Biochemistry and Biophysics Reports 23 (2020) 100791

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

CD204-positive monocytes and macrophages ameliorate septic shock by suppressing proinflammatory cytokine production in mice

Yoshimi Uchida, Gen Nishitai, Kenta Kikuchi, Takumi Shibuya, Kenichi Asano, Masato Tanaka *

Laboratory of Immune Regulation, School of Life Sciences, Tokyo University of Pharmacy and Life Sciences, Japan

ARTICLE INFO

Keywords:
Sepsis
CD204
Macrophage scavenger receptor 1
Macrophage
Monocyte

ABSTRACT

Sepsis is defined as a life-threatening multiorgan dysfunction caused by dysregulated inflammatory response to infection. It remains the primary cause of death from infection if not diagnosed and treated promptly. Therefore, a better understanding of the mechanism for resolving inflammation is needed. Monocytes and macrophages play a pivotal role not only in the induction but also in the suppression of inflammation. However, a tissue-resident macrophage subset that regulates a hyperinflammatory state during sepsis has not been explored. Here we show that CD204$^+$ monocytes and/or macrophages rescued mice from endotoxin-induced septic shock. Serum and tissue proinflammatory cytokine levels were significantly upregulated in the absence of these cells. This study provided evidence that CD204$^+$ monocytes and/or macrophages ameliorate septic shock by suppressing proinflammatory cytokine production.

1. Introduction

Sepsis is defined as a multiorgan dysfunction caused by a dysregulated host response to an infection [1,2]. Despite recent therapeutic innovations, it can turn into a life-threatening condition known as septic shock if not diagnosed and treated promptly. Therefore, a precise understanding of sepsis pathophysiology is needed.

Macrophages are considered to play a pivotal role not only in the induction but also in the resolution of inflammation [3–5]. This concept is based on the fact that macrophages exhibit immunologically polarized states when stimulated *in vitro* [6,7], that is, classically activated or M1 macrophages produce large amounts of reactive oxygen species and proinflammatory cytokines and contribute to killing pathogens. On the other hand, alternatively activated or M2 macrophages produce anti-inflammatory cytokines that may support resolution. Much effort has been exerted to elucidate the characteristics of tissue macrophages on the basis of criteria established *in vitro*. In many cases, however, macrophages in the human body exhibit a mixed phenotype having features of both M1 and M2. The exploration of immunoregulatory macrophages *in vivo* is under way.

To examine the kinetics of immunoregulatory macrophages *in vivo*, we used an M2 marker CD204/Macrophage scavenger receptor 1 (Msr1)/Class A scavenger receptor (SRA). This receptor was initially described as an acetylated low-density lipoprotein receptor [8] that is predominantly expressed by subsets of macrophages including tumor-infiltrating macrophages in human and mouse. The abundance of CD204$^+$ macrophages is correlated with low five-year survival rates in breast cancer [9] or oral squamous cell carcinoma [10] patients, suggesting their role in suppressing anti-tumor immunity. However, the tissue distribution and the roles of CD204$^+$ macrophages have not been analyzed *in vivo*.

In this study, we globally analyzed the distribution of CD204$^+$ macrophages among various organs in mouse. By generating CD204-diphtheria toxin receptor (DTR) knock-in mice, we revealed elevated TNFα and IL-6 levels in the absence of CD204$^+$ macrophages in a mouse model of sepsis. This finding suggests that CD204$^+$ macrophages contribute to the resolution of hyperinflammatory response by suppressing proinflammatory cytokine production.

2. Materials and methods

2.1. Mice

C57BL/6J (7- to 12-week-old) mice were purchased from CLEA Japan, Inc. All mice were housed under specific-pathogen-free conditions in the Tokyo University of Pharmacy and Life Sciences animal facility. All experiments using the mice described herein were approved by Tokyo University of Pharmacy and Life Sciences Animal Use Committee

* Corresponding author.
E-mail address: mtanaka@toyaku.ac.jp (M. Tanaka).

https://doi.org/10.1016/j.bbrep.2020.100791
Received 25 March 2020; Received in revised form 10 July 2020; Accepted 22 July 2020

(L17-23, 25, L18-22, 23 and L19-20) and performed in accordance with applicable guidelines and regulations.

2.2. Generation of CD204-DTR mice

A genomic clone containing the *Msr1* gene (encoding CD204 molecule) was obtained from BACPAC Resources CHORI (Oakland, CA, USA). To generate the targeting vector, the region from −5.4 to +6.5 kbp relative to the transcriptional start site of the *Msr1* gene was subcloned into the pBluescript II SK (+) vector. Then, an 8-bp fragment including the endogenous ATG start site in exon 1 was replaced with human DTR cDNA with a poly-A tail by a BAC recombineering technique. To allow selection for homologous recombinants, an FRT-flanked Neo cassette was cloned downstream of the human DTR gene. The thymidine kinase (TK) gene was inserted upstream of the 5′ arm to select against random integrants. To generate CD204-DTR mice, B6JN/1 ES cells were transfected with the linearized targeting vector by electroporation. G-418- and ganciclovir-resistant clones were screened for homologous recombination by Southern blot analysis with a 436-bp digoxigenin (DIG)-labeled DNA probe located outside the targeting vector using a DIG DNA Labeling Kit and Detection Kit (Roche, Basel, Switzerland). Germline chimeric mice were generated by aggregation methods. Chimeric mice with high embryonic stem cell contribution were crossed with C57BL/6J mice to produce $CD204^{+/DTR}$ mice. The $CD204^{+/DTR}$ mice were backcrossed to C57BL/6J mice for more than four generations, and wild-type (+/+) and heterozygous CD204-DTR (+/DTR) littermates were used for analysis.

2.3. Depletion of $CD204^{+}$ cells

For systemic $CD204^{+}$-cell depletion, CD204-DTR mice were injected intraperitoneally (i.p.) or intravenously (i.v.) with 500 ng/mouse of DT (Calbiochem, CA, USA or Sigma-Aldrich, MO, USA).

2.4. Sepsis model

For sepsis induction in mice, WT and CD204-DTR mice were injected i.v. with 2.5 mg/kg of LPS (O111:B4, Sigma-Aldrich) diluted in PBS.

2.5. ELISA

Serum TNFα and IL-6 levels were measured by ELISA MAX™ Standard Kits (BioLegend, CA, USA) according to the manufacturer's protocols.

2.6. Total RNA extraction for quantitative reverse transcription PCR

Liver, lung, colon, and spleen were resected from mice and soaked in RNAlater Stabilization Solution (Thermo Fisher Scientific, MA, USA) for 24 h at 4° C. Total RNA from these organs was extracted with an RNeasy Mini Kit (Qiagen, Hilden, Germany) or a FavorPrep Total RNA Extraction Column (Favorgen, Pingtung County, Taiwan) according to the manufacturers' protocols. For quantitative reverse transcription PCR (qRT-PCR), complementary DNAs (cDNAs) were synthesized using ReverTra Ace (Toyobo, Osaka, Japan). qRT-PCR was performed on cDNA using a THUNDERBIRD SYBR qPCR Mix (Toyobo). Expression levels were normalized to 18s ribosomal RNA. Primer sequences were as follows: IL-6: (Fwd) 5′-CTGGAGTACCATAGCTACC-3′, (Rev) 5′-CTGTTAGGAGAGCATTGGA-3′; TNFα: (Fwd) 5′-ACCCTCACACTCAGATCATC-3′, (Rev) 5′-GAGTAGACAAGGTACAACCC-3′; IL-1β: (Fwd) 5′-GGATGAGGACATGAGCACCT-3′, (Rev) 5′-AGCTCATATGGGTCCGACAG-3′; and 18s rRNA: (Fwd) 5′-CGGACAGGATTGACAGATTG-3′, (Rev) 5′-CAAATCGCTCCACCAACTAA-3′.

2.7. Antibodies for flow cytometry analysis

Anti-Ly6G (1A8), anti-Ly6C (HK1.4), anti-CD45.2 (104), anti-NK1.1 (PK136), anti-CD19 (6D5), anti-Thy1.2 (53–2.1), anti-CD11b (M1/70), anti-F4/80 (BM8), anti-CD64 (X54-5/7.1), and anti-CD16/32 (93) were purchased from BioLegend. Anti-CD11c (HL3) and anti-SiglecF (E50-2440) were purchased from BD Biosciences (CA, USA). Anti-CD115 (ASF98) and anti-MHC class II (M5/114.15.2) were purchased from Thermo Fisher Scientific. Anti-CD204 (REA148) and REA control (REA293) were purchased from Miltenyi Biotech (Bergisch Gladbach, Germany). Dead cells were excluded by DAPI (Dojindo, Kumamoto, Japan).

2.8. Preparation of immune cells for flow cytometry analysis

Immune cells in peripheral blood, bone marrow [11], lung [12], and colon [13] were prepared as described previously. Liver minced with scissors was digested with digestion mixture (100 μg/ml Liberase TM (Roche)/5 μg/ml DNase I (Sigma-Aldrich)/1xHBSS) for 25 min at 37° C with gentle shaking. Digested liver was passed through a 70 μm Cell Strainer (BD Biosciences). After centrifugation at 300x*g* for 4 min at 4° C, the supernatant was transferred to a new tube and centrifuged again at 100x*g* for 2 min to remove non-immune cells. This process was repeated five times. The final supernatant was centrifuged at 300x*g* for 4 min. Red blood cells (RBCs) within the pellet were lysed with 2 ml of lysing buffer (Pharm Lyse, BD Biosciences) and then diluted with FACS staining buffer (0.5% BSA/2 mM EDTA/PBS). The spleen was cut into four pieces and squeezed over a 70 μm Cell Strainer that was placed on a 6-cm dish. The filtrate was harvested in a 15 ml tube and centrifuged at 500x*g* for 5 min at 4° C. RBCs within the pellet were lysed with 2 ml of lysing buffer and diluted with FACS staining buffer.

2.9. Statistical analysis

The survival rate was compared between two groups using the log-rank test. Data were analyzed either by analysis of variance (ANOVA) followed by multiple comparison or by the unpaired *t*-test with GraphPad Prism (GraphPad Software, CA, USA). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Identification of $CD204^{+}$ immune cell types in vivo

First, we explored $CD204^{+}$ immune cell types in several major organs and peripheral blood by flow cytometry. In the steady state, CD204 was expressed by Kupffer cells in liver, $Ly6C^{hi}$ monocytes and $Ly6C^{lo}CD64^{+}$ macrophages in colon, and $Ly6C^{hi}CD115^{hi}$ monocytes and $Ly6C^{lo}CD115^{hi}$ monocytes in spleen (Fig. 1A). Both $CD11c^{+}CD11b^{-}CD64^{+}$ alveolar macrophages and $CD11c^{-}CD11b^{+}$MHC class II^{+} interstitial macrophages in lung were CD204-negative (Fig. 1A). In bone marrow and peripheral blood, $Ly6C^{hi}CD115^{hi}$ monocytes and $Ly6C^{lo}CD115^{hi}$ monocytes were $CD204^{+}$ but neutrophils, eosinophils, T cells, B cells, NK cells, and NKT cells did not express CD204 (Fig. 1A, B and C). These results indicate that CD204 expression is strictly limited to monocyte-macrophage lineage cells among leukocytes.

3.2. Generation of CD204-DTR mice

To analyze the function of $CD204^{+}$ monocytes and macrophages, we generated mice that harbor hDTR gene at the CD204 gene locus (CD204-DTR mouse, Fig. 2A). The targeting construct in CD204-DTR mouse genome was confirmed by Southern blot analysis (Fig. 2B). In these mice, we examined the depletion of $CD204^{+}$ cells by intraperitoneal diphtheria toxin (DT) administration. Twenty-four hours after DT injection, Kupffer cells (Fig. 3A); $Ly6C^{hi}CD115^{hi}$ monocytes and

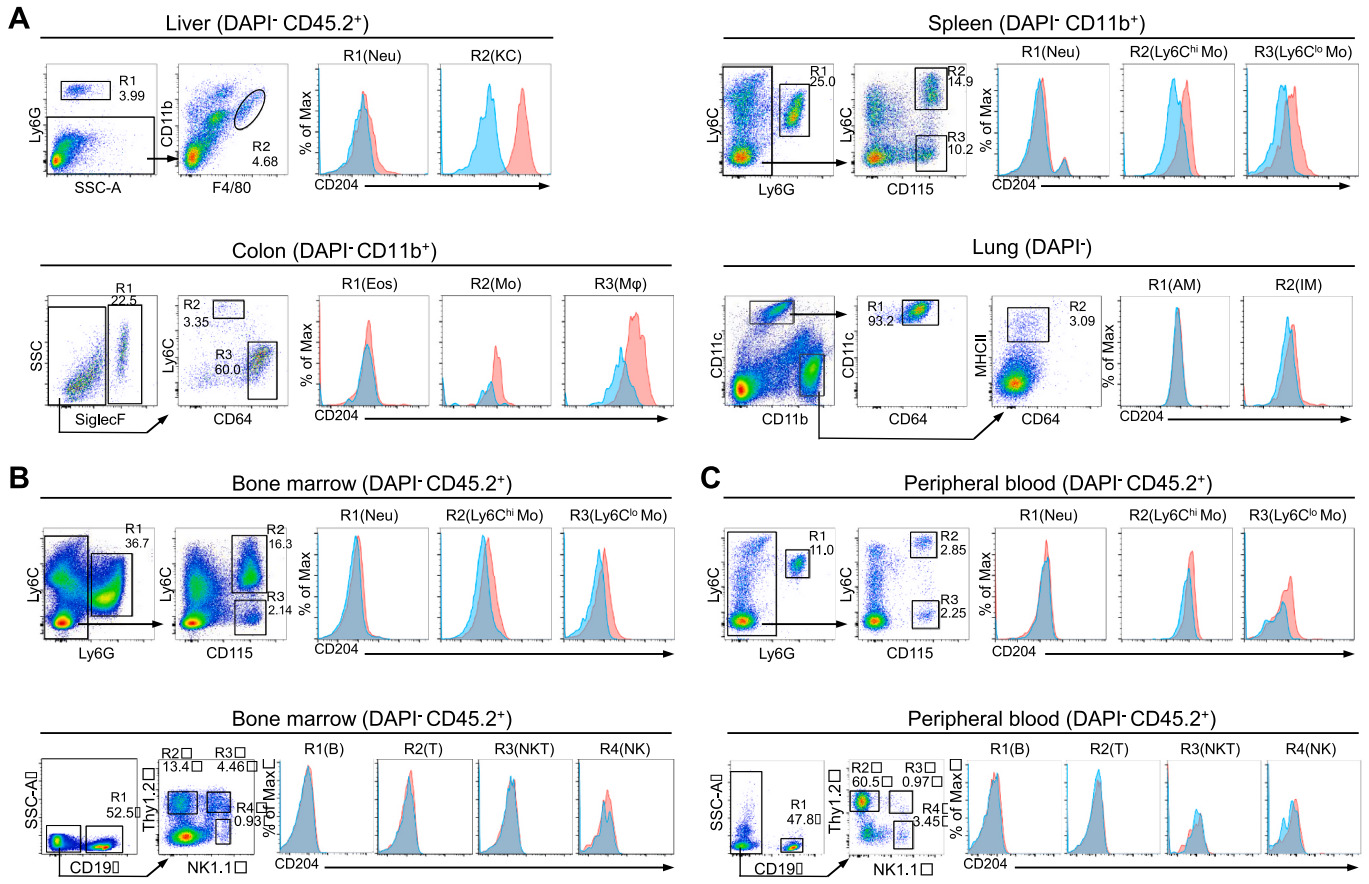


Fig. 1. Identification of CD204-expressing cells in various tissues. Flow cytometry analysis of various tissues (A), bone marrow (B), and peripheral blood (C). Stained with CD204 antibody is the shaded area in red. Stained with isotype control antibody is the shaded area in blue. Mφ, macrophage; KC, Kupffer cell; Mo, monocyte; Neu, neutrophil; Eos, eosinophil; AM, alveolar macrophage; IM, interstitial macrophage; B, B cell; T, T cell; NKT, natural killer T cell; NK, natural killer cell; MHCII, major histocompatibility complex II; SSC-A, side scatter. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).

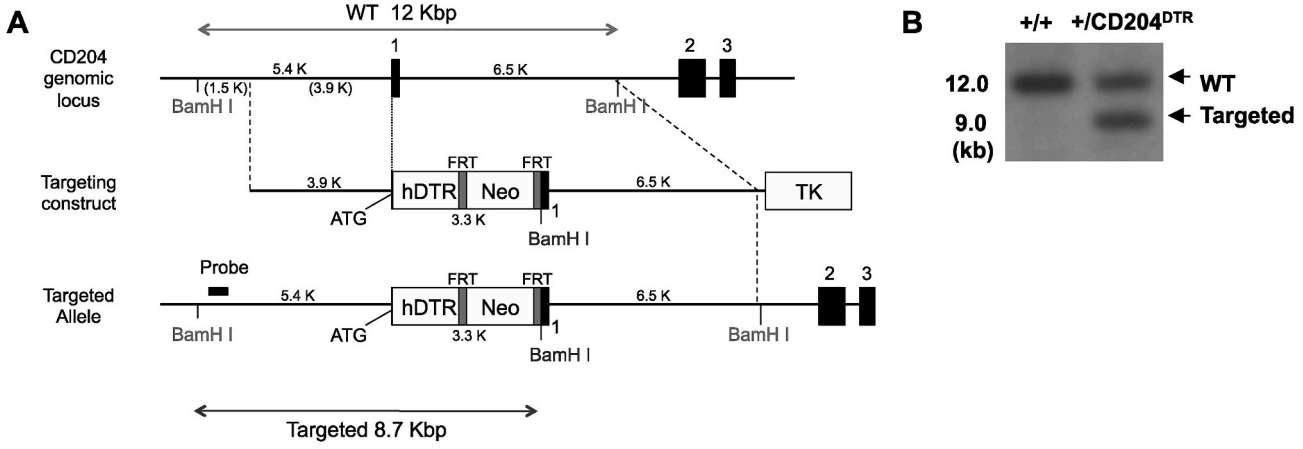


Fig. 2. Generation of CD204-DTR mice. (A) Schematic diagram of the CD204-DTR targeting construct. Exons of Msr1 gene (encoding CD204 molecule) are indicated by solid boxes. Human DTR (hDTR) cDNA, FRT-flanked neomycin resistance gene (Neo), and thymidine kinase gene (TK) are indicated by open boxes. The probe used for Southern blot analysis is indicated by a solid line together with the predicted hybridizing fragments. (B) Southern blot analysis of wild-type (+/+) and CD204-DTR (+/CD204^{DTR}) mouse genome using *Bam*HI digest in combination with the indicated probe.

Ly6C^{lo}CD115^{hi} monocytes in spleen (Fig. 3B); macrophages and monocytes in colon (Fig. 3C); and Ly6C^{hi}CD115^{hi} monocytes and Ly6C^{lo}CD115^{hi} monocytes in bone marrow and peripheral blood (Fig. 3D and E) were substantially decreased in DT-injected CD204-DTR mice compared with PBS-injected CD204-DTR mice. On the other hand, neutrophils, eosinophils, and lymphocytes were not depleted by DT administration in any of the organs analyzed and in peripheral blood (Fig. 3). Notably, lung macrophages that are CD204-negative did not decrease after DT injection (Fig. 3H). These results indicate that CD204^{+} monocytes and macrophages can be specifically depleted by DT

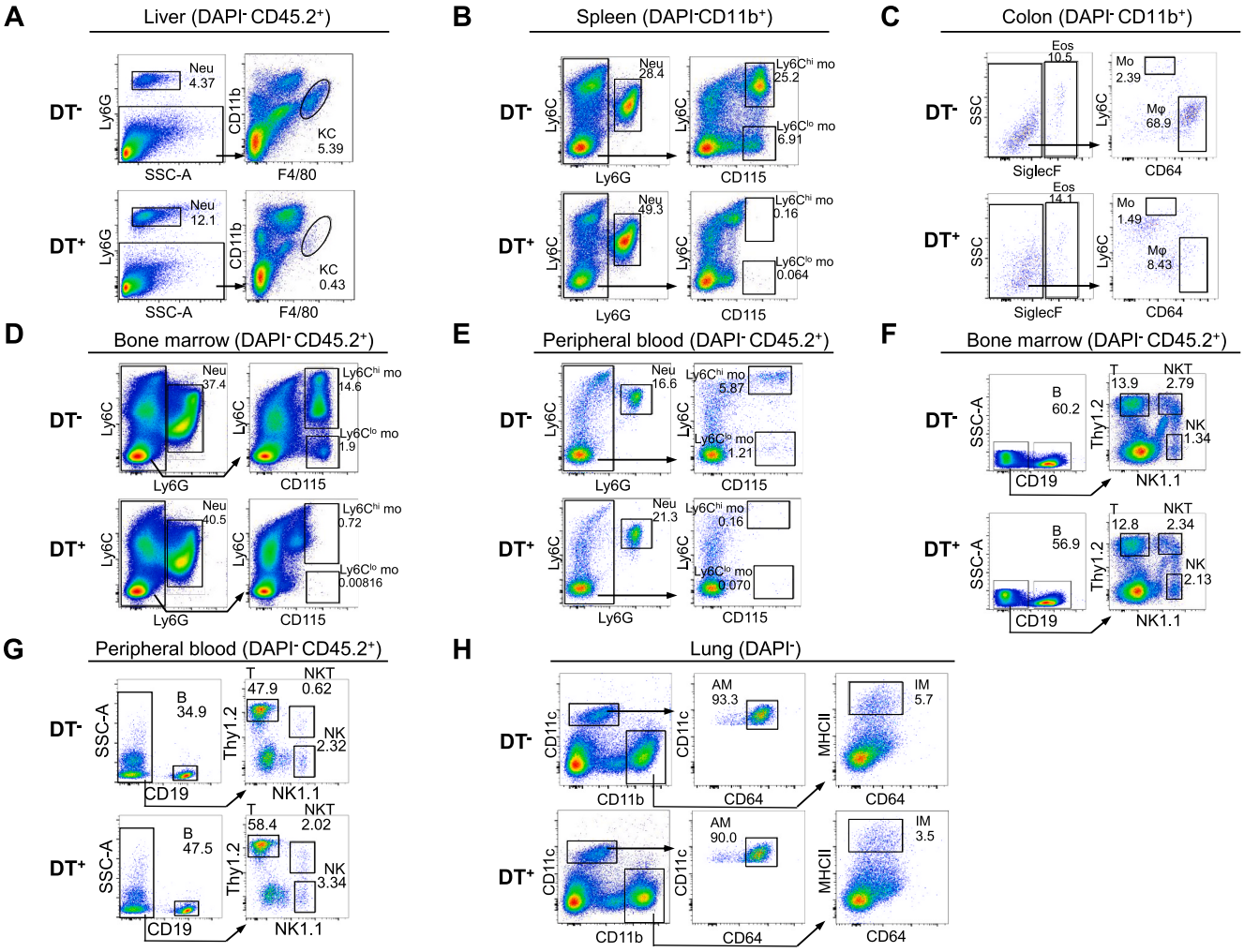


Fig. 3. Depletion of CD204$^+$ cells in DT-administered CD204-DTR mice. PBS or DT (500 ng/mouse) was i.p. injected into CD204-DTR (+/CD204DTR) mice. Various tissues and peripheral blood were obtained from these mice 24 h later. Liver (A), spleen (B), colon (C), bone marrow (D, F), peripheral blood (E, G), and lung (H). Mφ, macrophage; KC, Kupffer cell; Mo, monocyte; Neu, neutrophil; Eos, eosinophil; AM, alveolar macrophage; IM, interstitial macrophage; B, B cell; T, T cell; NKT, natural killer T cell; NK, natural killer cell.

administration in CD204-DTR mice.

3.3. CD204$^+$ cells protect mice from sepsis by suppressing proinflammatory cytokine production

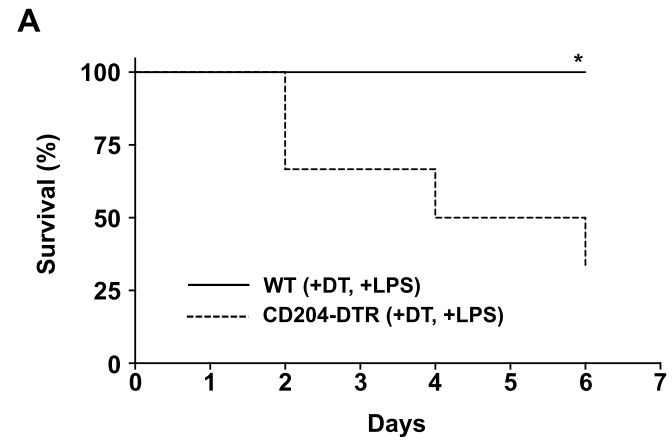
Having established an *in vivo* CD204$^+$ cell depletion system, we aimed to reveal the role of CD204$^+$ cells under an inflammatory condition. To this end, we injected DT into WT and CD204-DTR mice 24 h before systemic LPS injection. The injection of LPS caused hypotension and hypothermia characteristic of murine septic shock [14,15]. To our surprise, the survival rates of DT-injected CD204-DTR mice significantly decreased compared with those of DT-injected WT mice (Fig. 4A) or PBS-injected CD204-DTR mice (Fig. 4B). To investigate the reason for the increased mortality in CD204-DTR mice, we measured serum cytokine concentrations at different time points after LPS injection. Serum TNFα and IL-6 concentrations rapidly increased in the first 4 h and returned to basal levels as late as 16 h after LPS injection. We found that serum levels of TNFα at 2 h and IL-6 at 2 h, 4 h and 16 h were upregulated in CD204-DTR mice compared with WT mice (Fig. 4C). These results suggest that CD204$^+$ monocytes and/or macrophages suppress acute inflammatory response induced by LPS injection.

We further explored organs that produce increased levels of proinflammatory cytokines in CD204-DTR mice. qRT-PCR of tissues revealed that TNFα and IL-6 mRNA levels in spleen and TNFα mRNA level in lung were significantly upregulated in CD204-DTR mice compared with WT mice (Fig. 4D). IL-1β mRNA level was also upregulated in the lung and spleen although the difference was not statistically significant. Collectively, these results suggest that CD204$^+$ cells rescued mice from sepsis by regulating hyperinflammatory response.

4. Discussion

We were able to clarify two things in this study. Firstly, we revealed that CD204 expression is restricted to macrophages and monocytes in peripheral blood and some organs. Secondly, by depleting CD204$^+$ cells *in vivo* in CD204-DTR mice, we demonstrated that CD204$^+$ monocytes and/or macrophages protect mice from septic shock by suppressing TNFα and IL-6 production.

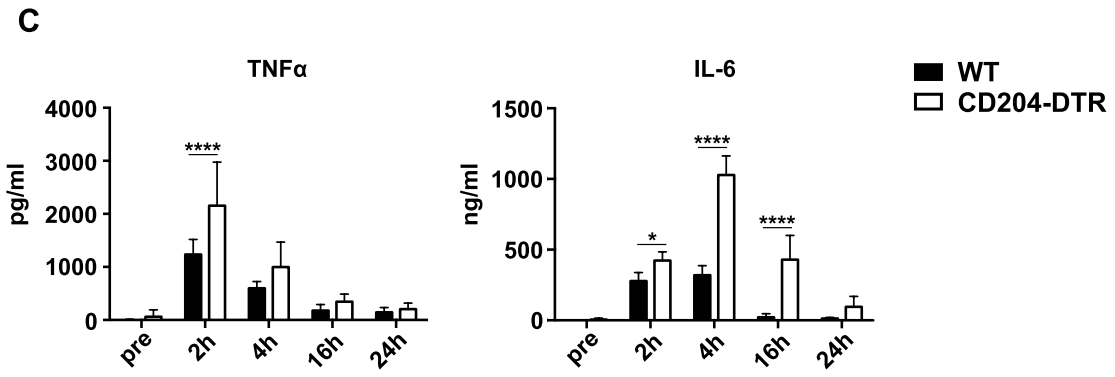
Drugs focusing on the regulation of hyperinflammatory immune response are being investigated for the treatment of septic shock [16]. Monocytes and macrophages are known to play important roles in resolving inflammation. We have recently reported that Ym1$^+$Ly6C^{hi} monocytes expand during the late phase of inflammation in bone marrow. Ym1$^+$ monocytes migrate to peripheral tissue where they promote tissue regeneration in a mouse colitis model [11]. Similarly, in an acute mucosal *Toxoplasma gondii* infection model, inflammatory



B

	Day 1	Day 2	Day 5
CD204-DTR (+PBS, +LPS)	4/4	4/4	4/4
CD204-DTR (+DT, +LPS)	0/3	0/3	0/3

Number of mice survived after LPS administration



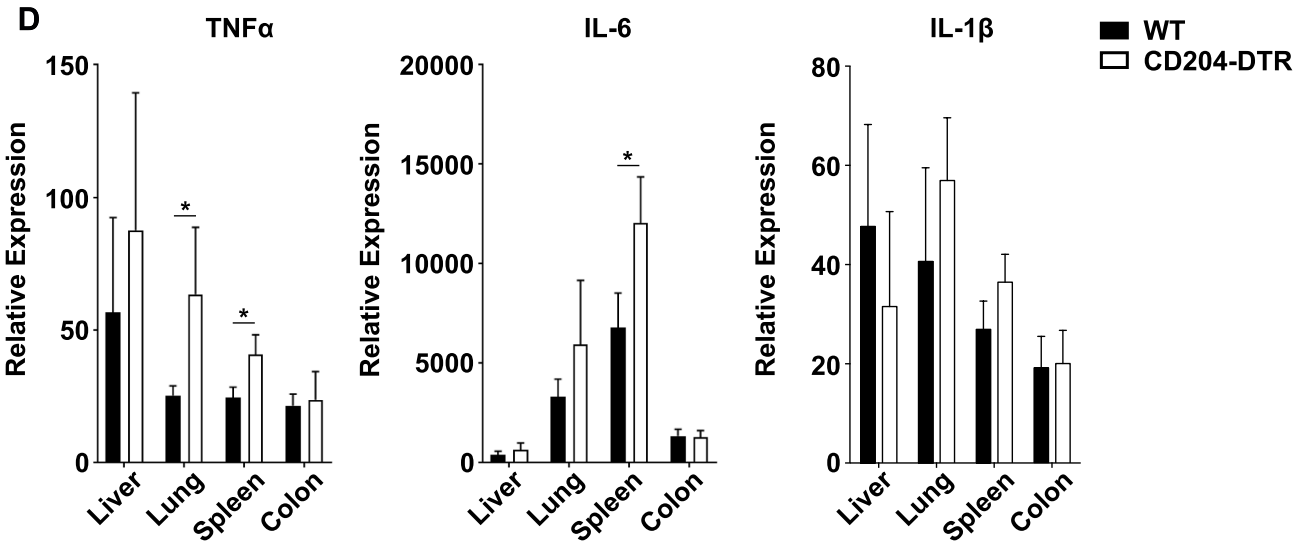


Fig. 4. Phenotype of CD204-DTR mice in septic condition. WT and CD204-DTR mice were intravenously injected with DT (500 ng/mouse) and LPS (2.5 mg/kg). (A) Survival rate of DT-injected WT and CD204-DTR mice (n = 6), log-rank test. (B) Survival of PBS- or DT-injected CD204-DTR mice. (C) Measurement of serum concentration of inflammatory cytokines. Peripheral blood obtained from these mice at the indicated hours after LPS injection (n = 4), two-way ANOVA with multiple comparisons. (D) Measurement of gene expression levels in various tissues by qRT-PCR. Liver, lung, spleen, and colon were collected from these mice 2 h after LPS injection (n = 4), Mann-Whitney t-test. Average values are shown with SD. *P < 0.05, ****P < 0.0001.

monocytes acquire a tissue-specific immunoregulatory phenotype that is associated with the increased production of lipid mediator prostaglandin E2 [17]. These findings suggest that some monocytes can suppress inflammatory response during inflammation. However, a subset of tissue resident macrophages that regulate immune response during sepsis was not identified.

We have shown that the transient and selective depletion of $CD204^+$ cells resulted in increased serum TNFα and IL-6 concentrations in LPS-induced septic shock. Moreover, the survival rate of septic mice was significantly decreased in the absence of $CD204^+$ cells. It was reported

that serum IL-6 concentration positively correlates with the severity of sepsis in human [18,19]. In addition, Marino's group demonstrated that TNFα-deficient mice are more resistant to LPS-induced sepsis than WT counterparts [20]. However, cells responsible for dysregulated production of pro-inflammatory cytokines in sepsis were up to now not identified. These reports and our findings indicate that CD204$^+$ monocytes and macrophages regulate host immune response by suppressing proinflammatory cytokine production. Tissue mRNA levels of TNFα and IL-6 were enhanced in spleen and lung of CD204$^+$ cell-depleted mice (Fig. 4D). However, we were unable to identify the cellular sources of the proinflammatory cytokines during sepsis. A recent study reported that marginal zone B cells in spleen produce IL-6 in the septic condition [21]. In addition, pulmonary endothelial cells, myocardial cells, and fibroblast cells can also produce TNFα and IL-6 [22]. These findings suggest that non-immune cells as well as B cells are responsible for the upregulated proinflammatory cytokine levels during sepsis. It remains to be determined whether CD204$^-$ macrophages produce larger amount of cytokines in the absence of CD204$^+$ macrophages. Exploration of the cellular source of TNFα and IL-6 during sepsis may yield novel therapeutic targets for septic shock. Although CD204 is predominantly expressed on macrophages and monocytes, *in vitro* observations suggested ectopic CD204 expression on endothelial cells [23], lung epithelial cells [24], and fibroblasts [25]. Therefore, we cannot exclude the possibility that CD204$^+$ non-parenchymal cells also contribute to the resolution of septic shock.

In conclusion, our study highlights the possibility that CD204$^+$ monocytes and/or macrophages ameliorate septic shock by directing the hyperinflammatory response toward resolution. As CD204$^+$ macrophages are closely associated with the prognosis of cancer patients [9,10,26], CD204-DTR mouse generated in this project may serve as a useful tool to understand the roles of CD204$^+$ monocytes and macrophages not only in sepsis but also in the pathophysiology of neoplasms.

Funding

This work was supported in part by Grants-in-Aid for Scientific Research (B) 17H04052 (to KA) and 17H04068 (to MT) from Japan Society for the Promotion of Science (JSPS), Scientific Research on Innovative Areas (Homeostatic regulation by various types of cell death) 26110006 (to MT) from the MEXT (Ministry of Education, Culture, Sports, Science and Technology), Japan, and AMED-CREST, AMED under grant number JP19gm1210002 (to MT).

Declaration of competing interest

The authors declare that no competing interests exist.

Acknowledgements

We thank Dr. Kohno for human DTR (HB-EGF) cDNA, Mrs. Kawana for secretarial assistance.

References

[1] M. Singer, C.S. Deutschman, C.W. Seymour, M. Shankar-Hari, D. Annane, M. Bauer, R. Bellomo, G.R. Bernard, J.D. Chiche, C.M. Coopersmith, R.S. Hotchkiss, M. M. Levy, J.C. Marshall, G.S. Martin, S.M. Opal, G.D. Rubenfeld, T. van der Poll, J. L. Vincent, D.C. Angus, The third international consensus definitions for sepsis and septic shock (Sepsis-3), J. Am. Med. Assoc. 315 (2016) 801–810.

[2] M. Cecconi, L. Evans, M. Levy, A. Rhodes, Sepsis and septic shock, Lancet 392 (2018) 75–87.

[3] A. Sica, A. Mantovani, Macrophage plasticity and polarization: in vivo veritas, J. Clin. Invest. 122 (2012) 787–795.

[4] F. Ginhoux, M. Guilliams, Tissue-resident macrophage ontogeny and homeostasis, Immunity 44 (2016) 439–449.

[5] T.A. Wynn, K.M. Vannella, Macrophages in tissue repair, regeneration, and fibrosis, Immunity 44 (2016) 450–462.

[6] C.D. Mills, K. Kincaid, J.M. Alt, M.J. Heilman, A.M. Hill, M-1/M-2 macrophages and the Th1/Th2 paradigm, J. Immunol. 164 (2000) 6166–6173.

[7] S. Gordon, F.O. Martinez, Alternative activation of macrophages: mechanism and functions, Immunity 32 (2010) 593–604.

[8] J.L. Goldstein, Y.K. Ho, S.K. Basu, M.S. Brown, Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition, Proc. Natl. Acad. Sci. USA 76 (1979) 333–337.

[9] Y. Miyasato, T. Shiota, K. Ohnishi, C. Pan, H. Yano, H. Horlad, Y. Yamamoto, M. Yamamoto-Ibusuki, H. Iwase, M. Takeya, Y. Komohara, High density of CD204-positive macrophages predicts worse clinical prognosis in patients with breast cancer, Canc. Sci. 108 (2017) 1693–1700.

[10] K. Kubota, M. Moriyama, S. Furukawa, H. Rafiul, Y. Maruse, T. Jinno, A. Tanaka, M. Ohta, N. Ishiguro, M. Yamauchi, M. Sakamoto, T. Maehara, J.N. Hayashida, S. Kawano, T. Kiyoshima, S. Nakamura, CD163(+)CD204(+) tumor-associated macrophages contribute to T cell regulation via interleukin-10 and PD-L1 production in oral squamous cell carcinoma, Sci. Rep. 7 (2017) 1755.

[11] N. Ikeda, K. Asano, K. Kikuchi, Y. Uchida, H. Ikegami, R. Takagi, S. Yotsumoto, T. Shibuya, C. Makino-Okamura, H. Fukuyama, T. Watanabe, M. Ohmuraya, K. Araki, G. Nishitai, M. Tanaka, Emergence of immunoregulatory Ym1(+)Ly6C (hi) monocytes during recovery phase of tissue injury, Sci. Immunol. 3 (2018).

[12] R.N. Hanna, C. Cekic, D. Sag, R. Tacke, G.D. Thomas, H. Nowyhed, E. Herrley, N. Rasquinha, S. McArdle, R. Wu, E. Peluso, D. Metzger, H. Ichinose, I. Shaked, G. Chodaczek, S.K. Biswas, C.C. Hedrick, Patrolling monocytes control tumor metastasis to the lung, Science 350 (2015) 985–990.

[13] K. Asano, N. Takahashi, M. Ushiki, M. Monya, F. Aihara, E. Kuboki, S. Moriyama, M. Iida, H. Kitamura, C.H. Qiu, T. Watanabe, M. Tanaka, Intestinal CD169(+) macrophages initiate mucosal inflammation by secreting CCL8 that recruits inflammatory monocytes, Nat. Commun. 6 (2015) 7802.

[14] M.P. Fink, Animal models of sepsis, Virulence 5 (2014) 143–153.

[15] A.J. Lewis, C.W. Seymour, M.R. Rosengart, Current murine models of sepsis, Surg. Infect. 17 (2016) 385–393.

[16] J. Rello, F. Valenzuela-Sanchez, M. Ruiz-Rodriguez, S. Moyano, Sepsis: a review of advances in management, Adv. Ther. 34 (2017) 2393–2411.

[17] J.R. Grainger, E.A. Wohlfert, I.J. Fuss, N. Bouladoux, M.H. Askenase, F. Legrand, L. Y. Koo, J.M. Brenchley, I.D. Fraser, Y. Belkaid, Inflammatory monocytes regulate pathologic responses to commensals during acute gastrointestinal infection, Nat. Med. 19 (2013) 713–721.

[18] J. Song, D.W. Park, S. Moon, H.J. Cho, J.H. Park, H. Seok, W.S. Choi, Diagnostic and prognostic value of interleukin-6, pentraxin 3, and procalcitonin levels among sepsis and septic shock patients: a prospective controlled study according to the Sepsis-3 definitions, BMC Infect. Dis. 19 (2019) 968.

[19] T. Shimazui, Y. Matsumura, T.A. Nakada, S. Oda, Serum levels of interleukin-6 may predict organ dysfunction earlier than SOFA score, Acute Med. Surg. 4 (2017) 255–261.

[20] M.W. Marino, A. Dunn, D. Grail, M. Inglese, Y. Noguchi, E. Richards, A. Jungbluth, H. Wada, M. Moore, B. Williamson, S. Basu, L.J. Old, Characterization of tumor necrosis factor-deficient mice, Proc. Natl. Acad. Sci. USA 94 (1997) 8093–8098.

[21] S. Honda, K. Sato, N. Totsuka, S. Fujiyama, M. Fujimoto, K. Miyake, C. Nakahashi-Oda, S. Tahara-Hanaoka, K. Shibuya, A. Shibuya, Marginal zone B cells exacerbate endotoxic shock via interleukin-6 secretion induced by Fcalpha/muR-coupled TLR4 signalling, Nat. Commun. 7 (2016) 11498.

[22] W. Schulte, J. Bernhagen, R. Bucala, Cytokines in sepsis: potent immunoregulators and potential therapeutic targets–an updated view, Mediat. Inflamm. 2013 (2013) 165974.

[23] M. Hashizume, M. Mihara, Blockade of IL-6 and TNF-alpha inhibited oxLDL-induced production of MCP-1 via scavenger receptor induction, Eur. J. Pharmacol. 689 (2012) 249–254.

[24] G.V. Limmon, M. Arredouani, K.L. McCann, R.A. Corn Minor, L. Kobzik, F. Imani, Scavenger receptor class-A is a novel cell surface receptor for double-stranded RNA, FASEB J 22 (2008) 159–167.

[25] S.J. DeWitte-Orr, S.E. Collins, C.M. Bauer, D.M. Bowdish, K.L. Mossman, An accessory to the 'Trinity': SR-As are essential pathogen sensors of extracellular dsRNA, mediating entry and leading to subsequent type I IFN responses, PLoS Pathog. 6 (2010), e1000829.

[26] Y. Komohara, K. Ohnishi, M. Takeya, Possible functions of CD169-positive sinus macrophages in lymph nodes in anti-tumor immune responses, Canc. Sci. 108 (2017) 290–295.