamma R surface expression and cytotoxic activity. Distinct regulation of antibody-dependent cellular cytotoxicity by IFN-gamma, IL-4, and IL-10. *J. Immunol.* **149**, 4048–4052 (1992).
47. R. Noelle, P. H. Krammer, J. Ohara, J. W. Uhr, E. S. Vitetta, Increased expression of ia antigens on resting B cells: An additional role for B-cell growth factor. *Proc. Natl. Acad. Sci. U.S.A.* **81**, 6149–6153 (1984).
48. C. Iking-Konert *et al*., Transdifferentiation of polymorphonuclear neutrophils to dendritic-like cells at the site of inflammation in rheumatoid arthritis: Evidence for activation by T cells. *Ann. Rheum. Dis.* **64**, 1436–1442 (2005).
49. L. Oehler *et al*., Neutrophil granulocyte-committed cells can be driven to acquire dendritic cell characteristics. *J. Exp. Med.* **187**, 1019–1028 (1998).
50. H. Nakamura *et al*., High serum and synovial fluid granulocyte colony stimulating factor (G-CSF) concentrations in patients with rheumatoid arthritis. *Clin. Exp. Rheumatol.* **18**, 713–718 (2000).
51. J. A. Walker, A. N. J. McKenzie, $T_H2$ cell development and function. *Nat. Rev. Immunol.* **18**, 121–133 (2018).
52. W. E. Paul, History of interleukin-4. *Cytokine* **75**, 3–7 (2015).
53. Y. Noma *et al*., Cloning of cDNA encoding the murine IgG1 induction factor by a novel strategy using SP6 promoter. *Nature* **319**, 640–646 (1986).
54. C. Doucet *et al*., Interleukin (IL) 4 and IL-13 act on human lung fibroblasts. Implication in asthma. *J. Clin. Invest.* **101**, 2129–2139 (1998).
55. L. E. M. Heeb, C. Egholm, D. Impellizzieri, F. Ridder, O. Boyman, Regulation of neutrophils in type 2 immune responses. *Curr. Opin. Immunol.* **54**, 115–122 (2018).

56. A. J. Harris *et al.*, IL4Rα signaling abrogates hypoxic neutrophil survival and limits acute lung injury responses in vivo. *Am. J. Respir. Crit. Care Med.* **200**, 235–246 (2019).
57. P. Fleming, A. M. Drucker, Risk of infection in patients with atopic dermatitis treated with dupilumab: A meta-analysis of randomized controlled trials. *J Am Acad Dermatol* **78**, 62–69 e1 (2018).
58. K. Nelms, A. D. Keegan, J. Zamorano, J. J. Ryan, W. E. Paul, The IL-4 receptor: Signaling mechanisms and biologic functions. *Annu. Rev. Immunol.* **17**, 701–738 (1999).
59. D. D. Donaldson *et al.*, The murine IL-13 receptor alpha 2: Molecular cloning, characterization, and comparison with murine IL-13 receptor alpha 1. *J. Immunol.* **161**, 2317–2324 (1998).
60. T. A. Waldmann, The multi-subunit interleukin-2 receptor. *Annu. Rev. Biochem.* **58**, 875–911 (1989).
61. W. Liao *et al.*, Priming for T helper type 2 differentiation by interleukin 2-mediated induction of interleukin 4 receptor alpha-chain expression. *Nat. Immunol.* **9**, 1288–1296 (2008).
62. G. Perona-Wright, K. Mohrs, K. D. Mayer, M. Mohrs, Differential regulation of IL-4Ralpha expression by antigen versus cytokine stimulation characterizes Th2 progression in vivo. *J. Immunol.* **184**, 615–623 (2010).
63. G. T. Consortium; GTEx Consortium, The Genotype-Tissue Expression (GTEx) project. *Nat. Genet.* **45**, 580–585 (2013).
64. R. M. Anthony, F. Wermeling, J. V. Ravetch, Novel roles for the IgG Fc glycan. *Ann. N. Y. Acad. Sci.* **1253**, 170–180 (2012).
65. S. Bournazos, T. T. Wang, R. Dahan, J. Maamary, J. V. Ravetch, Signaling by antibodies: Recent progress. *Annu. Rev. Immunol.* **35**, 285–311 (2017).
66. A. M. Boruchov *et al.*, Activating and inhibitory IgG Fc receptors on human DCs mediate opposing functions. *J. Clin. Invest.* **115**, 2914–2923 (2005).
67. S. Q. Hayat, M. Hearth-Holmes, R. E. Wolf, Flare of arthritis with successful treatment of Felty's syndrome with granulocyte colony stimulating factor (GCSF). *Clin. Rheumatol.* **14**, 211–212 (1995).
68. D. R. Herbert *et al.*, Alternative macrophage activation is essential for survival during schistosomiasis and downmodulates T helper 1 responses and immunopathology. *Immunity* **20**, 623–635 (2004).
69. S. K. Panda *et al.*, Green listed-a CRISPR screen tool. *Bioinformatics* **33**, 1099–1100 (2017).
70. J. G. Doench *et al.*, Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9. *Nat. Biotechnol.* **34**, 184–191 (2016).