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Myeloid-Specific Deletion of Mcl-1 Yields Severely Neutropenic Mice That Survive and Breed in Homozygous Form

Janka Zsófia Csepregi,*†,1 Anita Orosz,*†,1 Erik Zajta,‡ Orsolya Kása,*† Tamás Németh,*† Edina Simon,*† Szabina Fodor,§ Katalin Csonka,‡ Balázs L. Barátki,§ Dorottya Kövesdi,‡,‖ You-Wen He,‡ Attila Gácer,* and Attila Mócsai*†

Mouse strains with specific deficiency of given hematopoietic lineages provide invaluable tools for understanding blood cell function in health and disease. Whereas neutrophils are dominant leukocytes in humans and mice, there are no widely useful genetic models of neutrophil deficiency in mice. In this study, we show that myeloid-specific deletion of the Mcl-1 anti-apoptotic protein in Lys2-Cre/Cre;Mcl1floxflox (Mcl1Myelo) mice leads to dramatic reduction of circulating and tissue neutrophil counts without affecting circulating lymphocyte, monocyte, or eosinophil numbers. Surprisingly, Mcl1Myelo mice appeared normally, and their survival was mostly normal both under specific pathogen-free and conventional housing conditions. Mcl1Myelo mice were also able to breed in homozygous form, making them highly useful for in vivo experimental studies. The functional relevance of neutropenia was confirmed by the complete protection of Mcl1Myelo mice from arthritis development in the K/BxN serum-transfer model and from skin inflammation in an autoantibody-induced mouse model of epidermolysis bullosa acquisita. Mcl1Myelo mice were also highly susceptible to systemic Staphylococcus aureus or Candida albicans infection, due to defective clearance of the invading pathogens. Although neutrophil-specific deletion of Mcl-1 in MRP8-Cre;Mcl1floxflox (Mcl1ΔPMN) mice also led to severe neutropenia, those mice showed an overt wasting phenotype and strongly reduced survival and breeding, limiting their use as an experimental model of neutrophil deficiency. Taken together, our results with the Mcl1Myelo mice indicate that severe neutropenia does not abrogate the viability and fertility of mice, and they provide a useful genetic mouse model for the analysis of the role of neutrophils in health and disease. The Journal of Immunology, 2018, 201: 3793–3803.

Genetically manipulated mice lacking a certain hematopoietic lineage (1–11) have strongly contributed to our understanding of immune and inflammatory processes in health and disease. The best example is the deficiency of the recombination activating genes Rag1 or Rag2, which lack B and T lymphocytes and, therefore, are widely used to test the role of the adaptive immune response in vivo. Biological processes (10), Additional mutations result in the deficiency of B cells (2), T cell subtypes (3, 4), NK-cells (4), eosinophils (7), basophils (8), mast cells (9, 10), or certain macrophage lineages (11), allowing the analysis of those lineages in the immune and inflammatory process. The usefulness of such models is determined by the extent and selectivity of the deficiency of the given lineage as well as general characteristics, such as the survival and breeding of the mutant mice.

Neutrophils are the most abundant circulating leukocytes in humans and a predominant leukocyte population in experimental mice. Neutrophils are critically involved in the innate immune response, but they also contribute to tissue damage upon inappropriate activation of the cells (12–15). There are a number of mouse strains that show reduced numbers of neutrophils due to mutations in the genes encoding the Gfi1 transcription factor.

1J.Z.C. and A.O. contributed equally to this work.

ORCIDs: 0000-0002-8009-139X (J.Z.C.); 0000-0002-6839-964X (A.O.); 0000-0002-7493-9569 (O.K.); 0000-0001-6854-4301 (T.N.); 0000-0002-2224-0836 (D.K.).

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Address correspondence and reprint requests to Prof. Attila Mócsai, Department of Physiology, Semmelweis University School of Medicine, Tüzoló útca 37-47, Post Office Box 259, 1094 Budapest, Hungary. E-mail address: mocsai.attila@med.semmelweis-univ.hu

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Abbreviations used in this article: BHI, brain–heart infusion; CVII, type VII collagen; Mcl-1, myeloid cell leukemia 1; Mcl1Myelo, Lys2 Cre Cre Mcl1 flox/flox; Mcl1ΔPMN, MRP8-Cre;Mcl1 flox/flox; YPD, yeast extract/peptone/dextrose.

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(16–18), G-CSF (19), G-CSF receptor (20), or the Foxo3α tran-
scription factor (21). Unfortunately, all those models have sub-
stantial limitations, such as poor specificity (16, 17), partial
neutrophil deficiency, (18–21) or limited survival of the affected
animals (16, 17, 19, 20). In addition, although it is widely believed
that severe neutropenia is inconsistent with life, this has never
been appropriately tested in experimental mice.
Mcl-1 (myeloid cell leukemia 1) is an antiapoptotic member of
the Bcl-2 family protein present in various tissues (22, 23). We
have previously shown that Mcl-1 is required for the survival of
neutrophils (24), likely because these short-lived cells lack other
antiapoptotic Bcl-2 family members able to control the intrinsic
proapoptotic program of neutrophils (25). In contrast, the survival
of other myeloid cells, such as macrophages, does not rely on
Mcl-1 expression (24), likely because those cells also express
antiapoptotic proteins other than Mcl-1.

Given the critical role of Mcl-1 in neutrophil but not macrophage
survival, we hypothesized that myeloid-specific deletion of Mcl-1
would lead to selective loss of neutrophils but not of monocytes/
macrophages or nonmyeloid lineages. Indeed, Cre/lox–mediated
myeloid-specific deletion of Mcl-1 led to very severe neutropenia
without affecting other hematopoietic lineages. Surprisingly, the
survival and fertility of these mice was mostly normal, indicating
that mice are able to survive with very low circulating neutrophil
numbers. This mouse strain may be suitable for the analysis of the
role of neutrophils in various in vivo biological processes in health
and disease.

Materials and Methods

Animals
Mice carrying the Mcl1fllox/fox (Mcl1fox) floxed allele of the Mcl-1–
encoding gene (24) were crossed to mice carrying the LysMcre (Lys2);
also known as Ly6M-Cre) knock-in strain expressing the Cre recom-
binease in the entire myeloid compartment (26) to generate Lys2crecreMcl1fllox/fox
mutants (referred to as Mcl1Δmyelo mice). The mutations were mostly
maintained by breeding Mcl1Δmyelo mice with Ly52crecreMcl1fllox/fox mice, yielding
Mcl1Δmyelo homozygous animals and Ly52crecreMcl1fllox/fox litter-
gates. Several other breeding strategies (including breeding in the
Mcl1Δmyelo homozygous form) were also used (see Results). To generate a
more neutrophil-specific Mcl-1 deletion, Mcl1flfox mice were crossed to
MRP8-Cre transgenic animals (27) to generate MRP8-CreMcl1fox/fox
(mutants (referred to as Mcl1ΔPMN mice). G-CSF receptor–deficient (20)
(Csf3r−/−;Mim/Link; Csf3r−/−) mice were purchased from The Jackson
Lab. The genotype of all mice was tested by allele-specific PCR.

Mice were kept in individually sterile ventilated cages (Tecniplast), either
in a specific pathogen-free facility or an adjacent conventional facility. The
animals were obtained from our breeding colony.

All mice were on the C57BL/6 genetic background. Control C57BL/6
Laboratory. The genotype of all mice was tested by allele-specific PCR.

The following Abs (all from BD Biosciences, except 7/4 from Abcam
and IgM from Jackson Immonoresearch) were used for flow cytometry:

- CD45 (30-F11), CD11b (M1/70), CD11c/B220 (RA3-6B2), CD45.2 (104),
- Ly6C (AL-21), Ly6G (1A8), Siglec-F (E50-2440), Gr1 (RB6-8C5), 7/4
(ab53453), c-Kit (2B8), B20 (RA3-6B2), IgM (polyclonal, catalog num.
115-606-020), IgD (11-26.c2a), CD21 (7gb), and CD23 (B38).

Abs

The following Abs (all from BD Biosciences, except 7/4 from Abcam
and IgM from Jackson Immonoresearch) were used for flow cytometry:

- CD45.1 (70B4), CD11b (M1/70), CD11c/B220 (RA3-6B2), CD45.2 (104),
- Ly6C (AL-21), Ly6G (1A8), Siglec-F (E50-2440), Gr1 (RB6-8C5), 7/4
(ab53453), c-Kit (2B8), B20 (RA3-6B2), IgM (polyclonal, catalog num.
115-606-020), IgD (11-26.c2a), CD21 (7gb), and CD23 (B38).

A GENETIC MODEL OF NEUTROPHIL DEFICIENCY

Cell preparation, flow cytometry, and cytoxin

Blood samples were obtained from tail vein incisions, washed, stained, and
then resuspended in BD Biosciences FACSCalibur lysing solution. Bone marrow
and spleen cell samples were obtained by flushing the bone marrow or
flushing the spleen through a 70-μm cell strainer, followed by RBC lysis
buffer. LysM-Cre transgenic RBC Lysis Buffer, staining, and resuspension in PBS
containing 5% FBS. Samples were kept at 4°C during the entire procedure.
Specified volumes were used throughout, allowing a precise determination
of absolute cell counts.

Flow cytometry was performed using a BD Biosciences FACS Calibur
and analyzed by FCS Express 6 (De Novo Software). The different leuko-
cyte populations were identified within their typical forward and side
scatter profiles as follows: neutrophils as CD11b+Ly6C–Siglec-F–, mono-
cytes as CD11b+Ly6G+ Siglec-F–, eosinophils as Ly6G+ Siglec-F–, T cells
as CD3+, and B cells as B220+ cells. Blood monocyte subpopulations were
differentiated by Ly6C staining.

For cytoxin assays, bone marrow cells were obtained by flushing the
bone marrow, followed by RBC lysis with eBioscience RBC Lysis Buffer.
Cell counts were adjusted and cytospinned onto SuperFrost slides (Thermo
Fisher Scientific) for 5 min at room temperature using Shandon Cytospin 3
Cytocentrifuge cytoxin equipment. After drying, slides were stained with
the May–Grünewald method and analyzed by a Leica DMi6000b inverted
microscope.

In vitro culture and PCR analysis of macrophages

Bone marrow cells were obtained by flushing the bone marrow. Cells were
washed and resuspended in α-MEM supplemented with 10% FBS, 1%
penicillin/streptomycin, 10 mM HEPES (pH 7.4), 1% γ-glutamine, and
10 ng/ml recombinant murine M-CSF. Cells were plated on tissue culture–
treated plates and cultured for 3 d in a humidified CO2 incubator. Cells in
suspending were then collected, centrifuged, and resuspended in the above-
mentioned medium containing 40 ng/ml recombinant murine M-CSF. Four
days later, adherent cells were collected and prepared for flow cytometry
using the F4/80 marker or isolation of genomic DNA. For Mcl1 genomic
PCR analysis, the 5’-GTT CTG TCT CCT ACT TTA CTG TAF-3’
forward primer was used along with the 5’-TCT ACA ACT GGC AAG TCT-3’
reverse primer (Mcl1Δα; ~ 600 bp product length) and the 5’-CTC CTA ACC ACT GGT CAC ATC C-3’ reverse primer
(Mcl1WT or Mcl1fox allele; ~ 260- and 380-bp product length, respec-
tively). For Ilb2 (CD18) PCR analysis, the 5’-GCC CAC ACT CAT TGC
TGG TTG-3’ forward primer was used along with the 5’-CCC GGC AAC
TGC TGA CTT TGT-3’ reverse primer (Ilb2Δα allele; ~ 480-bp product
length).

Thioglycolate-induced peritonitis

Peritonitis was induced by i.p. injection of 1 ml 3% thioglycolate
(Loilöfich) or PBS. After 4 h, mice were euthanized, and the peritoneum
was flushed by 5 ml ice-cold PBS containing 5% FBS. The lavage samples
were washed, resuspended in PBS containing 5% FBS, and maintained at
4°C until staining for flow cytometry.

Survival and fertility

An online database (specific pathogen-free facility) and hand-written re-
cords (conventional facility) were used for the analysis of the survival,
fertility, and breeding behavior of our mice. Data were analyzed using a
custom-made software. Body weight of a smaller cohort was measured once
weekly from the age of 2 wk.

KB×N serum transfer arthritis

Serum from KRN transgene-positive (arthritic) KB×N mice was obtained as described previously
(28, 29). Arthritis was induced by i.p. injection of 300 μl KB×N (ar-
thritic) or KB×N (control) serum, followed by daily scoring of clinical signs
of arthritis and measurement of ankle thickness for 2 wk as described
previously (29–32).

Autoantibody-induced skin blistering model

The murine model of human epidermolysis bullosa acquista was triggered by
systemic administration of rabbit polyclonal Abs against type VII
collagen (CVII) as described previously (31–34). Twelve milligrams of
pathogenic IgG in PBS per mouse or PBS alone was injected s.c. under
isoflurane anesthesia every second day between 0 and 8 d (60 mg total IgG
per mouse). The disease onset and progression were followed by clinical
assessment every second day as described previously (31, 32, 34).
In vivo infection models

Staphylococcus aureus strain ATCC25923 and Candida albicans strain SC5314 originated from the Szeged Microbial Collection (World Federation of Culture Collections no. 987).

*S. aureus* was maintained on brain–heart infusion (BHI) agar and grown overnight at 37°C in liquid BHI medium prior to experiments. Mice were infected i.p. with 2 × 10^7 or 1 × 10^7 *S. aureus* bacteria in 100 µl PBS per mouse for survival assays and bacterial burden assessment, respectively.

*C. albicans* was maintained on yeast extract-peptone/dextrose (YPD) agar and grown overnight at 30°C in liquid YPD medium prior to experiments. Mice were infected i.v. through the tail vein with 1 × 10^7 yeast cells in 100 µl PBS per mouse.

Bacterial and fungal burdens were determined by a conventional CFU counting method 12 h post infection. Kidneys, spleens, livers, and brains were plated in serial dilutions on BHI or YPD agar plates and incubated for 1 d at 37°C.

Presentation of data and statistical analysis

Experiments were performed the indicated number of times. Bar graphs and kinetic curves show mean and SEM of all mice or samples from the indicated number of independent experiments. Tissue cell numbers were calculated for the entire spleen, the entire peritoneum, or the bone marrow of both femurs and both humeri combined. Statistical analysis was performed with StatSoft Statistica software. The analysis of blood, bone marrow, and splenic leukocyte populations and bacterial or fungal CFU counts was performed by Student t test. Peritonitis, arthritis, and dermatitis experiments were analyzed by two-way factorial ANOVA. A Mann–Whitney U test was used to analyze the body-weight curves. Survival studies were analyzed by the Kaplan–Meier method and logrank statistics. A p value < 0.05 was considered statistically significant.

Results

Myeloid-specific deletion of *Mcl-1* leads to severe neutropenia

To test the effect of myeloid-specific deletion of *Mcl-1*, we have generated *Mcl1^ΔMyelo* mice, which leads to Cre-mediated deletion of *Mcl1* in the myeloid compartment. Control mice included wild type C57BL/6 animals, *Ly6G^Cre/Cre* or *Mcl1^flox/flox* single-gene mutants, or *Ly6G^Cre/Cre*Mcl1^flox/+* littermate controls.

Whereas the peripheral blood of wild type animals contained a clear population of neutrophils (Ly6G+ cells with intermediate forward scatter and high side scatter characteristics), this population was not reduced as much in *Mcl1^ΔMyelo* mice (Fig. 1A, 1B). This was in line with our previously reported experiments with these animals (24, 35). No signs of neutropenia were observed in mice carrying mutations only in the *Lyz2* or *Mcl1* gene (Supplemental Fig. 1A). Severe neutropenia was also confirmed by staining peripheral blood neutrophils using the 7/4 or RB6-8C5 (Gr1) markers (Supplemental Fig. 1C, 1D).

Specificity of the effect of the *Mcl1^ΔMyelo* mutation

We next tested the effect of the *Mcl1^ΔMyelo* mutation on other leukocyte lineages. As shown in Fig. 1D and 1E, circulating neutrophil count in the peripheral blood of *Mcl1^ΔMyelo* mice was reduced by 98.1% relative to wild type animals (*p* = 0.73) and a moderate although statistically significant reduction of Ly6C+ monocyte counts (*p* = 0.0039). No substantial differences in those lineages were observed when only the *Lyz2* or *Mcl1* genes were mutated (Supplemental Fig. 1A, 1B). No changes in RBC count or blood hemoglobin concentration was observed in *Mcl1^ΔMyelo* mice either (data not shown).

Analysis of tissue leukocytes and in vitro–differentiated macrophages

We next tested the effect of the *Mcl1^ΔMyelo* mutation on tissue leukocyte numbers. As shown in Fig. 2A, the number of Ly6G+ neutrophils in the bone marrow was strongly reduced in the *Mcl1^ΔMyelo* animals (96% reduction; *p* = 1.1 × 10^-4). This is also reflected in the strong reduction of the number of cells with neutrophil-like donut-shaped nuclear morphology in cytopsin preparations of bone marrow cells (Supplemental Fig. 2A). More detailed analysis of Ly6G expression (Supplemental Fig. 2B) in the bone marrow has revealed that although the Ly6G^high population was practically absent in *Mcl1^ΔMyelo* mice the Ly6G^med/low* populations were not reduced, suggesting that the *Mcl1^ΔMyelo* mutation does not eradicate the myeloid progenitor or early neutrophil lineage cell compartment.

In contrast to neutrophils, no reduction of monocytes or T cells could be observed in *Mcl1^ΔMyelo* mice (Fig. 2B; *p* = 0.20 and 0.48, respectively). However, the number of bone marrow B cells was clearly reduced (*p* = 4.0 × 10^-4), despite the fact that circulating B cell numbers were not affected (compare Figs. 1E, 2B). Further analysis of the B cell compartment revealed that this reduction affected all tested B cell populations (proB/preB1, immature, and recirculating B cells; Supplemental Fig. 2C). The fact that even the recirculating B cell counts were reduced despite normal circulating (Fig. 1D, 1E) and splenic (Supplemental Fig. 2D) B cell numbers suggests that the reduced bone marrow B cell counts are likely due to a disturbed bone marrow B cell niche (rather than an intrinsic B cell defect) and that this bone marrow phenotype is well compensated in the periphery. Finally, the analysis of bone marrow macrophages and dendritic cells did not reveal any difference between wild type and *Mcl1^ΔMyelo* mice either (Supplemental Fig. 2E).

We have also tested various splenic leukocyte populations. As shown in Fig. 2C, splenic neutrophil numbers were strongly reduced in *Mcl1^ΔMyelo* animals (93% reduction; *p* = 1.5 × 10^-7). However, as shown in Fig. 2D, the number of splenic T or B cells was not affected (*p* = 0.77 and 0.092, respectively). Further analysis of splenic B cells (Supplemental Fig. 2D) also failed to reveal a defect in any of the splenic B cell populations tested. Additional studies on splenic macrophages and dendritic cells failed to reveal any reduction in their numbers in *Mcl1^ΔMyelo* mice (Supplemental Fig. 2F). However, the number of splenic macrophages was significantly increased in *Mcl1^ΔMyelo* animals (Supplemental Fig. 2F), which correlated with the size of the spleen in those mice (i.e., the difference disappeared after normalization for the weight of the spleen). Therefore, we believe that the increased macrophage number is related to splenomegaly in those mice (see below), reflecting the fact that macrophages represent one of the predominant cell types in this organ.

The number of tissue neutrophils under inflammatory conditions was assessed in thioglycolate-induced peritonitis. As shown in Fig. 2E, thioglycolate injection triggered a robust neutrophil infiltration in wild type animals, whereas no such infiltration could be observed in *Mcl1^ΔMyelo* mice (97% reduction; *p* = 1.3 × 10^-4). Therefore, the severe neutrophil deficiency in *Mcl1^ΔMyelo* mice is also evident under inflammatory conditions.

We have also tested the in vitro differentiation of macrophages from *Mcl1^ΔMyelo* bone marrow cells. We did not observe any difference between the number of bone marrow–derived macrophages generated from wild type or *Mcl1^ΔMyelo* bone marrow cells.
Supplemental Fig. 2G), and the morphology and F4/80 expression profile was also similar between those genotypes (data not shown). In contrast, PCR analysis of genomic DNA confirmed effective deletion of the Mcl1 flox allele in bone marrow–derived macrophage cultures, whereas only a marginal deletion (likely because of the presence of tissue macrophages or osteoclasts) was seen in tail biopsy samples (Supplemental Fig. 2H; see further explanation in the figure legend). Those results indicate that Mcl1 deletion does not affect the proliferation, differentiation, or overall morphology of macrophages.

Survival of Mcl1ΔMyelo mice

Although it is generally believed that severe neutropenia is inconsistent with life, this has never been tested in mice, in part because of the limitations of currently existing neutropenic mouse models (16–20). Therefore, we tested the survival of the Mcl1ΔMyelo mice during a prolonged period of time.

Surprisingly, and in contrast to our previous assumptions, the survival of Mcl1ΔMyelo mice under specific pathogen-free conditions was not dramatically different from that of wild type animals (Fig. 3A). Although there was a moderate reduction of the survival of Mcl1ΔMyelo mice compared with wild type animals (84% versus 92% at 6 mo and 66% versus 78% at 12 mo of age, respectively) and this was statistically highly significant (p < 0.00001) due to the very large number of mice tested (>600 per genotype), this difference was not at all dramatic, especially at the early age range when most animal experiments are performed.

The effect of the Mcl1ΔMyelo mutation under more real-world conditions was tested on a smaller cohort of mice in a conventional animal facility (Fig. 3B). Importantly, the survival of Mcl1ΔMyelo animals was again only slightly below that of the wild type mice (88 and 93% at 6 mo of age, respectively; p = 0.032), indicating that the survival of Mcl1ΔMyelo mice is not dramatically affected even under conventional conditions.

We did not see any substantial difference between the general appearance or behavior of wild type and Mcl1ΔMyelo mice (data not shown). Body weight measurements revealed a slight reduction in Mcl1ΔMyelo mice (Fig. 3C, 3D; p = 0.22 and 2.0 × 10⁻⁶ for males and females, respectively). The only consistent difference found during dissection was splenomegaly in Mcl1ΔMyelo animals, which...
Neutrophils are the major players in the host defense against skin and respiratory tract infection, abscess formation, and S. aureus infections by neutrophil-mediated autoantibody-induced inflammation models.

Mice were first subjected to K/B×N serum-transfer arthritis, an autoantibody-induced in vivo arthritis model (36, 37) previously suggested to be mediated by neutrophils (38, 39). As shown in Fig. 4A, K/B×N serum injection triggered robust arthritis in wild type mice, whereas McI1^ΔMyelo mutants appeared to be completely protected. Kinetic analysis of clinical score (Fig. 4B; p = 4.2 × 10^{-5}) and ankle thickness (Fig. 4C; p = 0.0059) has confirmed those findings. The protection of McI1^ΔMyelo mice was not due to deletion of LysM by the Lyz2^Cre/Cre knock-in mutation because K/B×N serum-transfer arthritis developed normally in Lyz2^Cre/Cre mice (Supplemental Fig. 3).

Neutrophils have been proposed to be critical for the development of anti-CVII Ab–induced dermatitis, a mouse model of the rare human-blistering skin disease epidermolysis bullosa acquisita (33, 40, 41). Anti-CVII Abs triggered severe skin inflammation in wild type mice, whereas no signs of the disease could be observed in McI1^ΔMyelo animals (Fig. 4D). Kinetic analysis revealed that McI1^ΔMyelo mice were completely protected from skin inflammation, both in terms of the affected body surface (Fig. 4E; p = 3.8 × 10^{-11}) and of a more elaborate clinical scoring system (Fig. 4F; p = 3.9 × 10^{-10}).

Taken together, our results indicate that McI1^ΔMyelo mice are completely protected from two separate, neutrophil-mediated autoantibody-induced inflammation models.

**Increased susceptibility to bacterial and fungal infection**

Although McI1^ΔMyelo mice resisted the microbial burden of their commensal flora (Fig. 3), we wanted to test their susceptibility to experimentally induced infections. Therefore, we subjected our mice to systemic S. aureus or C. albicans infection.

Neutrophils are the major players in the host defense against infections by S. aureus, a Gram-positive pathogen able to cause skin and respiratory tract infection, abscess formation, and bacteremia/sepsis (42, 43). As shown in Fig. 5A, whereas wild type animals survived i.p. infection with 2 × 10^7 S. aureus, more
than 80% of McI1\textsuperscript{\textsc{DMyelo}} mice succumbed to the same infectious challenge \((p = 1.0 \times 10^{-5})\). Analysis of the bacterial burden 12 h after the infection with \(1 \times 10^7\) bacteria revealed a more than 100-fold increase of bacterial colony counts in the spleen \((p = 0.0015)\), kidneys \((p = 0.023)\), and liver \((p = 9.0 \times 10^{-2})\) and significant increases in the brain \((p = 0.028)\) and in the blood \((p = 0.0038)\) but not in the peritoneum \((p = 0.098)\) of \(\text{McI1}\textsuperscript{\textsc{DMyelo}}\) mice (Fig. 5B, 5C).

Neutrophils are among the critical immune cells protecting the host from infection by \textit{C. albicans}, a fungal pathogen able to cause superficial or systemic infections and one of the most prevalent causes of hospital-acquired infections (44). As shown in Fig. 5D, i.v. infection with \(10^5\) \textit{C. albicans} caused lethality in 27% of wild type animals, whereas the same infection caused rapid lethality in 95% of \(\text{McI1}\textsuperscript{\textsc{DMyelo}}\) mice \((p < 0.0001)\). Analysis of the fungal burden at 12 h revealed a more than 10-fold increase in fungal counts in the liver \((p = 1.0 \times 10^{-4})\) of \(\text{McI1}\textsuperscript{\textsc{DMyelo}}\) mice, with moderate increase also in the spleen \((p = 0.0096)\) and in the kidneys \((p = 5.2 \times 10^{-7})\) but not in the brain \((p = 0.97)\) of the animals (Fig. 5E).

Taken together, \(\text{McI1}\textsuperscript{\textsc{DMyelo}}\) mice are highly susceptible to infectious challenge by bacterial or fungal pathogens, such as \textit{S. aureus} or \textit{C. albicans}, likely because of defective neutrophil-mediated elimination of the pathogens.

\section*{Analysis of McI1\textsuperscript{\textsc{DMyelo}} bone marrow chimeras}

It is often difficult to obtain larger homogeneous cohorts of mice for in vivo experiments from small breeding colonies. When studying neutrophil function, this problem may be overcome by transplanting bone marrow cells to larger cohorts of recipient mice. To test that possibility, we transplanted wild type or \(\text{McI1}\textsuperscript{\textsc{DMyelo}}\) bone marrow cells into lethally irradiated wild type recipients carrying the CD45.1 allele. As shown in Fig. 6A, circulating neutrophil numbers of \(\text{McI1}\textsuperscript{\textsc{DMyelo}}\) bone marrow chimeras was strongly reduced compared with parallel-generated wild type chimeras (98.3% reduction; \(p = 1.8 \times 10^{-14}\)).
marrow chimeras were also completely protected from K/B\(3\) serum-transfer arthritis, both in terms of clinical score (\(p = 2.1 \times 10^{-5}\); Fig. 6B) and ankle thickness changes (\(p = 2.2 \times 10^{-6}\); Fig. 6C). Therefore, bone marrow transplantation can be used to generate larger cohorts of mice with neutropenia caused by the \(Mcl1\)\(^{D}\)Myelo mutation.

**Neutrophil-specific deletion of \(Mcl-1\) leads to neutropenia with severe survival defects**

The above experiments were performed using \(Mcl1\)\(^{D}\)Myelo mice in which \(Mcl-1\) was deleted from the entire myeloid compartment. To test the effect of \(Mcl-1\) deletion in a more neutrophil-specific manner, we have crossed the \(Mcl1\)\(^{flox/flox}\) mice to mice carrying the MRP8-Cre transgene, which drives Cre expression specifically in the neutrophil compartment (45).

\(Mcl1\)\(^{D}\)PMN mice showed dramatic (99.1%, \(p = 9.8 \times 10^{-12}\)) reduction of circulating neutrophil counts (Fig. 7A, 7B) that was even more severe than the reduction seen in \(Mcl1\)\(^{D}\)Myelo animals (98.1%; see Fig. 1). The \(Mcl1\)\(^{D}\)PMN mutation did not affect circulating monocyte (\(p = 0.60\)), eosinophil (\(p = 0.99\)), B cell (\(p = 0.21\)), or T cell (\(p = 0.58\)) numbers (Fig. 7C, 7D) or the distribution of monocytes into Ly6C\(^{+}\) (inflammatory) or Ly6C\(^{-}\) (patrolling) monocytes (\(p = 0.24\) and 0.26, respectively; Fig. 7E, 7F). Therefore, similar to the \(Mcl1\)\(^{D}\)Myelo mutation, the \(Mcl1\)\(^{D}\)PMN mutation also leads to severe and selective neutropenia.

Analysis of the survival of \(Mcl1\)\(^{D}\)PMN mice (Fig. 7G) revealed a steady and substantial loss of \(Mcl1\)\(^{D}\)PMN animals, leading to 58% survival at 6 mo and only 30% survival at 12 mo of age (\(p = 0.000001\)). \(Mcl1\)\(^{D}\)PMN mice were also clearly distinguishable from their wild type littermates and often showed a severe wasting phenotype (data not shown). \(Mcl1\)\(^{D}\)PMN mice also showed a very poor breeding productivity (Fig. 7H). Taken together, our data suggest that the limited survival and breeding capacity makes \(Mcl1\)\(^{D}\)PMN mice rather difficult to maintain. This is further complicated by the fact that such poor breeders need to be maintained in heterozygous form; therefore, all offspring need to be individually genotyped, and only a fraction of the pups (25% in the most sensible MRP8-Cre\(Mcl1\)\(^{flox/+}\) \(Mcl1\)\(^{flox/flox}\) breeding strategy) are expected to be of the desired \(Mcl1\)\(^{D}\)PMN genotype.

Bone marrow transplantation experiments revealed that \(Mcl1\)\(^{D}\)PMN bone marrow chimeras also showed a severe wasting phenotype and succumbed to death 3–8 wk after transplantation (data not shown). Although initial results indicated complete protection of \(Mcl1\)\(^{D}\)PMN mice from K/B\(X\)N serum-transfer arthritis, the limited availability and fragile health status of those mice did not allow us to complete a sufficient number of those experiments (data not shown). The same issue also prevented us from performing more detailed analysis of the tissue leukocyte populations in \(Mcl1\)\(^{D}\)PMN mice. Nevertheless, it is interesting to note that in contrast to \(Mcl1\)\(^{D}\)Myelo mice the few \(Mcl1\)\(^{D}\)PMN animals we were able to dissect did not show an overt splenomegaly phenotype (data not shown).

**FIGURE 4.** Autoantibody-induced arthritis and skin-blistering disease in \(Mcl1\)\(^{D}\)Myelo mice. (A–C) Wild type (WT) or \(Mcl1\)\(^{D}\)Myelo mice were injected with control (B\(X\)N) or arthritic (K/B\(X\)N) serum on day 0. Arthritis development was followed by photographing on day 7 (A), clinical scoring of the hind limbs (B) and ankle thickness measurement (C). (D–F) Skin-blistering disease was triggered in wild type (WT) or \(Mcl1\)\(^{D}\)Myelo mice by systemic injection of control IgG or CVII-specific (anti-CVII) Abs. Skin disease was followed by photographing on day 14 (D) and clinical assessment of the total body surface affected (E) and the overall disease severity (F). Images are representative of and quantitative data show mean and SEM from five to nine control and 9 to 15 arthritic serum–treated individual mice per group from three independent experiments (A–C), or from three to four control and three to four anti-CVII-treated mice per genotype from two independent experiments (D–F).
A Survival curves show the data of 16 (as a neutropenia model. Csf3r (20) as a reference neutropenic mouse strain. Our results also indicate that mice are able to survive analyzing the role of neutrophils in in vivo processes in health and homozygous form. Those mice may, therefore, be highly useful in the RB6-8C5 or NIMP-R14 anti-Gr1 or the 1A8 anti–Ly6G Abs) showed only partial neutropenia (Fig. 4A, 4B), which was not nearly as severe and consistent as in Mcl1−/− mice following i.p. injection with 2 × 10^6 (A) or 10^7 (B and C) S. aureus bacteria. (D and E) Survival curves (D) or analysis of the fungal burden from the indicated tissues (E) of WT and Mcl1ΔMyclo mice following i.v. injection with 10^7 C. albicans. Survival curves show the data of 16 (A) or 19–22 (D) mice per group from three independent experiments. Bar graphs show mean and SEM from 9 to 10 (B and C) or 10 to 11 (E) mice per group from three (B and C) or four (E) independent experiments.

Taken together, our results indicate that although the Mcl1ΔPMN mutation leads to severe and specific neutropenia, the poor and fragile health status, limited survival and fertility, and non-homozogous nature of those animals makes them hardly suitable for larger-scale in vivo experiments.

Partial neutropenia in G-CSF receptor–deficient mice
We have also tested G-CSF receptor–deficient (Csf3r−/−) mice (20) as a reference neutrophilic mouse strain. Csf3r−/− mice showed only partial neutropenia (p = 1.1 × 10^-4; Supplemental Fig. 4A, 4B), which was not nearly as severe and consistent as in Mcl1ΔMyclo or Mcl1ΔPMN animals (Figs. 1, 7). Similar to the Mcl1ΔMyclo and Mcl1ΔPMN mutations, the Csf3r−/− mutation did not affect other circulating leukocyte populations either (Supplemental Fig. 4C, 4D). Although the survival of Csf3r−/− mice was not substantially reduced, we also had difficulties breeding Csf3r−/− mice in homozygous form (data not shown). Those results indicate severe limitations of the Csf3r−/− mutation as a neutropenia model.

Discussion
Our results indicate that Mcl1ΔMyclo mice lacking Mcl-1 in the myeloid lineage are severely neutropenic but survive and breed in homozygous form. Those mice may, therefore, be highly useful in analyzing the role of neutrophils in in vivo processes in health and disease. Our results also indicate that mice are able to survive almost normally when the circulating neutrophil numbers are reduced to <2% of their normal values, necessitating the re-evaluation of the role of neutrophils in rodent survival.

Currently available tools for reducing neutrophil numbers have substantial limitations. Although Ab-mediated depletion (e.g., by the RB6-8C5 or NIMP-R14 anti-Gr1 or the IA8 anti-Ly6G Abs) has clear benefits, such as easy availability and suitability to be used on transgenic strains without breeding delay, it suffers from limited specificity (especially when using anti-Gr1 Abs), very high reagent costs, and the temporary nature of the depletion. Prior reports of neutropenic mice (16–21) also revealed phenotypes that strongly limit their use as in vivo neutropenia models. Besides severe neutropenia, Gf1-deficient mice also show various defects in the T and B cell compartment and have a median survival time of ~8–10 wk (16, 17), in line with the severe neutropenia and lymphocyte defects caused by dominant negative GFI1 mutations in human patients (46). The so-called “Genista” mice carrying a chemically induced Gfi1 mutation show incomplete neutrophil deficiency and are only partially protected in a neutrophil-dependent in vivo inflammation model (18). Mice lacking G-CSF (19) or the G-CSF receptor (20) are only moderately neutropenic (see also Supplemental Fig. 4), and the latter strain also shows breeding defects (data not shown). Deficiency of the Foxo3A transcription factor causes accelerated neutrophil apoptosis at the site of inflammation but does not affect circulating neutrophil numbers (21). In contrast to those genetic and pharmacological models, the Mcl1ΔMyclo mice show consistent, severe, and fairly specific neutropenia and survive and breed in homozygous form, making them quite useful as an in vivo neutropenia model.

The specificity of reduced neutrophil numbers in Mcl1ΔMyclo mice is due to two factors: the deletion of the antiapoptotic Mcl-1 protein in the entire myeloid lineage (including macrophages) and the specific requirement for Mcl-1 for the survival of neutrophils but not of the cells of the monocyte/macrophage lineage (24, 47). This is also indicated by the normal number and overall appearance of macrophages differentiated from Mcl1ΔMyclo bone marrow cells, despite effective deletion of the Mcl1lox allele (Supplemental Figs. 2G, 2H). We have also tested Mcl1ΔPMN mice in which Mcl1 deletion is achieved by the MRP8-Cre transgene, which is more specific for neutrophils than the Lyz2Cre knock-in
mutant (45). Although the Mcl1ΔPMN mutations also strongly reduced circulating neutrophil counts and appeared to be specific over several other leukocyte lineages (Fig. 7), the limited survival and poor breeding of those mice make them very difficult to use as an in vivo neutropenia model. Although it is at present unclear why the Mcl1ΔMyelo and Mcl1ΔPMN mice have different survival and breeding characteristics, one of the possible explanations is that the remaining ~2% of neutrophils in Mcl1ΔMyelo mice is sufficient to control the commensal flora, whereas the ~1% of remaining neutrophils in the Mcl1ΔPMN mutants is below the threshold of neutrophil levels required for normal survival. It would theoretically also be possible that the survival of the Mcl1ΔMyelo mice is due to some genetic drift in our mouse colony, although our heterozygous breeding strategy argues against that possibility. Understanding the exact reason for the different survival of Mcl1ΔMyelo and Mcl1ΔPMN mice would require substantial additional experiments, including detailed apoptosis and in vitro progenitor differentiation/proliferation assays.

Although the Mcl1ΔMyelo mutation causes severe neutropenia both in the peripheral blood and in various tissues (Figs. 1, 2), it is at present not entirely clear at which stage the mutation interferes with neutrophil development and/or survival. The fact that the number of Ly6Gmed/dim cells in the bone marrow is not reduced (Supplemental Fig. 2B) suggests that the Mcl1ΔMyelo mutation affects cells in the latest stage of neutrophil development. This is also supported by the fact that HoxB8-transduced myeloid progenitors were unable to engraft the bone marrow of Mcl1ΔMyelo mice (A.O. and A.M., unpublished observations), suggesting that the myeloid progenitor niche is preoccupied by endogenous cells in those animals.

Although the Mcl1ΔMyelo mutation proved to be fairly specific for neutrophils, we have consistently observed reduced B lineage cell numbers in the bone marrow of Mcl1ΔMyelo mice (Fig. 2B). The relevance of this finding is at present unclear, especially given the normal circulating (Fig. 1E) and splenic (Fig. 2D) B cell counts. More detailed analysis of the B cell compartment (Supplemental Fig. 2C, 2D) has revealed that even recirculating B cell numbers are reduced in the bone marrow of Mcl1ΔMyelo animals, suggesting disturbance of the bone marrow B cell niche. Alternatively, this observation may be due to the expression of LysM in the early B cell lineage as indicated by the ImmGen database (www.immgen.org). It should also be noted that more splenic macrophages (Supplemental Fig. 2F) were observed in Mcl1ΔMyelo mice, which likely reflects splenomegaly in those animals.

To our knowledge, this is the first detailed characterization and validation of the Mcl1ΔMyelo mice as a suitable experimental neutropenia model. In particular, our study provides the most detailed lineage analysis of those animals, reports large-scale assessment of their survival and fertility, and validates the mutant mice on known neutrophil-dependent in vivo inflammation and infection models. To our knowledge, we also provide the first detailed analysis of Mcl1ΔPMN mice and a side-by-side comparison of the Mcl1ΔMyelo, Mcl1ΔPMN, and Csf3r−/− mutants. It should, nevertheless, be noted that we have already used the Mcl1ΔMyelo model in the recent past to test the role of neutrophils in various disease models, such as graft-versus-host disease (48), contact hypersensitivity (35), gout (49), and experimental lupus (50). All those reports and further ongoing studies have confirmed the usefulness of this model for the in vivo analysis of neutrophil function.

Taken together, our results indicate that the unique combination of severe and fairly specific neutropenia, mostly normal survival, and capability for breeding in homozygous form make the Mcl1ΔMyelo mutation highly suitable for the analysis of the role...
of neutrophils in in vivo models of normal and pathological processes in experimental mice. Our results also indicate that rodents are able to survive and breed when their circulating neutrophil counts are dramatically reduced.

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Disclosures
The authors have no financial conflicts of interest.

References
1. Spanopoulou, E. 1996. Cellular and molecular analysis of lymphoid development using Rag-deficient mice. Int. Rev. Immunol. 13: 257–288.
2. Kitamura, D., J. Roers, R. Kühn, and K. Rajewsky. 1991. A B cell-deficient mouse by targeted disruption of the membrane exon of the immunoglobulin μ chain gene. Nature 350: 423–426.
3. Mombaerts, P., A. R. Clarke, M. A. Radnicki, J. Iacomini, S. Itohara, J. J. Lafaille, L. Wang, Y. Ichikawa, R. Jaenisch, M. L. Hooper, et al. 1992. Mutations in T-cell antigen receptor genes α and β block thymocyte development at different stages. Nature 356: 228–231.
4. Itohara, S., P. Mombaerts, J. Lafaille, J. Iacomini, A. Nelson, A. R. Clarke, M. L. Hooper, A. Farr, and S. Tonegawa. 1993. T cell receptor δ gene mutant

FIGURE 7. Neutrophil-specific deletion of Mcl-1 leads to neutrophil deficiency with survival and breeding defects. (A) Flow cytometric histograms of Ly6G staining of wild type (WT) and Mcl1ΔPMN mouse peripheral blood leukocytes. (B) Quantitative analysis of the number of mature neutrophils (CD11b+Ly6G+Siglec-F cells) in WT and Mcl1ΔPMN mice. Flow cytometric profiles (C) and quantitative analysis (D) of other leukocyte populations (red, neutrophils; green, monocytes; blue, eosinophils; magenta, B cells; orange, T cells). Flow cytometric histograms (E) and quantitative analysis (F) of monocyte subpopulations. (G) Survival of WT and Mcl1ΔPMN mice under specific pathogen-free conditions. (H) Breeding behavior of WT and Mcl1ΔPMN mice. Flow cytometry dot plots and histograms are representative of and quantitative data show mean and SEM from, 10–22 (A–D) or 8–14 (E and F) mice per group from four (A–D) or three (E and F) independent experiments. Survival curves (G) show the data of 138–469 mice per genotype. Eighty-six breeding pairs were used for the analysis of breeding behavior (H).
mice: independent generation of αβ T cells and programmed rearrangements of αβ TCR genes. Cell 72: 337–348.

5. Kim, S., K. Izuka, H. L. Aguila, I. L. Weissman, and W. M. Yokoyama. 2000. In vivo natural killer cell activities revealed by natural killer cell-deficient mice. Proc. Natl. Acad. Sci. USA 97: 2731–2736.

6. Satoskar, A. R., L. M. Stamm, X. Zhang, M. Okano, J. R. David, C. Terhorst, and B. Wang. 1999. NK cell-deficient mice develop a Th1-like response but fail to mount an efficient antigen-specific IgG2a antibody response. J. Immunol. 163: 5298–5302.

7. Yu, C., A. B. Cantor, H. Yang, C. Browne, R. A. Wells, Y. Fujiwara, and S. H. Orkin. 2002. Targeted deletion of a high-affinity GATA-binding site in the GATA-1 promoter leads to selective loss of the eosinophil lineage in vivo. J. Exp. Med. 195: 1387–1395.

8. Wada, T., K. Ishiwata, H. Koseki, T. Ishikura, T. Ugajin, N. Ohnuma, K. Obata, R. Ishikawa, S. Yoshikawa, K. Mukai, et al. 2010. Selective ablation of basophils in mice reveals their nonredundant role in acquired immunity against ticks. J. Clin. Invest. 120: 2867–2875.

9. Feyrerabend, T. B., A. Weiser, A. Tietz, M. Stassen, N. Harris, M. Kopf, P. Radermacher, P. Möller, C. Benoist, D. Mathis, et al. 2011. Cre-mediated cell ablation contests mast cell contribution in models of antibody- and T cell-mediated autoimmune inflammation. Immunity 35: 832–844.

10. Dudek, A., J. Dudek, J. Scholten, A. Petzold, S. Surianarayanan, A. Köhler, K. Peschke, D. Vöhringer, C. Waskow, T. Krieg, et al. 2011. Mast cells are key promoters of contact allergy that mediate the adjuvant effects of hapten. Immunity 34: 973–986.

11. Duffield, J. S., S. J. Forbes, C. M. Constantinescu, S. Clay, M. Partolina, S. Vuthoori, S. Wu, R. Lang, and J. P. Iredale. 2005. Selective depletion of macrophages reveals distinct, opposing roles during liver injury and repair. J. Clin. Invest. 115: 56–65.

12. Mantovani, A., M. A. Cassatella, C. Costantini, and S. Jaillon. 2011. Neutrophils in the activation and regulation of innate and adaptive immunity. Nat. Rev. Immunol. 11: 519–531.

13. Mocsai, A. 2013. Diverse novel functions of neutrophils in immunity, inflammation, and beyond. J. Exp. Med. 210: 1283–1299.

14. Jaillon, S., M. R. Galdiero, D. Del Prete, M. A. Cassatella, C. Garlanda, and S. Jaillon. 2011. Neutrophils in mice reveals their nonredundant role in acquired immunity against ticks. J. Clin. Invest. 120: 2867–2875.

15. Ne´meth, T., O. Virtic, C. Sitaru, and A. Mo´csai. 2017. The Syk tyrosine kinase is required for skin inflammation in an in vivo mouse model of epidermolysis bullosa acquista. J. Invest. Dermatol. 137: 2131–2139.

16. Karsunky, H., C. Mauero¨der, M. J. Podolska, M. H. Biermann, et al. 2017. Ex-GATA-1 promoter leads to selective loss of the eosinophil lineage in vivo. J. Exp. Med. 212: 195–222.

17. Krieg, T. M., C. L. Kjelland, S. H. Orkin. 2002. Targeted deletion of a high-affinity GATA-binding site in the Gfi-1 promoter in neutrophil differentiation. Nat. Genet. 35: 89–91.

18. Ordon˜ez-Rueda, D., F. Jonsson, D. A. Mancardi, W. Zhao, A. Malzac, Y. Liang, K. Peschke, D. Vöhringer, C. Waskow, T. Krieg, et al. 2011. Mast cells are key promoters of contact allergy that mediate the adjuvant effects of hapten. Immunity 34: 973–986.

19. Michels, J., P. W. Johnson, and G. Packham. 2005. Mcl-1.

20. Jonsson, H., P. Allen, and S. L. Peng. 2005. Inflammatory arthritis requires Foxo3a and autoimmune arthritis in the absence of p190RhoGAP: generation and analysis of a novel null mutation in mice. J. Immunol. 185: 3064–3075.

21. Karsunky, H., C. Mauero¨der, M. J. Podolska, M. H. Biermann, et al. 2017. Ex-GATA-1 promoter leads to selective loss of the eosinophil lineage in vivo. J. Exp. Med. 212: 195–222.

22. Michels, J., P. W. Johnson, and G. Packham. 2005. Mcl-1.

23. Jonsson, H., P. Allen, and S. L. Peng. 2005. Inflammatory arthritis requires Foxo3a and autoimmune arthritis in the absence of p190RhoGAP: generation and analysis of a novel null mutation in mice. J. Immunol. 185: 3064–3075.

24. Jonsson, H., P. Allen, and S. L. Peng. 2005. Inflammatory arthritis requires Foxo3a and autoimmune arthritis in the absence of p190RhoGAP: generation and analysis of a novel null mutation in mice. J. Immunol. 185: 3064–3075.

25. Jonsson, H., C. Mauero¨der, M. J. Podolska, M. H. Biermann, et al. 2017. Ex-GATA-1 promoter leads to selective loss of the eosinophil lineage in vivo. J. Exp. Med. 212: 195–222.