Chronic interleukin-1 exposure drives haematopoietic stem cells towards precocious myeloid differentiation at the expense of self-renewal

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Haematopoietic stem cells (HSCs) maintain lifelong blood production and increase blood cell numbers in response to chronic and acute injury. However, the mechanism(s) by which inflammatory insults are communicated to HSCs and their consequences for HSC activity remain largely unknown. Here, we demonstrate that interleukin-1 (IL-1), which functions as a key pro-inflammatory ‘emergency’ signal, directly accelerates cell division and myeloid differentiation of HSCs through precocious activation of a PU.1-dependent gene program. Although this effect is essential for rapid myeloid recovery following acute injury to the bone marrow, chronic IL-1 exposure restricts HSC lineage output, severely erodes HSC self-renewal capacity, and primes IL-1-exposed HSCs to fail massive replicative challenges such as transplantation. Importantly, these damaging effects are transient and fully reversible on IL-1 withdrawal. Our results identify a critical regulatory circuit that tailors HSC responses to acute needs, and is likely to underlie deregulated blood homeostasis in chronic inflammation conditions.

All lineages of haematopoietic cells, including those of the immune system, arise from a rare population of self-renewing HSCs residing in the bone marrow (BM) of adult mammals1. Blood production by HSCs is regulated by the concerted action of cell-intrinsic transcription factors such as PU.1 and GATA-1, and cell-extrinsic determinants produced by the stromal and haematopoietic components of the BM niche, which together regulate HSC self-renewal and specify lineage commitment2,3. Although normally maintained in a largely quiescent or dormant state, most HSCs can be rapidly activated to proliferate and differentiate in response to acute needs such as regenerative challenges including myeloablation and transplantation, and physiological insults that induce an inflammatory state4–6. Inflammation is a critical physiological process that mediates host defence against invading pathogens, injury and other insults, and is characterized by rapid mobilization and overproduction of specialized immune cells, particularly myeloid cells7. Inflammation is communicated to the haematopoietic system, and HSCs in particular, either by direct sensing through Toll-like receptors (TLRs), or indirectly through a series of pro-inflammatory cytokines8–10. In particular, interferons (IFNs), both type-I (IFN-α/β) and type-II (IFN-γ), and tumour necrosis factor alpha (TNFα) directly impact HSC fate during an inflammatory response11–14 and drive HSC specification during embryonic development15,16. Pro-inflammatory cytokines are therefore exciting new regulators of HSC function17, with much remaining to be understood regarding how inflammatory insults tailor blood production under homeostatic and disease conditions.

Interleukin-1 (IL-1) was the first interleukin identified and is the founding member of a group of 11 cytokines (the IL-1 family), with a central role in responses to infections or sterile insults18,19. IL-1 consists of two related genes (Il1a and Il1b) with distinct regulation but similar biological activities19, which bind a broadly expressed surface receptor (IL-1R) and trigger downstream transcriptional responses through the adaptor protein MyD88 and a broad range of signalling pathways including NF-κB, p38 MAPK, JNK and AP-1 (ref. 20). IL-1 is a key ‘emergency’ signal that rapidly activates host defence and repair in many tissues, including the blood system, but also drives tissue dysfunction in the context of chronic inflammation and autoimmune diseases18,19. Acute IL-1 signalling is

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IL-1 accelerates HSC differentiation along the myeloid lineage. (a) Representative expansion in liquid culture (n=3 biological replicates per group). (b) CFSE dilution assays after 60 h. Data represent one of two replicate experiments. Grey histogram shows –IL-1β HSCs at 24 h. (c–e) Continuous single-cell tracking experiments (n=47 and 51 HSCs per group) experimental design (c), single-cell pedigrees of median division times (d), and box plot quantification of division times (e). Results show median (lines) and 10–90th percentile (whiskers). (f–i) Colony-forming unit (CFU) assays in methylcellulose: experimental design (f), single-cell clonogenic assays (n=3 replicate experiments with 60 HSCs per group) (g), representative colony type (scale bar, 100 μm) and morphology (scale bar, 10 μm; arrow indicates a macrophage) (h), and replating (repl.) experiments (i). Data represent one of two replicate experiments. Colonies were scored after 7 days. MkE, megakaryocyte/erythrocyte; M, macrophage; G, granulocyte; GM, granulocyte/macrophage; GMMkE, mix colony. (j,k) Myeloid differentiation in liquid culture (n=6 biological replicates per group) experimental design with representative FACS plots (j), and quantification of myeloid marker expression (k). Source data for a are shown in Supplementary Table 1. Data are means ± s.d.; **P<0.01, ***P<0.001. P values in a were determined by one-way ANOVA with Dunnett’s test, in g by paired Student’s t-test, and in e and k by Mann–Whitney U-test. Exact P values, number of replicates used to derive statistical data (n) and statistical tests used are shown in Supplementary Table 2.

RESULTS

IL-1 accelerates HSC differentiation

To investigate IL-1 effects, we isolated HSCs (Lin–c-Kit+Sca-1+Flk2–CD48–CD150+) from wild-type mice and monitored their expansion in liquid culture with or without (+) IL-1α or IL-1β (25 ng ml⁻¹). Notably, HSCs cultured with IL-1 differentiated and expanded significantly faster than untreated HSCs over an 8-day period (Fig. 1a), which seemed to result from faster division rates as measured by carboxyfluorescein succinimidyl ester (CFSE) dilution assay after 60 h (Fig. 1b). To confirm accelerated cell division in HSC cultures, we used an automated single-cell

associated with increased myeloid cell production both in culture and in vivo in response to infection, irradiation or myeloablative chemotherapy. Many of the inflammatory disease conditions associated with chronic IL-1 production such as rheumatoid arthritis, obesity and type-2 diabetes also feature severe haematological complications, including overproduction of tissue-damaging myeloid cells, loss of naive lymphoid cell production and chronic anaemia. However, the mechanism by which IL-1 contributes to deregulated blood output in these conditions, and the functional consequences of both acute and chronic IL-1 exposure on HSC fate, is largely unknown.

Table 2.
tracking approach to continuously monitor cell division over a 6-day period (Fig. 1c)\(^2\). Remarkably, although the timing of the exit from quiescence first division seemed relatively unaffected in IL-1-treated HSCs, the kinetics of the subsequent differentiation divisions were significantly compressed (Fig. 1d,e). This effect was specific to HSCs, as expansion, survival and proliferation were unchanged in IL-1-treated granulocyte/macrophage progenitors (GMPs: Lin\(^{-}\)c-Kit\(^{+}\)Sca-1\(^{-}\)CD34\(^{-}\)Flk2\(^{-}\)) and multipotent progenitors (MPPs), including myeloid-biased MPP2 (Lin\(^{-}\)c-Kit\(^{-}\)Sca-1\(^{-}\)Flk2\(^{-}\)CD48\(^{+}\)CD150\(^{+}\)) and MPP3 (Lin\(^{-}\)c-Kit\(^{+}\)Sca-1\(^{-}\)Flk2\(^{-}\)CD48\(^{+}\)CD150\(^{-}\)) or lymphoid-primed MPP4 (Lin\(^{-}\)c-Kit\(^{-}\)Sca-1\(^{-}\)Flk2\(^{-}\))\(^{2,30}\) which all express IL-1R at similar levels to HSCs (Supplementary Fig. 1a-e). These results indicate that IL-1 specifically targets HSCs and accelerates their division kinetics.

To directly address the effects of IL-1 on HSC differentiation, we performed colony formation assays in methylcellulose ± IL-1β (Fig. 1f). Strikingly, IL-1-treated HSCs produced almost exclusively myeloid-committed granulocyte/macrophage (GM)-type colonies containing abundant macrophages, in contrast to untreated HSCs, which had a higher proportion of immature multilineage colonies containing mostly immature myeloblasts and mast cells (Fig. 1g,h). We also re-plated the progeny of these colonies once in methylcellulose without IL-1β, and observed near exhaustion of colony-forming ability in cells from IL-1-treated HSC cultures (Fig. 1i). Moreover, HSCs grown in liquid culture with IL-1β rapidly lost expression of the immaturity markers c-Kit and Sca-1, and gained expression of the myeloid differentiation markers Mac-1 and FcγR, resulting in greatly increased absolute numbers of c-Kit\(^{+}\) progenitors and mature myeloid cells in IL-1-treated HSC cultures (Fig. 1j,k and Supplementary Fig. 1f). This pro-myeloid differentiation effect was diminished in MPPs and completely absent in GMPs (Supplementary Fig. 2a-e). Collectively, these results demonstrate that IL-1 accelerates the production of mature myeloid cells by HSCs.

**Precocious activation of a PU.1 molecular circuit**

To understand the effects of IL-1 at the molecular level, we used quantitative PCR with reverse transcription (qRT–PCR) and a custom-made Fluidigm PCR array to analyse gene expression in HSCs cultured ± IL-1β for 12 or 24 h (Fig. 2a and Supplementary Fig. 3a). Remarkably, we observed a strong induction of the transcription factor PU.1 (Spi1) and its target genes Csf2ra (GM-CSFR) and Csf1r (M-CSFR) in IL-1-treated HSCs (Fig. 2b and Supplementary Fig. 3b). In contrast, lymphoid and megakaryocyte/erythrocyte (MkE) lineage genes were largely unaffected, suggesting that IL-1 functions by activating, rather than suppressing, lineage-specific gene programs in HSCs\(^{31}\). Consistent with the effects of IL-1 on cell division, we also observed activation of the cell cycle machinery and decreased expression of the quiescence-enforcing cyclin D1 (Ccnb1) and p57 (Cdkn1c) genes, alongside induction of p21 (Cdkn1a), another PU.1 target (Fig. 2b). We confirmed rapid upregulation of M-CSFR and two other PU.1-dependent myeloid surface markers, Mac-1 and CD18, in IL-1-treated HSCs (Fig. 2c), and found limited or absent activation of PU.1 and its target genes in IL-1-treated MPPs and GMPs (Supplementary Fig. 3b). Moreover, using HSCs isolated from PU.1-eYFP reporter mice, we confirmed fast upregulation of PU.1 activity on IL-1 exposure, with uniformly high PU.1 levels in IL-1-treated PU.1-eYFP HSCs before their first division in single-cell tracking experiments (Fig. 2d,e and Supplementary Fig. 3c). We also uncovered a small subpopulation of PU.1HSCs in untreated cultures whose progeny showed differentiation kinetics similar to IL-1–treated HSCs (Supplementary Fig. 3d). In particular, both had a delayed first division when compared with untreated PU.1HSCs, consistent with the decreased BrdU incorporation observed after 24 h in IL-1-treated HSCs (Supplementary Fig. 3e). The subsequent divisions of untreated PU.1HSCs were also accelerated, thus directly linking elevated PU.1 activity with the overall effect of IL-1 in accelerating division kinetics. This is in contrast to the recently reported function of steady-state PU.1 levels in limiting HSC cell cycle re-entry\(^{32}\) and is likely to reflect differences in gene dosage. In fact, high PU.1 levels similarly delayed HSC first division while simultaneously priming their progeny to undergo accelerated proliferation through induction of myeloid lineage determinants and cell cycle activators including Cdk4, Myc, Ccnb and Ccn1 (Fig. 2b). To directly demonstrate the importance of PU.1 for IL-1 effects, we isolated HSCs from PU.1\(^{32}\) hypomorphic mice lacking the PU.1 upstream regulatory element (URE) and expressing PU.1 at 10–20% normal levels\(^{33}\).

Strikingly, myeloid differentiation, including Mac-1 expression, was severely attenuated in PU.1\(^{32}\) HSC cultures (Fig. 2f), confirming the requirement for PU.1 in IL-1-driven HSC differentiation. Moreover, to establish that PU.1 upregulation is sufficient to drive accelerated HSC differentiation, we overexpressed PU.1 in HSCs using a validated lentiviral vector\(^{34}\). Similar to IL-1-treated HSCs, PU.1-overexpressing HSCs showed accelerated gain of the myeloid markers Mac-1/FcγR relative to control HSCs (Fig. 2g). These results establish that IL-1 acts instructively and ‘primes’ HSCs to undergo accelerated myeloid differentiation through precocious activation of a PU.1-dependent molecular circuit.

**Mechanism of PU.1 activation**

To gain insight into the mechanism of PU.1 activation by IL-1, we first addressed its dependency on IL-1R signalling. As expected, Il1r\(^{–/–}\) HSCs did not show accelerated myeloid differentiation in the presence of IL-1β (Fig. 3a), and IL-1-treated Il1r\(^{–/–}\)::PU.1-eYFP HSCs failed to upregulate PU.1 (Fig. 3b). We then exposed PU.1-eYFP HSCs cultured for 12 h ± IL-1β (25 pg ml\(^{-1}\), a lower dose titrated for sensitivity), to inhibitors of various signalling pathways acting downstream of IL-1R (Fig. 3c,d and Supplementary Fig. 3g). Interestingly, whereas blockade of p38, MEK and PKC partially decreased IL-1-induced PU.1 levels, inactivation of IKK almost entirely abolished PU.1 activity, consistent with the ability of its downstream target, NF-κB, to bind the PU.1 promoter\(^{35}\). On the other hand, PI(3)K and mTOR inhibition had no effect, and blockade of Src kinase, which drives PU.1 activation downstream of M-CSFR\(^{36}\), only partially inhibited IL-1 effects. As M-CSFR expression was increased in IL-1-treated HSCs, we also tested whether IL-1 could directly activate an M-CSF autocrine loop to induce PU.1. However, treatment with an anti-M-CSFR blocking antibody did not attenuate IL-1-mediated PU.1 activation in PU.1-eYFP HSCs, despite preventing M-CSF-mediated macrophage differentiation in BM cultures (Fig. 3e and Supplementary Fig. 3h). Furthermore, the effect of IL-1 required constant IL-1R signalling and was lost if IL-1 was washed out...
Haematopoietic remodelling following IL-1 exposure

To investigate the effects of IL-1 on haematopoiesis in vivo, we injected mice intraperitoneally with 0.5 μg IL-1β for 1 day (acute treatment) up to 20 consecutive days (chronic exposure; Fig. 4a). As expected, IL-1-treated mice exhibited a rapid and sustained increase in circulating myeloid cells, with concomitant decreases in lymphoid cells and erythrocytes (Fig. 4b,c and Supplementary Fig. 4a). In the BM, 1 day acute IL-1 treatment did not significantly alter the overall cellularity or lineage distribution of mature cell populations, save for a small but significant increase in Mac-1+ Gr-1int pre-granulocytes/monocytes (preGrs; Supplementary Fig. 4b–d). However, acute IL-1 treatment already resulted in a rapid MPP expansion and substantial erosion of common lymphoid progenitors (CLPs: Lin−Flk2+IL-7Rα+c-Kit+Scal−1+; Supplementary Fig. 4c,d), suggesting quick myeloid priming on IL-1 exposure. These changes were maintained and associated with a clear rebalancing of lineage output following 20 days of chronic IL-1 exposure, with significant expansion of Mac-1+ Gr-1+ pre-granulocytes (Grs) and corresponding loss of B220+ CD19− B cells (Fig. 4d). This was associated with a specific amplification of GMPs and myeloid-biased MPP2 and MPP3 subsets (Fig. 4e), suggesting direct activation of a myeloid differentiation axis at the level of HSCs. Consistently, we observed a rapid and sustained increase in the metabolically activated MPP1 (Lin−c-Kit+Sca-1+Flk2−CD48−CD150+CD34+), which

Figure 2 IL-1 induces precocious activation of a PU.1 gene program in HSCs. (a,b) Gene expression analyses (using the Fluidigm platform) of lineage determinant and cell cycle genes: heatmap (a), and expression of individual genes (n=8 pools of 100 cells for each condition; bars are means) (b). Results are expressed as fold changes compared with levels in −IL-1β HSCs (set to 1). MIFE: megakaryocyte/erythroid. (c) Expression of PU.1 targets (n=5 biological replicates per group; M-CSFR day 4, n=7). Results are expressed as mean fluorescence intensity (MFI) levels. (d) Representative histogram and PU.1 levels in PU.1-eYFP HSCs (n=6 biological replicates per group; day 4, n=4). (e) Representative images and PU.1 levels in individual PU.1-eYFP HSCs before first division (n=33–37 HSCs per group; scale bar, 10 μm). Results are expressed in arbitrary units (a.u.) and box plots show the median (lines) with the 10–90th percentile (whiskers). (f) Experimental design and myeloid marker expression in control (Ctrl) and PU.1-overexpressing HSCs (n=3 biological replicates per group). (g) Experimental design, representative FACS plots and myeloid marker expression in lentivirally transduced (GFP+) Ctrl or PU.1-overexpressing HSCs (n=3 biological replicates per group for day 4; day 1 represents the mean of two biological replicates per group). Source data for d,f and g are shown in Supplementary Table 1. Data are means ± s.d.; *P < 0.05, **P < 0.01, ***P < 0.001. P values in b–e were determined by Mann–Whitney U-test, in f by one-way ANOVA with Dunnett’s test, and in g by paired Student’s t-test. Exact P values, number of replicates used to derive statistical data (n) and statistical tests used are shown in Supplementary Table 2.
is produced by dormant long-term HSCs (HSCLT; Lin−c-Kit+Sca-1+Flk2+CD48−CD150−CD34−)29, and solely accounted for the overall expansion of phenotypic HSCs observed following 20 days of chronic IL-1 exposure (Fig. 4g and Supplementary Fig. 4e). Importantly, HSC activation and BM remodelling was not observed in IL-1-treated Il1r1−/− mice, confirming its dependency on IL-1R signalling (Supplementary Fig. 4e). Moreover, using a sensitive in vivo CFSE dilution assay27 and 7 consecutive days of IL-1β treatment, we demonstrated that fewer IL-1R-deficient HSCs remain in an undivided state compared with untreated HSCs27 (Fig. 4h,i and Supplementary Fig. 5a,b). This confirms in vivo that IL-1 exposure directly increases HSC division rates, which is likely to fuel the MPP overproduction observed even after acute treatment. In addition, we showed persistent PU.1 activation in a substantial fraction of HSCs from PU.1−eYFP mice on both acute and chronic IL-1 treatment (Fig. 4j). We also found decreased Cebpa levels and increased Runx1 expression in HSCs exposed to IL-1 for 20 days (Fig. 4k), which is strikingly similar to the molecular changes observed in regenerating HSCs that overproduce myeloid-biased MPP2/3 as they re-build the myeloid lineage30. PU.1 was also activated in MPP4 from IL-1-treated PU.1−eYFP mice (Supplementary Fig. 5c), which is likely to reflect the myeloid lineage reprogramming of this lymphoid-primed MPP subset on IL-1 exposure. This is consistent with the observed loss of lymphoid output and previously reported inhibition of lymphopoiesis by IL-1 (ref. 38). Collectively, these results indicate that in vivo IL-1 exposure triggers a rapid PU.1-mediated myeloid differentiation program in HSCs, which amplifies myeloid cell production at the expense of the other blood lineages.
Acute IL-1 stimulation contributes to myeloid recovery

To investigate the importance of IL-1 for blood regeneration, we focused on 5-fluorouracil (5-FU)-mediated myeloablation, as we found that both IL-1α and IL-1β levels were significantly increased in BM plasma, but not in blood serum, whereas no other pro-inflammatory cytokines were induced following one injection of 150 mg kg

−1 body weight (BW) 5-FU (Fig. 5a and Supplementary Fig. 6a). To identify the cellular source(s) of IL-1, we analysed Il1a and Il1b expression by qRTPCR in immature BM myeloid and lymphoid cells, as well as in BM stromal populations

19, specifically osteoblastic lineage cells (OBCs: Lin−CD45−Sca-1−CD51+)

, multipotent stromal cells (MSPs: Lin−CD45−Sca-1−CD51+) and endothelial cells (ECs: Lin−CD45−Sca-1−CD31+) (Supplementary Fig. 6b). At steady state, Il1a was expressed largely by BM CD45+ T cells, and Il1b by Grs (Fig. 5b). However, on 5-FU treatment, Il1a was strongly induced in myeloid cells and ECs, but no increase in Il1b expression was detected, suggesting that it might be released by dying Grs on inflammasome activation

20. Strikingly, myeloid recovery was significantly and specifically delayed in 5-FU-injected Il1r1−/− mice (Fig. 5c), emphasizing the importance of IL-1R signalling for myeloid recovery following BM injury. However, HSCs isolated from Il1r1+/+ and Il1r1−/− mice 10 days post-5-FU treatment, and at the peak of IL-1 production, showed equivalent engraftment and long-term multilineage reconstitution on transplantation (Fig. 5d–g). This indicates that acute exposure to IL-1 and transient activation of pro-myeloid differentiation pathways required for optimal blood recovery does not affect HSC self-renewal activity. In addition, Il1r1−/− mice had unaffected basal myeloid lineage priming in the absence of tonic IL-1 signalling (Supplementary Fig. 6c,d). Together, these results demonstrate that although IL-1 signalling is dispensable in HSCs

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– IL-1β

+ IL-1β

Cells μl−1 PB (×103)

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Myeloid

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IL-1 enforces HSC myeloid differentiation in vivo. (a–c) Peripheral blood (PB) parameters in mice injected ± IL-1β for 20 days; experimental design (a), percentage of myeloid (Mac-1+Gr-1+) and lymphoid (B220+ and CD3+ T cells) cells over time (n = 5 mice per group; ∗ versus −IL-1β; † versus day 0) (b), and complete blood counts after 20 days (c). My, myeloid; Ly, lymphoid; RBC, red blood cell; Plt, platelet. (d–g) Size of the indicated BM compartments, aside from a specific decrease in myeloid-biased MPP3 that may represent impaired basal myeloid lineage priming in the absence of tonic IL-1 signalling (Supplementary Fig. 6c,d). Together, these results demonstrate that although IL-1 signalling is dispensable in HSCs...
at steady state, it becomes important in physiological 'emergency' conditions when acute IL-1 production rapidly activates HSC myeloid differentiation to enhance blood regeneration. They also identify ECs as one of the key producers of IL-1 following haematopoietic damage, consistent with a local delivery of IL-1 to quiescent HSCs lodged in their perivascular BM niches42.

**Chronic IL-1 exposure impairs HSC function**

To better understand the consequence of chronic IL-1 exposure, we used HSCs isolated from mice injected with IL-1β for 20 days. First, we confirmed that IL-1-treated HSCs had unaffected clonogenic efficiency in methylcellulose and normal survival as measured by cleaved caspase-3 (CC3) levels compared with naive HSCs...
isolated from PBS-treated mice (Supplementary Fig. 7a). We then transplanted both naive and IL-1-treated HSCs into sublethally irradiated congenic recipients that were subsequently injected ± IL-1β for another 30 days to track lineage reconstitution and BM HSC chimaerism (Fig. 6a). We observed accelerated donor blood output from naive HSCs exposed to IL-1 after transplantation (Fig. 6b), which is consistent with earlier literature describing enhanced myeloid regeneration in similar conditions. In contrast, donor blood output rapidly eroded in mice transplanted with IL-1-treated HSCs on continued IL-1 exposure (Fig. 6b and Supplementary Fig. 7b). In both cases, IL-1 treatment following transplantation completely blocked donor lymphoid output and rebalanced blood production towards enhanced myelopoiesis (Fig. 6c and Supplementary Fig. 7b). However, chimaerism analyses uncovered a near complete exhaustion of donor HSCs in the BM of mice transplanted with IL-1-treated HSCs, even in recipients not subsequently injected with IL-1 (Fig. 6d and Supplementary Fig. 7c). To determine whether these effects were due to IL-1-mediated PU.1 activation, we transplanted lentivirus-transduced HSCs overexpressing PU.1 into sublethally irradiated recipient mice and tracked these mice for 30 days (Fig. 6e). Strikingly, compared with control-transduced HSCs, we observed rapid erosion of donor blood output and complete exhaustion of PU.1-overexpressing HSCs in the BM (Fig. 6f and Supplementary Fig. 7d), a behaviour similar to IL-1-exposed HSCs. Together, these short-term lineage-tracking results indicate that although IL-1 treatment always enhances myeloid cell production regardless of its duration, it also severely compromises HSC function and blood regeneration in conditions of chronic exposure, probably through hyperactivation of PU.1.

To confirm the functional impairment of HSCs chronically exposed to IL-1, we transplanted naive and IL-1-treated HSCs into lethally irradiated recipient mice to assess long-term engraftment and self-renewal activity after 4 months, and also performed limiting dilution analyses with unfractionated BM cells to address HSC function independently of surface markers (Fig. 7a and Supplementary Fig. 8a). In both cases, we observed significantly decreased donor chimaerism and reduced lymphoid output from IL-1-treated HSCs (Fig. 7b,c and Supplementary Fig. 8b,c), reflecting compromised self-renewal activity. To investigate whether long-term IL-1 treatment could lead to HSC exhaustion, we also injected mice ± IL-1β for a total of 70 days (Fig. 7d). Remarkably, even after such a long exposure, the HSC pool remained numerically intact though with an elevated frequency and number of myeloid-primed CD41+ HSC43–45 and expansion of myeloid-biased MPP2/3 (Supplementary Fig. 8d,e). We also found that 70-day IL-1-treated HSC47 had unaffected survival in methylcellulose (Supplementary Fig. 8f), but significantly impaired self-renewal activity following transplantation (Fig. 7e,f). These results demonstrate that chronic IL-1 exposure restricts HSC lineage output and severely compromises HSC regenerative function, thus priming IL-1-exposed HSCs to fail replicative challenges such as transplantation.

**IL-1 effects are reversible on withdrawal**

Last, we tested whether the damaging effects of chronic IL-1 exposure were retained by the HSC pool. We first re-transplanted into
Figure 7 Chronic IL-1 exposure impairs HSC self-renewal. (a–c) Long-term engraftment of HSCs from mice injected ± IL-1β for 20 days and transplanted into lethally irradiated (IR) recipients (n=10 mice per group): experimental design (a), donor chimaerism (b) and lineage distribution in PB over time (c). (d–f) Long-term engraftment of HSCLT from mice injected ± IL-1β for 70 days (n=9 – IL-1β and 10 +IL-1β mice per group): experimental design (d), donor chimaerism (e) and lineage distribution in PB over time (f). (g–i) Secondary transplantation (2° txpl) of HSCs re-isolated from primary transplanted mice (1° txpl, shown in a) reconstituted with HSCs from mice injected ± IL-1β for 20 days (n=8 mice per group): experimental design (g), donor chimaerism (h) and lineage distribution in PB over time (i). Source data for b, c, e, f and i are shown in Supplementary Table 1. Data are means ± s.d.; *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001. P values in b, e, f and i were determined by Mann–Whitney U-test, and in c and f by Student’s t-test. Exact P values, number of replicates used to derive statistical data (n) and statistical tests used are shown in Supplementary Table 2.

Lethally irradiated recipient mice donor HSCs collected from mice initially reconstituted with naive and IL-1-exposed HSCs (20 days or 70 days; Fig. 7g and Supplementary Fig. 8g). Remarkably we observed a complete recovery relative to naive controls in donor chimaerism and lineage output in HSCs derived from IL-1-treated donor mice, regardless of the length of the original IL-1 exposure (Fig. 7h,i and Supplementary Fig. 8h,i). These results suggest that the remaining portion of the IL-1-exposed HSC pool was not functionally compromised. To assess whether IL-1 effects were reversible under native conditions, we used mice that were injected ± IL-1β for 20 days and allowed to rest without further treatment for 8 weeks (Fig. 8a). After the rest period, blood parameters had fully normalized to untreated levels with a complete restoration of myeloid and B cell numbers (Fig. 8b). Moreover, most of the BM changes had also reverted to untreated levels, with normal numbers of HSCs and MPPs, including MPP1, and restoration of the CLP compartment (Fig. 8c,d). The only persisting differences were increased GMP and decreased MPP4 numbers, which may reflect residual myeloid priming effects of the initial IL-1 treatment. Most importantly, donor chimaerism and lymphoid output were indistinguishable from untreated HSCs, indicating full restoration of self-renewal activity (Fig. 8e,f). Taken together, these results demonstrate that the damaging effects of chronic IL-1 exposure on HSC regenerative functions are fully reversible on interruption of IL-1 exposure.

DISCUSSION

Our results demonstrate that IL-1 directly regulates HSC fate and instructs quick myeloid differentiation through precocious activation...
of an instructive PU.1 gene program and exclusive production of myeloid cells in ‘emergency’ situations such as myeloaablation and transplantation (Fig. 8g)66. These results imply that previous models, in which sustained low PU.1 levels initiate eventual myeloid commitment in progenitors45, do not apply in stress conditions. IL-1 has long been known to inhibit lymphopoiesis and erythropoiesis38,48,49 although, paradoxically, high PU.1 levels also play a key role in B cell development46. It is therefore likely that rapid PU.1 induction in HSCs by IL-1 drives myelopoiesis while preventing the establishment of other gene programs specifying lymphopoiesis and erythropoiesis. Interestingly, PU.1 activation proceeds through IKK kinase, which is the primary activator of NF-kB, a known IL-1 target that is also downstream of TLRs20. As TLRs also induce HSC myeloid differentiation29, it is possible that PU.1 serves as a convergence point for these pro-inflammatory signals. Interestingly, the transcription factor C/EBPβ also regulates ‘emergency’ myeloid differentiation in response to IL-3 or GM-CSF in haematopoietic progenitors41, or to IFN-γ stimulation in HSCs52. It will therefore be interesting to investigate whether activation of PU.1 and activation of C/EBPβ represent distinct or converging mechanisms accelerating HSC myeloid differentiation in response to inflammation.

Our results also demonstrate that chronic IL-1 exposure significantly impairs HSC function. Notably, the numerical HSC pool is not depleted, consistent with previous results of long-term in vivo exposure to IFN-α13. This may reflect the recently described ability of the MPP compartment to ‘buffer’ myeloid demand independently of HSCs53,54, or the replenishment of an IL-1-responsive HSC subset from quiescent HSC55 similar to the IFN-responsive CD41+ HSCs recently involved in emergency megakaryopoiesis56. This latest interpretation is supported by our observation that a fraction of, rather than all, HSCs activate PU.1 following IL-1 exposure, and the partial, rather than complete, functional impairment of IL-1-exposed...
HSCs. In this context, neither CD34 nor CD41 expression seems to separate IL-1-responsive from IL-1-non-responsive HSCs, and further analyses will be required to identify ways to separate these two functional subsets. Interestingly, the deleterious effects of chronic IL-1 exposure on HSC function are largely resolved following IL-1 withdrawal, suggesting that they are not permanently ‘imprinted’ onto the HSC pool through epigenetic or other means. As IL-1 receptor blockers including anakinra are highly efficacious treatments for a wide range of chronic inflammatory diseases featuring deregulated blood production, restoration of HSC function could be a key benefit of IL-1 blockade. Moreover, it is likely that IL-1 could play a similar dual role, both beneficial and pathogenic, in a variety of tissues by directly reprogramming their stem cell populations. Collectively, our findings demonstrate that IL-1 acts as a double-edged sword for HSC function, promoting myeloid regeneration without functional cost to HSCs during acute need, but significantly impairing their self-renewal and lineage output following chronic exposure. Modulation of IL-1 signalling, particularly its duration, may therefore be an important approach for broadly improving stem cell health and tissue function in the context of chronic inflammation or physiological ageing.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Supplementary Information is available in the online version of the paper

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AUTHOR CONTRIBUTIONS

E.M.P. performed all of the experiments with assistance from C.M.-B. and S.F.D.L. helped with the single-cell continuous tracking experiments that were performed in T.S. laboratory. R.L. helped with the Il1r1−/− and 5-FU analyses, I-M.T. with the in vitro cultures, C.P.C. with analysis of single-cell tracking experiments, L.Y.K. and M.G.M. with the in vivo CFSE dilution experiments, B.W. and U.S. with the PU.1.GFP experiments, and C.N. provided PU.1→EYFP reporter mice. E.M.P., C.M.-B., S.F. and E.P. designed the experiments and interpreted the results. E.M.P. and E.P. wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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1. Orkin, S. H. & Zon, L. I. Hematopoiesis: an evolving paradigm for stem cell biology. Cell 132, 631–644 (2008).

2. Iwasaki, H. & Akashi, K. Myeloid lineage commitment from the hematopoietic stem cell. Immunity 26, 726–740 (2007).

3. Scheper, K., Campbell, T. C. & Passegue, E. Normal and leukemic stem cell niches: insights and therapeutic opportunities. Cell Stem Cell 16, 254–267 (2015).

4. Wilson, A. et al. Hematopoietic stem cell reversely switch from dormancy to self-renewal during homeostasis and repair. Cell 135, 1118–1129 (2009).

5. Pietras, E. M., Warr, M. R. & Passegue, E. Cell cycle regulation in hematopoietic stem cells. J. Cell Biol. 195, 709–720 (2011).

6. Takizawa, H., Boettcher, S. & Manz, M. G. Demand-adapted regulation of early hematopoiesis in infection and inflammation. Blood 119, 2991–3002 (2012).

7. Medzhitov, R. Origin and physiological roles of inflammation. Nature 454, 428–435 (2008).

8. Nagai, Y. et al. Toll-like receptors on hematopoietic progenitor cells stimulate innate immune system replenishment. Immunity 24, 801–812 (2006).

9. Scheutypegel, L. G. & Link, D. C. Regulation of hematopoietic stem cell activity by inflammation. Front. Immunol. 4, 204 (2013).

10. Mirandel, C., Passegué, E. & Pietras, E. M. Pro-inflammatory cytokines: emerging players regulating HSC function in normal and diseased hematoepoiesis. Exp. Cell Res. 329, 248–254 (2014).

11. Essers, M. et al. IFNα activates dormant haematopoietic stem cells in vivo. Nature 458, 904–908 (2009).

12. Baldridge, M. T., King, K. Y., Boles, N. C., Weckberg, D. C. & Goodell, M. A. Quiescent haematopoietic stem cells are activated by IFN-γ in response to chronic infection. Nature 465, 793–797 (2010).

13. Pietras, E. M. et al. Re-entry into quiescence protects hematopoietic stem cells from the killing effect of chronic exposure to type I interferons. J. Exp. Med. 211, 245–262 (2014).

14. Pronk, C. J., Veby, O. P., Bryder, D. & Jacobsen, S. E. Tumor necrosis factor restricts hematopoietic stem cell activity in mice: involvement of two distinct receptors. J. Exp. Med. 208, 1563–1570 (2011).

15. Espin-Palazon, R. et al. Proinflammatory signaling regulates hematopoietic stem cell emergence. Cell 159, 1070–1085 (2014).

16. Li, Y. et al. Inflammatory signalling regulates embryonic hematopoietic stem and progenitor cell production. Genes Dev. 28, 2597–2612 (2014).

17. King, K. Y. & Goodell, M. A. Inflammatory modulation of HSCs: viewing the HSC as a fundamental immune response. Nat. Rev. Immunol. 11, 685–692 (2011).

18. Sims, J. E. & Smith, D. E. The IL-1 family: regulators of immunity. Nat. Rev. Immunol. 10, 89–102 (2010).

19. Dinarello, C. A., Simon, A. & van der Meer, J. W. Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. Nat. Rev. Drug Discov. 11, 633–652 (2012).

20. Martin, M. U. & Wesche, H. Summary and comparison of the signaling mechanisms of the Toll/Interleukin-1 receptor family. BBA Mol. Cell Res. 1592, 263–280 (2002).

21. Morrissey, P., Charrier, K., Bressler, L. & Alper, A. The influence of IL-1 treatment on the reconstitution of the hematopoietic and immune systems under sublethal irradiation. J. Immunol. 140, 4204–4210 (1988).

22. Damia, G. et al. Prevention of acute chemotherapy-induced death in mice by recombinant human interleukin 1-β: protection from hematological and nonhematological toxicities. Cancer Res. 52, 4082–4089 (1992).

23. Hestdal, K. et al. Interleukin-1 (IL-1) directly and indirectly promotes hematopoietic cell growth through type I IL-1 receptor. Blood 84, 125–132 (1994).

24. Ueda, Y., Cain, D. W., Kuraoka, M., Kondo, M. & Kelsoe, G. IL-1R type-I dependent hematopoietic stem cell proliferation is necessary for inflammatory granulopoiesis and neutrophilic neutrophilia. J. Immunol. 182, 6477–6484 (2009).

25. Smith, M. A., Knight, S. M., Maddison, P. J. & Smith, J. G. Mechanism of anaemia in rheumatoid arthritis: effect of the blunted response to erythropoietin and of interleukin 1 production by marrow macrophages. Ann. Rheum. Dis. 51, 753–757 (1992).

26. Dinarello, C. A. Blocking IL-1 in systemic inflammation. J. Exp. Med. 201, 1355–1359 (2005).

27. Cain, D., Kondo, M., Chen, H. & Kelsoe, G. Effects of acute and chronic inflammation on B-cell development and differentiation. J. Invest. Dermatol. 129, 266–277 (2009).

28. Rieger, M. A., Hoppe, P. S., Smejkal, B. M., Eitelhuber, A. C. & Schroeder, T. Hematopoietic cytokines can instruct lineage choice. Science 325, 217–218 (2009).

29. Cabanas-Wallscheid, N. et al. Identification of regulatory networks in HSCs and their immediate progeny via integrated proteome, transcriptome, and DNA methylation analysis. Cell Stem Cell 15, 507–522 (2014).

30. Pietras, E. M. et al. Functionally distinct subsets of lineage-biased multipotent progenitors control blood production in normal and regenerative conditions. Cell Stem Cell 17, 35–46 (2015).

31. Enver, T. & May, G. Lineage specification: reading the instructions may help! Curr. Biol. 23, R662–R665 (2013).

32. Stabler, P. B. et al. Sustained PU.1 levels balance cell-cycle regulators to prevent exhaustion of adult hematopoietic stem cells. Mol. Cell 49, 934–946 (2013).

33. Schroeder, S. J. Diverse activators of the NLRP3 inflammasome promote IL-1β production in rheumatoid arthritis. J. Exp. Med. 208, 273–284 (2011).

34. Rasmussen, K. IL-1 inhibits differentiation in long term bone marrow cultures. J. Immunol. 6, 1191–1197 (1988).

35. Scheper, K. et al. Myeloproliferative neoplasia remodels the endostal bone marrow niche into a self-reinforcing leukemic niche. Cell Stem Cell 13, 285–299 (2013).

36. Cullen, S. P., Kearney, C. J., Clancy, D. M. & Martin, S. J. Diverse activators of the NLRP3 inflammasome promote IL-1β secretion by triggering necrosis. Cell Rep. 11, 1535–1548 (2015).
41. Glaccum, M. B. et al. Phenotypic and functional characterization of mice that lack the type I receptor for IL-1. *J. Immunol.* **159**, 3364–3371 (1997).
42. Mendelson, A. & Frenette, P. S. Hematopoietic stem cell niche maintenance during homeostasis and regeneration. *Nat. Med.* **20**, 833–846 (2014).
43. Gekas, C. & Graf, T. CD41 expression marks myeloid-biased adult hematopoietic stem cells and increases with age. *Blood* **121**, 4463–4472 (2013).
44. Yamamoto, R. et al. Clonal analysis unveils self-renewing lineage-restricted progenitors generated directly from hematopoietic stem cells. *Cell* **154**, 1112–1126 (2013).
45. Miyawaki, K. et al. CD41 marks the initial myeloid-erythroid specification in adult mouse hematopoiesis: redefinition of murine common myeloid progenitor. *Stem Cells* **33**, 976–987 (2015).
46. Manz, M. G. & Boettcher, S. Emergency granulopoiesis. *Nat. Rev. Immunol.* **14**, 302–314 (2014).
47. Dakic, A., Wu, L. & Nutt, S. A. Is PU.1 a dosage-sensitive regulator of haemopoietic lineage commitment and leukaemogenesis? *Trends Immunol.* **28**, 108–114 (2007).
48. Adamson, J. W. The anemia of inflammation/malignancy: mechanisms and management. *Hematol. Am. Soc. Hematol. Educ. Program.* **2008**, 159–165 (2008).
49. Zhang, P. et al. PU.1 inhibits GATA-1 function and erythroid differentiation by blocking GATA-1 DNA binding. *Blood* **96**, 2641–2648 (2000).
50. Zhao, J. L. et al. Conversion of danger signals into cytokine signals by hematopoietic stem and progenitor cells for regulation of stress-induced hematopoiesis. *Cell Stem Cell* **14**, 445–459 (2014).
51. Hirai, H. et al. C/EBPβ is required for ‘emergency’ granulopoiesis. *Nat. Immunol.* **7**, 732–739 (2006).
52. Matalon, K. A., Shen, C. C., Challen, G. A. & King, K. Y. Type II interferon promotes differentiation of myeloid-biased hematopoietic stem cells. *Stem Cells* **32**, 3023–3030 (2014).
53. Sun, J. et al. Clonal dynamics of native haematopoiesis. *Nature* **514**, 322–327 (2014).
54. Busch, K. et al. Fundamental properties of unperturbed haematopoiesis from stem cells in vivo. *Nature* **518**, 542–546 (2015).
55. Haas, S. et al. Inflammation-induced emergency megakaryopoiesis driven by hematopoietic stem cell-like megakaryocyte progenitors. *Cell Stem Cell* **17**, 422–434 (2015).
Methods

Mice. Congenic wild-type C57Bl/6 mice of both genders were bred in house and used for these studies. Il1r1−/− mice and P unmistakably EYP mice (gift from C. Nerlov, Oxford University, UK) were on a pure C57Bl/6 background, and P 1.4+ mice were on a mixed C57Bl/6 background. All experiments were performed in accordance with UCSF IACUC-approved protocols.

In vivo assays. For in vivo IL-1 treatment, mice were injected intraperitoneally (i.p.) with 0.5 μg IL-1p (Peprotek) in 100 μl PBS/0.2% BSA or 100 μl PBS/0.2% BSA alone once daily for up to 70 days in primary mice and 30 days in transplanted mice. For myeloablation treatment, mice were injected once i.p. with 150 mg/kg 5-fluorouracil (5-FU, Sigma-Aldrich) in PBS. For transplantation experiments, 8–12-week-old CD45.1 C57Bl/6 mice of both genders were used. For identification of CD41FITC (eBioscience, 11-0341-85) and either SAPECy7 (eBioscience, 25-4317-82) or Flk2Bio/SAPECy7, and the second one Fc Rhodamine 680 (BioLegend, 557330) were used. For secondary transplantations, 2,500–5,000 cells per well were added to 96-well U-bottom plates, and 30% of the wells were stained with anti-biotin microbeads (Miltenyi Biotec) in accordance with UCSF IACUC-approved protocols.

Flow cytometry. BM stem and progenitor populations were analysed and/or isolated as described previously76. For cell sorting, BM cells were obtained by flushing the bone marrow cavities from femurs and tibiae of euthanized mice. Cells were filtered through a 70-μm cell strainer, centrifuged to pellet the cells, resuspended in 5 μl per cell of media, and injected into the cytometer. The flow cytometer used was a FACSAria II or III (Becton Dickenson) using double sorting, and cell analyses were performed using a FACS LSR II (Becton Dickenson). Additional antibody information, including clones and dilutions, is listed in Supplementary Table 3.

Cell culture. All cultures were performed at 37°C in a 5% CO2 water jacket incubator (Thermo Scientific). Cells were grown in StemPro34 medium (Invitrogen) supplemented with penicillin (50 U ml−1)/streptomycin (50 μg ml−1) and l-glutamine (2 mM), SCF (25 ng ml−1), FLS (25 ng ml−1), IL-11 (25 ng ml−1), IL-3 (10 ng ml−1), GM-CSF (10 ng ml−1), Epo (4 U ml−1) and Tpo (25 ng ml−1) (Peprotek). IL-1 or IL-1p (Peprotek) was added at 25 ng ml−1 and M-CSF (Peprotek) at 100 ng ml−1 where indicated. For expansion assays, cells (400 per well) were directly sorted into 96-well plates and cultured for up to 8 days. For surface marker tracking experiments, cells (2,500–5,000 per well) were directly sorted into 96-well plates and cultured for up to 8 days. Cytokines were refreshed every other day. The percentage of the total well volume and replacing with fresh media, and cultures were split as needed. For cleared caspase-3 and ATP activity assays, cells (400 per well) were directly sorted into 384-well white luminescence culture plates containing 40 μl of media. Either directly after sorting or after 12 h an equal volume of Caspase-Glo 3/7 or CellTiter Glo substrate (Promega) was added to wells and the assay was performed according to the manufacturer’s instructions. For pathway blockade assays, HSCs were cultured for 12 h ± 25 μg ml−1 IL-1p or with 5 μM of PD-0325901 (MEK inhibitor; Shanghai Chemokine Co., CAS 391210-10-9), 10 μM rottlerin (PKC inhibitor; Tocris, 1610), 10 μM SB203580 (p38 inhibitor; Millipore, 553989), 10 μM BMS-345541 (IKK inhibitor; Sigma-Aldrich, B9935) and 10 μM PP2 (Src inhibitor; Calbiochem, 592567).

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50 µm LY294002 (PI3K inhibitor; Sigma-Aldrich, 9901), and 200 µm rapamycin (mTOR inhibitor; Sigma-Aldrich, R8781). For reprogramming assays, blocking agents (100 µM 5-Fluorodeoxyuridine (5-FUDR), 200 µM Trichostatin A (TSA), 3 µM Lysinuric protein intolerance (LPI), 10 µM Chloroquine Dihydrochloride (CQ), and 10 µM 5-Fluoro-2'-deoxyuridine (FUDR)) were added to each well at the beginning of the culture period (i.e. just before the day of plating). The medium was replaced every 2 days.

Gene expression. For direct qRT–PCR analysis, $1 \times 10^6$ cells were plated in 6-well plates for 12h on 0.1% gelatin. On the next day, cells were either exposed to 1 µg/ml puromycin for 2 days or set up as described in the puromycin treatment. RNA was isolated according to the manufacturer’s instructions, treated with DNAse I (Invitrogen) and reverse-transcribed using a Superscript III kit (Invitrogen). Two hundred cell-equivalents of cDNA per sample were run in triplicate in 384-well qRT–PCR plates (Applied Biosystems) on an ABI 7900HT Fast Real-time PCR System (Applied Biosystems) using 2× Sybr Green master mix (Applied Biosystems). Ct values were normalized to Gusb and relative changes were calculated using the ΔΔCt method. For Fluidigm gene expression analyses, pools of 100 cells were directly sorted per well of a 96-well plate containing 5 µl of 2× CellsDirect reaction buffer (Invitrogen) and snap-frozen on dry ice until use. RNA was reverse-transcribed using Superscript III (Invitrogen) and subjected to 18 rounds of pre-amplification using a custom-designed set of target-specific primers as described previously^56. Pre-amplification products were subsequently treated with Exonuclease I (New England Biolabs) to remove excess primers and diluted in DNA dilution buffer (Clontech). Pre-amplified cDNAs and custom-designed primer sets were loaded onto a Fluidigm 96.96 Dynamic Array IFC and run on a BioMark System (Fluidigm) using SsoFast Sybr Green for detection (Bio-Rad). Data were analysed using Fluidigm software and normalized to Gusb expression. For analysis of Il1a and Il1b expression, pools of 100 cells were directly sorted into 2× CellsDirect reaction buffer and pre-amplified as described above, and 1 µl of pre-amplified cDNA per sample was run in triplicate in 384-well qRT–PCR plates using KAPA Fast Sybr Green Master Mix (KAPA Biosystems) on the ABI 7900HT Fast Real-time PCR System. Ct values were normalized to Actb and relative changes were calculated using the ΔΔCt method. Primer information, including sequences and NCBI gene IDs, is included in Supplementary Table 4.

Cytokine analyses. For PB serum, blood was collected from euthanized mice through cardiac puncture, allowed to coagulate at room temperature for 30 min, and subsequently spun down at 12,000 g for 10 min to remove blood cells. For BM plasma, the four long bones (two femurs and two tibiae) of the same mice were flushed with 150–200 µl HBSS/2% FBS using a 0.3 cc insulin syringe with a 28g needle and spun at 500g for 5 min to remove BM cells. Supernatants were further clarified by spinning down at 12,000g for 10 min, and samples were subsequently stored at −20°C until use. For cytokine measurement, 50 µl of 2×-diluted sample was analysed with a Luminex Cytokine Mouse 20-plex panel (Life Technologies) using a BioPlex instrument (Bio-Rad) according to the manufacturer’s instructions. M-CSF levels were analysed using an antibody sandwich ELISA kit (Raybiotech) according to the manufacturer’s instructions.

Statistics and reproducibility. All experiments were performed in triplicate and repeated as indicated. N indicates the numbers of independent experiments performed. For in vivo experiments, sample sizes were predetermined on the basis of estimation of the minimum number of animals required to obtain biologically meaningful results (at least 80% power). For other experiments no statistical method was used to predetermine sample size, and replicate experiments were performed on the basis of the variability of results obtained, as well as experiment type. Data were expressed as mean ± standard deviation (s.d.). Statistical analyses were performed using Prism 5.0 software (GraphPad). Pairwise statistical significance was evaluated by two-tailed Mann–Whitney U-test or Student’s t-test. Statistical significance between multiple groups was evaluated by one-way ANOVA with Dunnett’s or Tukey’s test for multiple comparisons. The experiments were not randomized. The investigators were not blinded to allocation during experiments and outcome assessment. P values ≤0.05 were considered statistically significant. Source data are provided in Supplementary Table 1. Exact P values and statistical tests used are shown in Supplementary Table 2.

56. Hu, Y. & Smith, K. ELDA: Extreme limiting dilution analysis for comparing depleted and enriched populations in stem cell and other assays. J. Immunol. Methods 346, 70–78 (2009).
57. Chow, A. et al. Bone marrow CD169+ macrophages promote the retention of hematopoietic stem and progenitor cells in the mesenchymal stem cell niche. J. Exp. Med. 208, 261–271 (2011).
58. Mohrin, M. et al. Hematopoietic stem cell quiescence promotes error-prone DNA repair and mutagenesis. Cell Stem Cell 7, 174–185 (2010).
59. Flach, J. et al. Replication stress is a potent driver of functional decline in ageing haematopoietic stem cells. Nature 512, 198–202 (2014).
Supplementary Figure 1 Gating strategy and effect of IL-1 on progenitor cell proliferation and survival. (a) Gating strategy and representative FACS plots of stained c-Kit-enriched wild-type (WT) BM cells. MPP2 and MPP3 are often isolated as a combined MPP2/3 population for experiments. (b) Il1r1 expression in the indicated populations. Results are dye intensity levels from microarray analyses\(^{30}\), (n = 5 MPP4, n = 4 HSC and GMP, remainder n = 3). (c) Representative expansion in liquid culture in one of two independent experiments performed in triplicate. (d) Cleaved caspase-3 (CC3) activity expressed as relative luminescence units, RLU) after 12 hours culture in one of two independent experiments performed in triplicate (e) Experimental design and quantification of BrdU incorporation after 24 hours culture in one of two independent experiments performed in triplicate. (f) Mean absolute numbers of the indicated phenotypic cell populations in HSC cultures over time in two independent experiments. Source data for b are shown in Supplementary Table 1. Data are means, and shown in b as means ± S.D. Exact P-values, number of replicates used to derive statistical data (n) and statistical tests used are shown in Supplementary Table 2.
**Supplementary Figure 2** Effect of IL-1 on progenitor cell differentiation.

(a–b) Myeloid differentiation in cultured MPP2/3, MPP4 and GMPs: (a) representative FACS plots, and (b) quantification of surface marker expression (n = 4 biological replicates/group). (c) Single cell clonogenic colony forming unit (CFU) assays in methylcellulose (n = 60 cells/group). Colonies were scored after 7 days. MkE: megakaryocyte/erythrocyte; M: macrophage; G: granulocyte; GM: granulocyte/macrophage; GMMkE: mix colony. (d) Representative colony-type (scale bar, 100 μm) and colony-morphology (scale bar, 10 μm; arrows indicate macrophages). (e) Representative FACS plots of myeloid differentiation in cultured HSCs and GMPs (n = 3 biological replicates). Source data for b are shown in Supplementary Table 1. Data in b are means ± S.D.; * p ≤ 0.05; **p ≤ 0.001. P-values in b were determined by Mann-Whitney u test. Exact P-values, number of replicates used to derive statistical data (n) and statistical tests used are shown in Supplementary Table 2.

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**Notes:**

- The image contains graphs and tables showing the effects of IL-1 on progenitor cell differentiation.
- The graphs illustrate the differentiation of MPP2/3, MPP4, and GMPs under IL-1 stimulation.
- The quantification of surface marker expression and clonogenic colony formation are shown.
- Representative FACS plots and colony morphology are provided.
- The source data for statistical analysis are provided in Supplementary Tables 1 and 2.
**SUPPLEMENTARY INFORMATION**

**Supplementary Figure 3** PU.1 activation by IL-1. (a-b) qRT-PCR analyses of myeloid lineage genes the indicated populations after 12 hours culture (n = 4 biological replicates/group for HSC, MPP4 and GMP, n = 3 for MPP2/3): (a) experimental design, and (b) expression of individual genes. Results are expressed as fold changes compared to levels in each -IL-1β population (set to 0). (c) PU.1 levels in individual PU.1-eYFP HSCs at 0 and 15 hours culture (n = 140 and 186 individual HSC/group). Results are expressed in arbitrary units (AU) with box plots showing median (lines) and 10-90th percentile (whiskers). (d) Division kinetics of PU.1-eYFP HSCs from Fig. 1e re-analysed based on pre-division PU.1lo (≤ 4 AU) and PU.1hi (> 4 AU) expression levels. (e) Experimental design, representative histograms and quantification of BrdU incorporation in HSCs after 24 hours culture (n = 3 biological replicates/group). (f) PU.1 levels in PU.1-eYFP HSCs after 12 hours culture with the indicated concentrations of IL-1β. (g) Representative histograms showing PU.1 levels in PU.1-eYFP HSCs after 12 hours culture ± IL-1β (25 pg/ml) and the indicated inhibitors. Results with II1rt1-/-:PU.1-eYFP HSCs are shown as negative controls. (h) Validation of the blocking activity of the anti-M-CSFR antibody (αMR) in BM cells cultured for 5 days ± M-CSF (M, 100 ng/ml). Source data for b and e are shown in Supplementary Table 1. Data are means ± S.D.; * p ≤ 0.05; *** p ≤ 0.001. P-values in b and e were determined by paired Student’s t-test, and in c-d by one-way ANOVA with Tukey’s test. Exact P-values, number of replicates used to derive statistical data (n) and statistical tests used are shown in Supplementary Table 2.
**Supplementary Figure 4** Gating strategies and effect of acute *in vivo* IL-1 treatment. a-c, Gating strategies with representative FACS plots for the different hematopoietic stem, progenitor and mature cell populations analysed in peripheral blood (PB) and bone marrow (BM): (a) PB of mice injected ± IL-1β for 20 days, (b) and (c) BM of mice injected ± IL-1β for 1 day. Number in each gate indicates population frequency. (d) BM cellularity and size of the indicated BM populations in mice injected ± IL-1β for 1 day (n = 15 –IL-1β and 13 +IL-1β mice/group). (e) Numbers of $HSC^{LT}$ and MPP1 in $Il1r1^{+/+}$ and $Il1r1^{-/-}$ mice in mice injected ± IL-1β for 1 day (n = 15 –IL-1β and +IL-1β $HSC^{LT}$ mice, 5 –IL-1β and 6 +IL-1β $HSC^{LT}$ mice/group). Data are means ± S.D.; *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001. P-values in d and e were determined by Mann-Whitney u-test. Exact P-values, number of replicates used to derive statistical data (n) and statistical tests used are shown in Supplementary Table 2.
Supplementary Figure 5  In vivo HSC division tracking and specificity of PU.1 activation. (a) Representative FACS plots showing CFSE dilution in donor cells following 7 days treatment of the recipient mice ± IL-1β. (b) Gating strategy for identification of HSCLT in CFSE-labelled donor cells. (c) PU.1 levels in the indicated populations from PU.1-eYFP mice injected ± IL-1β for 1 or 20 days (n = 4 –IL-1β and 5 +IL-1β mice/group for day 1; n = 5 –IL-1β and 3 +IL-1β mice/group for day 20). Source data for c are shown in Supplementary Table 1. Data are means ± S.D.; * p ≤ 0.05. P-values in c were determined by Mann-Whitney u test. Exact P-values, number of replicates used to derive statistical data (n) and statistical tests used are shown in Supplementary Table 2.
Supplementary Figure 6 Cytokine production following 5-FU myeloablation, identification of IL-1 producing cells and haematopoiesis in Il1r1−/− mice. (a) Cytokine levels in BM plasma (top) and blood serum (bottom) of 5-FU-treated mice (mice/group: n = 7 day 0-8; n=8 day 10; n=6 day 12; n=3 day 14; for M-CSF n=4 day 0-12; n=3 day 14). (b) Experimental design and gating strategy used to isolate BM stromal ECs, MSCs and OBCs, and hematopoietic granulocytes (Gr), monocytes (Mon), macrophages (Mφ), B cells and CD4+ T cells. Representative FACS plots of untreated d0 mice are shown. (c) Representative FACS plots and quantification of Ki67/DAPI cell cycle distribution in HSCs from Il1r1+/+ and Il1r1−/− mice (n = 4 Il1r1+/+ and 6 Il1r1−/− mice/group). (d) BM cellularity and size of the indicated BM populations in Il1r1+/+ and Il1r1−/− mice (n = 20 mice/group; for HSC1T and MPP1, n = 16 Il1r1+/+ and 13 Il1r1−/− mice/group) Source data for a are shown in Supplementary Table 1. Data are means ± S.D.; * p≤0.05; ** p≤0.01; *** p≤0.001. P-values in a were determined by one-way ANOVA with Dunnet’s test, and in c and d by Mann-Whitney u test. Exact P-values, number of replicates used to derive statistical data (n) and statistical tests used are shown in Supplementary Table 2.
Supplementary Figure 7 Short-term in vitro and in vivo tracking of HSCs.
(a) Representative methylcellulose colony forming-unit (CFU) and cleaved caspase-3 (CC3; expressed as relative luminescence units: (RLU) assays. Results are representative of one of two independent experiments performed in triplicate. b–c, Terminal analyses of mice transplanted with HSCs exposed ± IL-1β for 20 days in donor mice and another 30 days in recipient mice shown in Fig. 6a–d): representative FACS plots showing donor chimerism, and myeloid (My) and lymphoid (Ly) donor lineage distribution in (b) PB and (c) BM HSCs 30 days post-transplant. (d) Representative FACS plots showing the frequency of GFP+ control and PU.1-transduced HSCs prior to transplantation (d0 input) and after 30 days post-transplant in the BM (d30). Data in a are shown as means.
SUPPLEMENTARY INFORMATION

Supplementary Figure 8 Functional analysis of IL-1-exposed HSCs. a–c, Limiting dilution analysis (LDA) of unfractionated BM cells from mice injected ± IL-1β for 20 days (10^5 cell dose: n = 4 –IL-1β and 4 +IL-1β mice/group; 10^6 cell dose: n = 4/mice/group; 10^7 cell dose: n = 4 –IL-1β and 3 +IL-1β mice/group): (a) experimental design, (b) LDA graph (values: estimated HSC frequency; solid lines: dose response curve; dashed lines: upper and lower confidence intervals), and (c) engraftment results at 16 weeks post-transplant. Mice with ≥ 0.5% donor chimerism were considered engrafted. d–f, Analyses of mice injected ± IL-1β for 70 days n = 5 mice/group): (d) representative FACS plots and HSCLT number fractionated by CD41 expression (n = 4 –IL-1β and 5 +IL-1β mice/group), (e) size (means) and 95% confidence intervals) and (f) donor contribution to colonies (means and 95% confidence intervals). 

g, h, Secondary transplantation (2° txpl) of HSCs re-isolated from primary transplanted mice (1° txpl, shown in Fig. 7d–f) reconstituted with HSCLT from mice injected ± IL-1β for 70 days (n = 5 mice/group); (g) experimental design, (h) donor contribution to colonies and (i) lineage distribution in PB over time. Source data for b–d are shown in Supplementary Table 1. Data are means ± S.D. except when indicated. 

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Supplementary Table Legends

Supplementary Table 1 | Statistics source data.
Supplementary Table 2 | Exact p-values and statistical tests used in experiments.
Supplementary Table 3 | Antibodies used.
Supplementary Table 4 | Primers used for qRT-PCR and Fluidigm analysis.