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Brief Conclusive Report

Granulocyte functions are independent of arginine availability

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ABSTRACT

Arginine depletion via myeloid cell arginase is critically involved in suppression of the adaptive immune system during cancer or chronic inflammation. On the other hand, arginine depletion is being developed as a novel anti-tumor metabolic strategy to deprive arginine-auxotrophic cancer cells of this amino acid. In human immune cells, arginase is mainly expressed constitutively in PMNs. We therefore purified human primary PMNs from healthy donors and analyzed PMN function as the main innate effector cell and arginase producer in the context of arginine deficiency. We demonstrate that human PMN viability, activation-induced IL-8 synthesis, chemotaxis, phagocytosis, generation of ROS, and fungicidal activity are not impaired by the absence of arginine in vitro. Also, profound pharmacological arginine depletion in vivo via ADI-PEG20 did not inhibit PMN functions in a mouse model of pulmonary invasive aspergillosis; PMN invasion into the lung, activation, and successful PMN-dependent clearance of *Aspergillus fumigatus* and survival of mice were not impaired. These novel findings add to a better understanding of immunity during inflammation-associated arginine depletion and are also important for the development of therapeutic arginine depletion as anti-metabolic tumor therapy. *J. Leukoc. Biol.* 96: 1047–1053; 2014.

Introduction

PMNs are our crucial first line of defense against invading microbes. In case of infection, they emigrate from the bloodstream via specialized interactions with endothelial cells, follow chemotactic gradients toward the epicenter of infection, and combat microbes by phagocytosis and with a variety of oxidative and nonoxidative effector mechanisms. Oxidative PMN effector functions are initiated by NADPH oxidase, a multiprotein complex that converts molecular oxygen to O_2^-, which is metabolized further to H_2O_2 and via myeloperoxidase to hypochlorous acid [1]. PMN can also degranulate and release granule-associated, potentially antimicrobial effector molecules or serine proteases [1]. This rather unidirectional view on PMN, as terminally differentiated effector cells with essentially one final task (antimicrobial killing), has diversified enormously. It became clear that: 1) PMNs also serve as immunoregulatory cells that interact with essentially all other cells of the immune system to orchestrate a proper immune response [2], and 2) PMNs can also suppress immunity [3]. One central, potentially immunosuppressive effector molecule is the enzyme arginase I, which hydrolyzes arginine to ornithine and urea. Arginase I is constitutively expressed in human PMNs [4] and the related population of granulocytic MDSCs, which have characteristics of immature or activated granulocytic cells [5]. Local or systemic arginase-induced arginine deficiency via PMNs or MDSCs is often encountered in cancer patients [6–9] or during chronic inflammation [10] and can profoundly suppress the adaptive immune response by inhibiting T cell proliferation and cytokine synthesis [5–8, 10–15]. In contrast to its immunosuppressive consequences, arginine depletion might also have an anti-tumoral potential, especially against tumors that

Abbreviations: ADI=arginine deiminase, ADI-PEG20=pegylated (20 kDa) arginine deiminase, BALF=bronchoalveolar lavage fluid, CD62L=CD62 ligand, DCFH-DA=dichlorofluorescein diacetate, IPA=invasive pulmonary aspergillosis, i.t.=intratracheally, MDSC=myeloid-derived suppressor cell, MFI=mean fluorescence intensity, nor-NOHA=N-ω-hydroxy-nor-L-arginine, O_2^-=superoxide anion, PI=propidium iodide, PMN=polymorphonuclear neutrophil granulocyte, PMN-S=sonicate of human polymorphonuclear neutrophil granulocyte, ROS=reactive oxygen species, SFI=specific fluorescence index, XTT=(2, 3)-bis-(2-methoxy-4-nitro-5-sulfenyl)-(2H)-tetrazolium-5-carboxanilide

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are unable to resynthesize their own arginine from the precursor amino acid citrulline via argininosuccinate synthase [16]. The most promising drug candidate for pharmacological arginine depletion is the nonmammalian enzyme ADI, which catabolizes arginine to citrulline and ammonia [16]. ADI-PEG20 constitutes ADI conjugated to polyethylene glycol and is a compound with much reduced antigenicity and a prolonged half-life. Pharmacological arginine depletion via ADI-PEG20 is already in Clinical Phase I–III cancer trials against melanoma [17, 18] and hepatocellular carcinoma [19].

Given the profound suppressive effect of arginine deficiency on adaptive immunity, we now studied the function of PMN, the quantitatively and functionally most important cell type of innate immunity, in the context of arginine depletion. We demonstrate that the absence of arginine has no influence on PMN viability and key effector functions of human PMN: cytokine synthesis, oxidative burst, chemotactic movement, phagocytosis, and fungicidal-killing capacity all remain uncompromised in vitro. Also, in a murine model of IPA, we demonstrate in vivo that PMN emigration from the blood into the lung, local activation and fungicidal function, and consequently, viability of infected mice are also unimpaired in the setting of ADI-PEG20-mediated complete arginine depletion. In summary, our data demonstrate that in contrast to the adaptive immune system, granulocyte-based innate immune function is not inhibited by arginine deficiency. This novel finding adds to a better understanding of immunity during cancer inflammation-associated arginine depletion and is also important for the development of therapeutic arginine depletion as anti-metabolic tumor therapy.

MATERIALS AND METHODS

Human subjects

All work involving human subjects was carried out in accordance with the Declaration of Helsinki. Human studies with healthy normal blood donors were approved by the Ethics Committee of the University of Heidelberg and were conducted with the understanding and informed consent from all subjects.

Reagents and media

If not otherwise stated, chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). ADI-PEG20 was from Polaris Pharmaceuticals (San Diego, CA, USA), nor-NOHA (1 mM) was from Bachem (Weil am Rhein, Germany). RPMI-1640 medium, without arginine, was from PromoCell (Heidelberg, Germany) and supplemented as described [12, 20, 21].

Mice

C57BL/6 mice, at 8 weeks, were obtained from the animal facility of the University of Mainz, and all animal procedures were performed in accordance with the institutional guidelines and approved by the responsible national authority (National Investigation Office Rheinland-Pfalz; Approval ID: AZ 23 177-07/G11-1-034).

Isolation of human PBMCs and PMNs, generation of PMN-S, and measurement of arginase activity

Total granulocytes were isolated from peripheral heparin-anticoagulated blood of healthy donors, as described previously [4]. Purity and viability of isolated PMN were always >95%. PMN-S were generated as described [12], protein concentration and arginase activity were determined, as described previously [4], and aliquots were frozen at −80°C until further use.

Real-Time RT-PCR, ELISA, apoptosis and cell death, and phagocytosis

RT-PCR was performed exactly as described previously [12]. IL-8 protein in supernatants of stimulation cultures was measured by specific capture ELISA (BD Biosciences, San Jose, CA, USA). Apoptosis and cell death of PMN were measured by flow cytometry after staining with Annexin-V and PI, as described [12, 20, 21]. Human PMN phagocytic activity was quantified by a commercial PhagoTest kit (Orpegen Pharma, Heidelberg, Germany).

Respiratory burst

Human PMN respiratory burst activity was quantified by three alternative methods: 1) H_2O_2 was detected by oxidation of DCFH-DA, as described [22]. SFI was obtained by subtraction of the background fluorescence of labeled cells incubated in medium alone and 2) in whole blood with a commercial PhagoBurst kit (Orpegen Pharma), and 3) superoxide generation was also measured by its ability to reduce cytochrome C, as described [23].

Fungal strains and cultivation conditions and PMN fungicidal activity

The *A. fumigatus* wild-type strain, ATCC 46645, was cultivated as described [22]. PMNs were preincubated in cell culture media (±arginine; 1 mM) for 2 h at 37°C and then cocultured with *A. fumigatus* conidia (ratio 333:1) in 1 ml HBSS (±arginine; 1 mM). After 4 h, PMNs were lysed, and conidia were grown for 20 h in *Aspergillus* minimal medium [22]. The viability of the resulting hyphae was determined with the XTT assay [4].

Chemotaxis assay

A modified Boyden chamber assay was used as described [24, 25]. As a bona fide chemokine, dialyzed (=arginine-free) and then yeast-activated normal human serum was used as source of C5a (diluted 1:10 in HBSS).

Mouse model of IPA

Mice received 10^7 *A. fumigatus* conidia i.t., as described previously [22]. The severity of systemic infection was examined using a scoring system as described [26]. PMN depletion was induced by injection of the anti-Gr-1 antibody (150 μg i.p., day 1, clone RB6-8C5). Characterization of in vivo fungal clearance in the lung as well as purification and analysis of cells in murine blood and BALF were done exactly as described previously [22].

Amino acid measurements

Arginine concentrations in human whole blood and PMN-S-incubated cell culture medium were measured following our published methodology [4, 20]. Arginine and citrulline in plasma, BAL, and lung tissue of the murine IPA model were measured via *o*-phthaldialdehyde derivatization and fluorescence detection by HPLC, as described previously [27].

Statistical analysis

Statistical analysis was done with GraphPad Prism (version 5.0a for MacOS X; GraphPad Software, San Diego, CA, USA). Comparison of two different parameters was done using paired Student's *t*-test; comparison of four different conditions was done using paired ANOVA with Bonferroni's post-test. In the IPA model, different experimental mouse groups were analyzed by a two-tailed Student's *t*-test for comparison between two groups and one-way ANOVA with Bonferroni's post-test for comparison among more than two groups.

RESULTS AND DISCUSSION

Arginine deficiency does not impair human PMN viability and IL-8 synthesis

We first analyzed the potential influence of arginine deficiency on human PMN viability by incubating freshly isolated PMN in cell-culture medium, with (1 mM) or without arginine (**Fig. 1A** and **B**). As expected, the frequency of dead (AnnexinV+, PI+; Fig. 1A) and apoptotic (AnnexinV+, PI−; Fig. 1B) cells increased strongly during in vitro culture. However, the absence of arginine clearly had no influence on the extent of cellular apoptosis or cell death. In further experiments, we saw that the proinflammatory cytokine IFN-γ decreased apoptosis and cell death compared with unstimulated cells, but there was again no influence of arginine deficiency on cellular viability or apoptosis at all time-points tested (data not shown).

One of the key features of the suppressed adaptive immune response upon arginine deficiency is the impaired production of cytokines by T lymphocytes [10]. We therefore quantified the production of IL-8, a central PMN cytokine [2, 28], on mRNA (Fig. 1C) and protein (Fig. 1D) level upon PMN activation by LPS. Whereas resting human PMN expressed very low levels of IL-8 mRNA and no detectable IL-8 protein, LPS stimulation led to a pronounced induction of IL-8 mRNA (Fig. 1C) and IL-8 protein (Fig. 1D). The absence of arginine during stimulation had no significant influence on this LPS- (Fig. 1C and D)- or IFN-γ + LPS (data not shown)-mediated induction. To allow comparison between the different experiments, the amount of IL-8 protein in the supernatants in arginine-containing medium at 18 h (corresponding to 2719±1970 pg/ml) was set to 100% in each experiment.

Chemotaxis of human PMN is not inhibited by arginine deficiency

To study chemotaxis, we first preincubated human PMN in HBSS, with or without 1 mM arginine. Chemotaxis was then induced by yeast-activated dialyzed (i.e., arginine-free) human serum, which contains the chemotactic factor C5a. We quantified PMN chemotaxis in the absence or presence of arginine in a modified Boyden chamber assay (**Fig. 2A**). Whereas the chemotactic stimulus led to a directed movement of the PMN, there was no significant difference between arginine-containing and -free conditions. To allow for extended periods of preincubation under different arginine concentrations and in a more physiological setting, we depleted human heparin-anticoagulated whole blood of arginine ex vivo by supplementation with a PMN-S of a defined arginase activity (1500 mU/ml), with or without concurrent supplementation of the arginase inhibitor nor-NOHA, based on our well-established in vitro model of PMN arginase-mediated arginine depletion [12, 20, 21] (Fig. 2B). We interpret the unexpectedly low arginine concentration in preincubated whole blood (without PMN-S) as a result of low concentrations of liberated endogenous arginases from PMN or red blood cells. In line with this hypothesis is the physiological arginine concentration measured in the presence of the arginase inhibitor nor-NOHA. In any case, this model of preincubated whole blood allowed us to study PMN chemotaxis ex vivo with PMNs that were subjected to different concentrations of arginine for several hours before being isolated from the blood and exposed to the chemotactic stimulus in the assay with controlled arginine concentrations (0 mM vs. 1 mM). Again, chemotactic stimulation led to a significantly increased directed movement of the PMN, and there was no significant difference regarding chemotaxis between arginine-containing and -free conditions (Fig. 2C).

No influence of arginine deficiency on phagocytosis of human PMN

Next, we studied phagocytic capacity of human PMN in whole blood or in blood that was depleted of arginine via PMN-S or in which this depletion was inhibited by nor-NOHA (see Fig. 2B). Phagocytosis was measured with PhagoTest, which determines the percentage of phagocytes that ingest FITC-labeled, opsonized *Escherichia coli* bacteria and their activity (number of bacteria/cell), as measured by FITC MFI. Both parameters were not significantly different depending on the availability of arginine: a representative flow cytometry blot is shown in

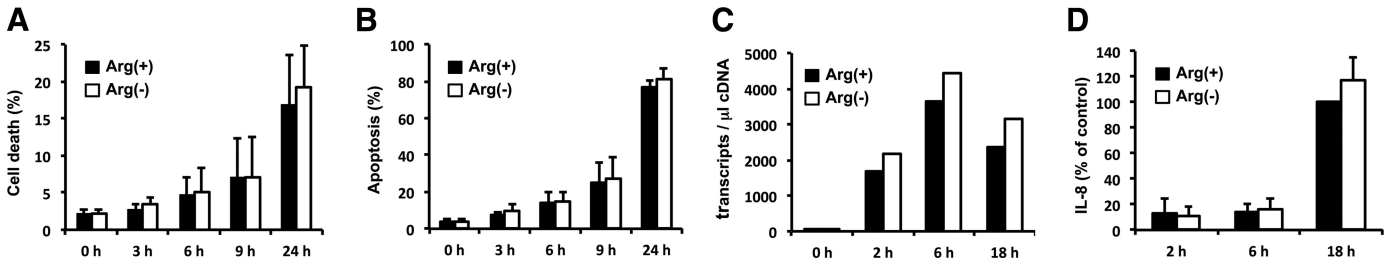

Figure 1. Human granulocyte viability and stimulated IL-8 production are not impaired in the absence of arginine. Human PMNs were purified from peripheral blood of healthy donors and incubated in normal cell culture medium (A and B) or upon stimulation with LPS (1 μg/ml; C and D) in the presence of 1 mM arginine [Arg(+)] or the absence of arginine [Arg(−)]. (A and B) At the indicated time-points, cells were harvested and stained with AnnexinV and PI. The frequency of dead (AnnexinV+, PI+) cells (A) and apoptotic (AnnexinV+, PI−) cells (B) was quantified by flow cytometry. Mean ± SD is shown of six individual experiments. (C) At the indicated time-points, cells were harvested, and IL-8 mRNA was quantified in cell lysates with quantitative real-time PCR. One representative experiment (total $n=2$) is shown. (D) IL-8 concentrations in supernatants of the indicated time-points were determined by ELISA. To allow comparison between the different independent experiments (total $n=5$), the amount of IL-8 protein in the supernatants in Arg(+) medium at 18 h was set to 100% in each experiment.

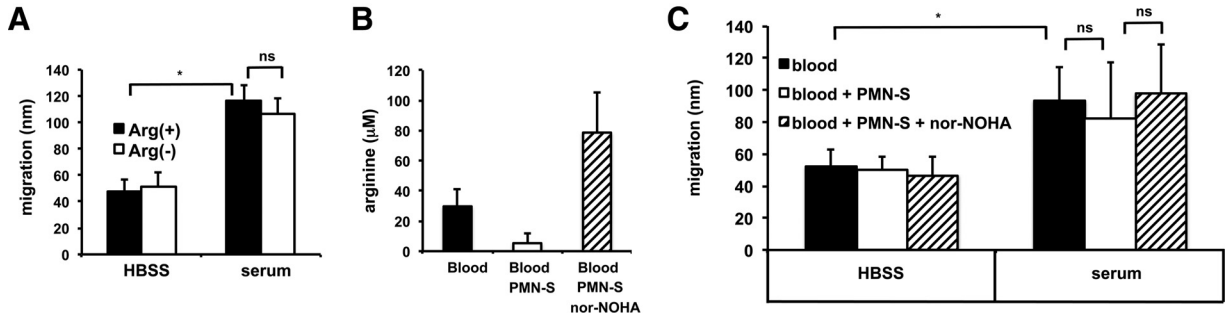

Figure 2. Human PMN chemotaxis is not disturbed by the absence of arginine. (A) Freshly isolated human PMNs were preincubated in HBSS in the absence of arginine (0 mM) or the presence of arginine (1 mM). Chemotaxis was induced by yeast-activated, dialyzed human serum, whereas HBSS served as a control for random migration. PMN chemotaxis was analyzed in a modified Boyden chamber assay (mean±SD of four separate experiments). (B) Whole blood was depleted of arginine by a PMN-S (arginase activity: 1500 mU/ml) ± supplementation of arginase inhibitor nor-NOHA. After 5 h at 37°C, arginine concentrations in the respective samples were measured (mean±SD of three experiments). (C) PMNs were isolated from whole blood that was pretreated as described in B. Chemotaxis was then induced and analyzed as described in A. Arginine (1 mM) was supplemented for the preincubation conditions "blood" and "blood + PMN-S + nor-NOHA" during the migration assay (mean±SD of three separate experiments). $*P < 0.001$.

Fig. 3A, and a summary of the results of three independent experiments is presented in Fig. 3B.

Unimpaired oxidative burst and fungicidal activity in vitro in human PMNs in the absence of arginine

Human PMN respiratory burst activity was quantified by three alternative methods. There was no difference in the kinetics

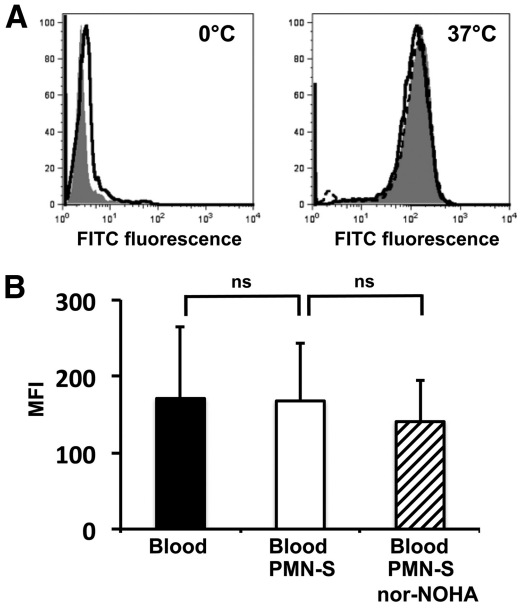

Figure 3. Human PMN phagocytosis is unimpaired in the absence of arginine. Human PMN phagocytosis capacity was measured with PhagoTest in whole blood that had been preincubated as described in Fig. 2. (A) A representative flow cytometry result for incubations at 0°C (control for unspecific attachment) and 37°C (experimental condition, active phagocytosis) is demonstrated, where the filled gray curves correspond to whole blood, the thick black lines to blood + PMN-S, and the dashed lines to blood + PMN-S + nor-NOHA. (B) A summary (mean FITC MFI±SD at 37°C) of the three independent experiments is shown.

and absolute amount of H_2O_2 production upon LPS stimulation of human PMN in the absence or presence of arginine (**Fig. 4A**). We also quantified O_2^- production of human PMN after ingestion of *E. coli* by flow cytometry, based on their oxidation of the fluorogenic substrate dihydrorhodamine 123 (Fig. 4B and C) or on PMA stimulation by cytochrome C reduction (Fig. 4D) Again, we measured no influence of arginine depletion on the percentage of PMN that produced O_2^- (Fig. 4B) and the quantity of O_2^- production/PMN (Fig. 4C) upon activation with *E. coli* or on cytochrome C reduction (Fig. 4D). We finally analyzed in vitro fungicidal activity of human PMN, which partially depends on ROS generation [4]. The killing of *A. fumigatus* (Fig. 4E) and *Candida albicans* (data not shown) was equally effective in vitro in the absence or presence of arginine.

Arginine depletion in vivo by ADI-PEG20: no impairment of PMN recruitment and effector function in a murine pneumonia model

We finally wanted to study a potential influence of the arginine limitation on PMN function in vivo in the setting of infection in our established murine model of IPA [22]. The clearing of *A. fumigatus* and survival of mice are critically dependent on the presence of PMN and PMN-associated oxidative effector functions [22]. Arginine depletion in vivo can be achieved in a very effective and long-lasting manner by i.p. application of ADI-PEG20. We first verified in our murine IPA model that application of ADI-PEG20 induces efficient arginine depletion in serum (<7 μM) with concurrent citrulline synthesis (>1 mM) in vivo, from 24 h to 96 h after a single i.p. injection of ADI-PEG20 (data not shown). Mice were then infected i.t. with 10×10^6 *A. fumigatus* conidia or water, 24 h after i.p. administration of 5 IU ADI-PEG20 and/or i.p. injection of anti-Gr-1 antibody (resulting in sustained neutropenia) [22]. Arginine depletion and pronounced citrulline synthesis upon ADI-PEG20 treatment were also measurable in *A. fumigatus*-infected mice in blood (**Fig. 5A**) and lung homogenates,

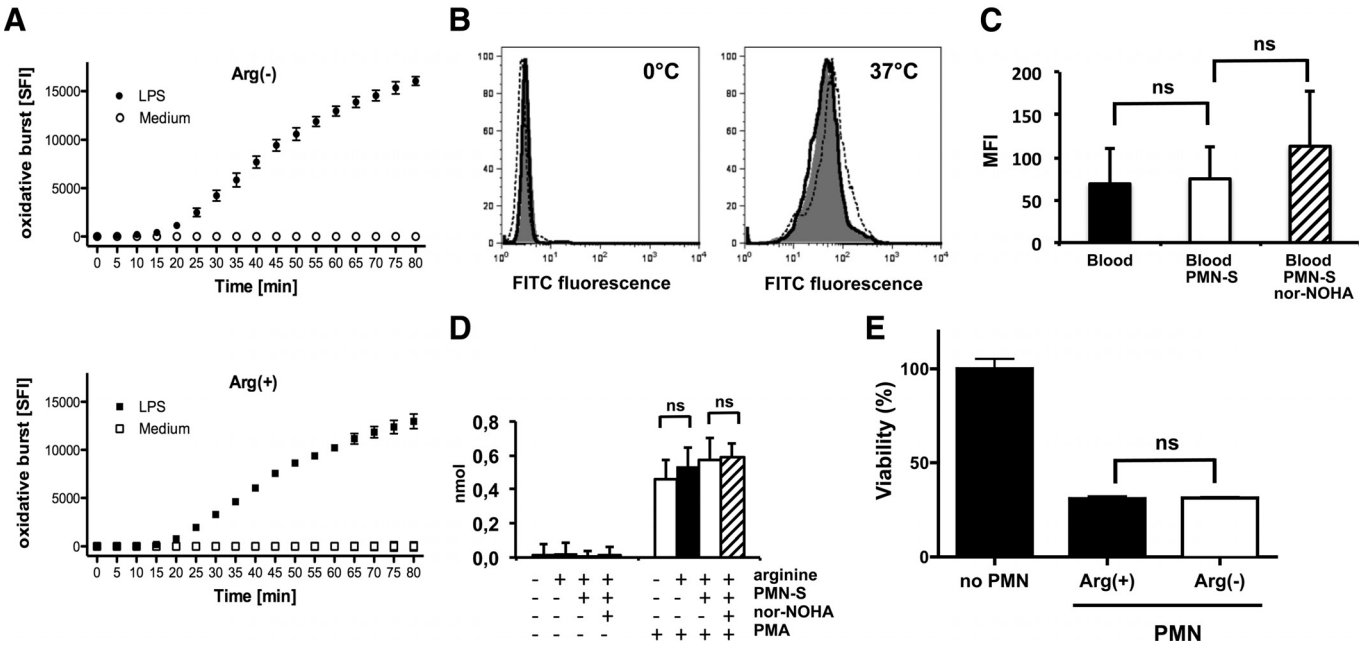

Figure 4. Arginine deficiency has no influence on human PMN oxidative burst and fungicidal activity. (A) PMNs (10^6/ml) were stimulated with LPS (1 μg/ml) in medium in the absence (0 mM) or presence (1 mM) of arginine or left untreated (Medium) as indicated. The oxidative burst activity was measured using DCFH-DA over time. One representative experiment (total of three) is shown. (B and C) Human whole blood was preincubated as described in Fig. 3 with PMN-S. After addition of opsonized *E. coli*, the generation of ROS by PMN was quantified by flow cytometry, based on their oxidation of the fluorogenic substrate dihydrorhodamine 123 (PhagoBurst). (B) A representative flow cytometry result for 10-min incubations at 0°C (control for unspecific fluorescence) and 37°C is demonstrated, where the filled gray curves correspond to whole blood, the thick black lines to blood + PMN-S, and the dashed lines to blood + PMN-S + nor-NOHA. (C) Mean FITC MFI ± SD of five independent experiments is shown. (D) Purified human PMNs were preincubated for 3 h in cell-culture media as indicated. Arg(−) medium as well as medium treated with PMN-S (for 24 h) do not contain measurable arginine concentrations. PMNs were then suspended in cytochrome C (1 mg/ml in HBSS), stimulated with PMA, and ROS generation was quantified photometrically after 90 min incubation by cytochrome C reduction. A summary (mean production of O_2^- in nmol±SD) of six independent experiments is shown. (E) Purified human PMN (10^6/ml) were incubated in Arg(+) or Arg(−) medium in the presence of *A. fumigatus* conidia for 4 h at 37°C. After lysis of PMN, conidia were cultivated for 20 h. Fungal viability (relative to *A. fumigatus* conidia without PMN) of *A. fumigatus* was evaluated with the XTT assay. A summary (mean±SD) of three independent experiments is shown.

i.e., locally in the infectious micromilieu (Fig. 5B). In parallel, an increase in neutrophils in the peripheral blood (Fig. 5C) and locally in the BALF (Fig. 5E) was detectable, whereas no PMNs were present in either compartment in anti-Gr-1-treated mice (Fig. 5C and E). ADI-PEG20 treatment neither had any negative influence on the peripheral neutrophilia (Fig. 5C) nor did it inhibit the influx of PMN into BAL (Fig. 5E). The comparison of local PMN activation status in the lungs of infected mice (Fig. 5F) with peripheral blood (Fig. 5D) revealed a strong up-regulation of CD11b as a marker for degranulation and nearly complete CD62L shedding of PMNs in the lungs with no influence of ADI-PEG20-mediated arginine depletion. Finally, we studied the influence of ADI-PEG20-mediated arginine depletion on the survival of *A. fumigatus*-infected mice. As already shown [22], control treatment with anti-Gr-1 antibody demonstrated the crucial importance of functional PMN for survival in this infectious model (Fig. 5G). In contrast, ADI-PEG20 treatment did not impair survival of mice (Fig. 5G). This finding was also corroborated by CFU quantification for fungal growth analysis from lung tissues of infected mice (Fig. 5H).

Myeloid cell arginase-induced arginine depletion has emerged as a fundamental principle of immune regulation [29], and its role in infection [10, 30, 31], cancer [6–8, 13], and allergic inflammation [32] is well established. Whereas inhibition of adaptive immunity by arginine deprivation is the common denominator of all of these various inflammatory scenarios, we demonstrate here that important functions of human and murine PMNs and therefore, innate immunity, are not impaired by the absence of arginine. This novel aspect of PMN biology is potentially important for PMN-mediated innate antimicrobial defense, PMN-based immunoregulation, and cancer-associated PMN. As human PMN-dominated inflammation is characterized by arginase liberation, measurable, e.g., in pus [12], with consecutive arginine depletion, PMNs face arginine deficiency regularly in the setting of arginase I liberation from their necrotic fellow PMN. Theoretically, it would potentially be a huge disadvantage if this self-inflicted arginine depletion would shut down antimicrobial PMN effector function. Our data demonstrate for the first time that this does indeed not happen: PMN antimicrobial effector functions—chemotaxis toward inflammatory stimuli, ROS produc-

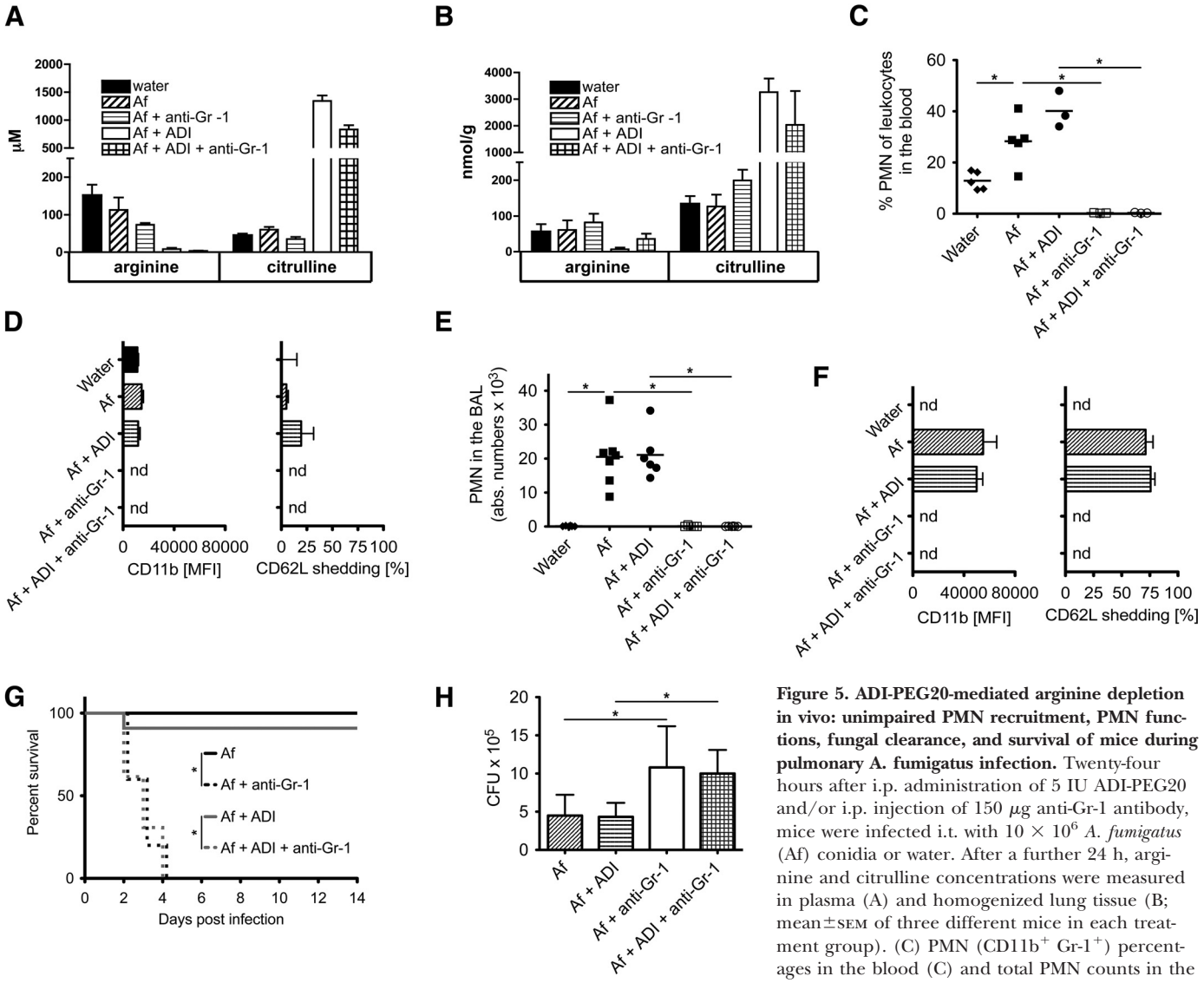

Figure 5. ADI-PEG20-mediated arginine depletion in vivo: unimpaired PMN recruitment, PMN functions, fungal clearance, and survival of mice during pulmonary A. fumigatus infection. Twenty-four hours after i.p. administration of 5 IU ADI-PEG20 and/or i.p. injection of 150 μg anti-Gr-1 antibody, mice were infected i.t. with 10×10^6 *A. fumigatus* (Af) conidia or water. After a further 24 h, arginine and citrulline concentrations were measured in plasma (A) and homogenized lung tissue (B; mean±SEM of three different mice in each treatment group). (C) PMN ($CD11b^+$ $Gr\text{-}1^+$) percentages in the blood (C) and total PMN counts in the BALF (E) were quantified by flow cytometry. Expression of the PMN degranulation marker CD11b and the shedding marker CD62L of $Gr\text{-}1^+$ cells was determined in blood (D) and BAL (F). For CD62L, the percentage of $Gr\text{-}1^+CD62L^-$ cells is depicted as "CD62L shedding," which was quantified in relation to the amount of CD62L expressing $Gr\text{-}1^+$ PMN in the blood of water-treated control mice (mean of two independent experiments). (G) Overall survival of mice was monitored for 14 days. Shown are the cumulative results of two independent experiments, with 10–12 mice in each group. (H) For analysis of fungal growth, CFUs were quantified 24 h after infection. Depicted are cumulative results of two independent experiments, with four to six mice in each group. $^*P < 0.05$; nd, not detectable.

tion, phagocytosis, and antifungal killing—are uncompromised in the complete absence of arginine in vitro or upon severe arginine depletion in vivo. PMNs also interact with other cells of the innate and adaptive immune system [3, 33], and they can, e.g., inhibit the proliferation and activation of T cells by arginine depletion via PMN arginase I [12, 34, 35] or H_2O_2 secretion [36, 37]. Whereas our data show clear differences in arginine dependence of PMN versus T cells [12] or NK cells [21], there are also common themes: cellular viability, cytokine and chemokine mRNA induction, IL-8 protein synthesis, as well as cytotoxicity and chemotaxis are unimpaired in the absence of arginine in T cells [14, 38] and in PMN (this manuscript). Finally, our results are relevant for the therapeutic use of novel, arginine-depleting, metabolic anti-tumor strategies [16]. Given the superb and long-lasting arginine depletion induced by ADI-PEG20 [18, 19], our data of unimpaired PMN functions in the absence of arginine are reassuring for clinicians and are also in agreement with the absence of relevant infectious problems in the published and ongoing clinical studies with ADI-PEG20.

With the consideration of the fundamental importance of cellular metabolic reorganization toward external nutrient signals and the emerging principle of amino acid deprivation via induction of amino acid-consuming enzymes, such as arginase [29], exciting times are ahead for the analysis of this fundamental principle of immune regulation.

AUTHORSHIP

K.K., C.M., and C.L-M. designed and performed experiments (human PMN). S.P. designed and performed experiments (murine PMN). A.H. designed and performed experiments (arginine measurements in murine PMN experiments). T.G. designed and performed experiments (cytokine mRNA quantification). J.B. provided ADI-PEG20 and assisted in writing the manuscript. C-D.L. designed and performed experiments (arginine measurements in human PMN experiments). P.K., I.M., and E.I.C. assisted in designing experiments and writing the manuscript. M.P.R. designed murine experiments and assisted in writing the manuscript. M.M. conceived of the total project, designed experiments, and wrote the manuscript.

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DISCLOSURES

J.B. is an employee of and has stock options in Polaris Pharmaceuticals, a subsidiary of Polaris Group.

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