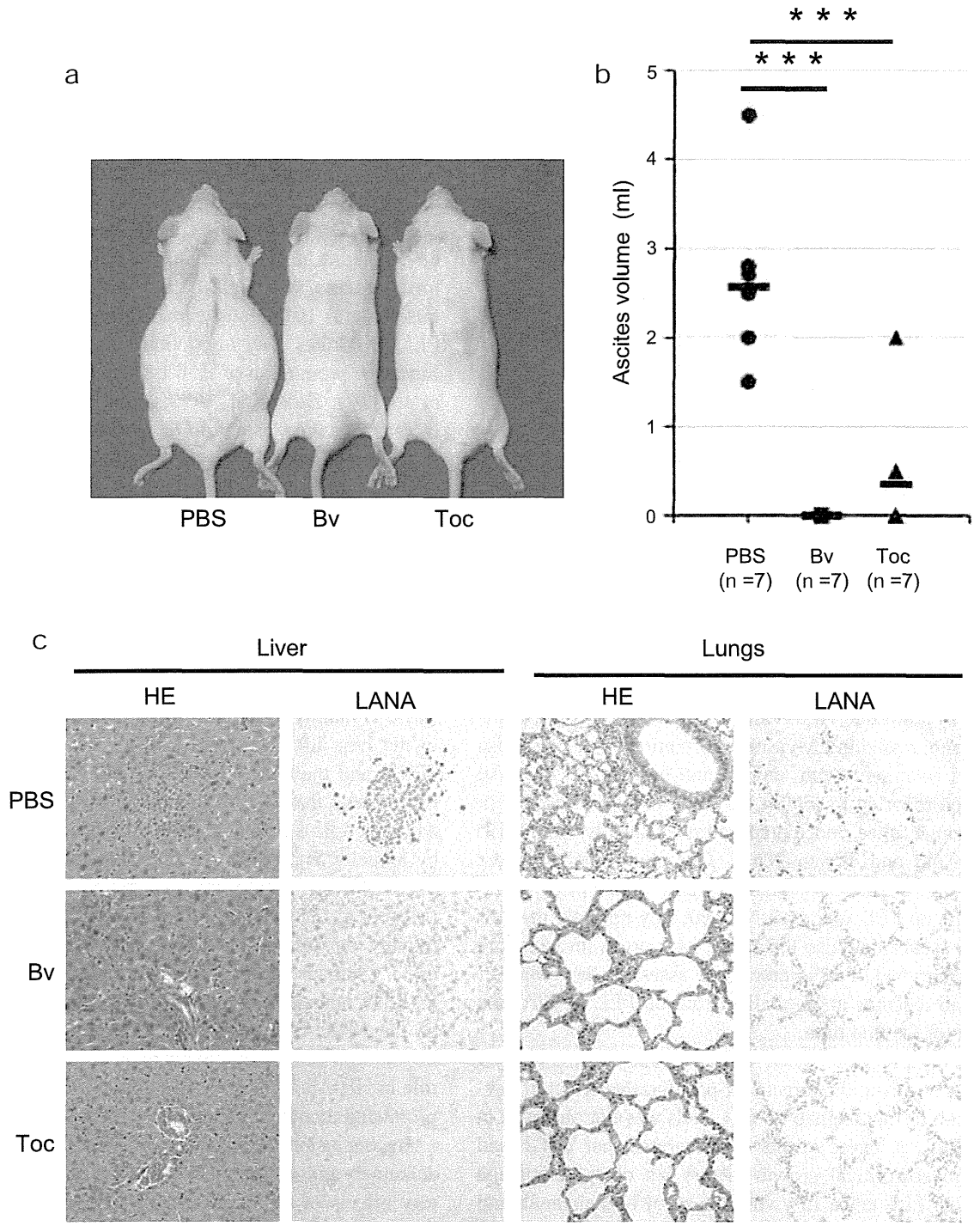

Fig. 5 Treatment of NOD/Rag-2/Jak3-deficient mice with bevacizumab (Bv) or tocilizumab (Toc) suppressed the development of PEL in vivo. **a** Photograph of untreated and treated ascites-bearing mice 4 weeks after inoculation with BCBL-1 intraperitoneally. **b** The volume of ascites 4 weeks after inoculation with BCBL-1 cells in mice is shown as the mean ± SD of 7 mice. $^{***}P < 0.001$ when compared with ascites volume. **c** Invasion of PEL cells into the organs of BCBL-1-inoculated mice on day 28. Hematoxylin–eosin staining and immunohistochemical staining using anti-LANA (PA1-73 N antibody) were performed to detect BCBL-1 in the liver and lungs

Discussion

PEL is a highly aggressive lymphoma that is resistant to conventional chemotherapy. Recent studies proposed new therapeutic strategies for PEL such as inhibition of NF-κB (Keller et al. 2000), activating TRAIL-mediated apoptosis by IFN-α and azidothymidine (Toomey et al. 2001; Wu et al. 2005), and inducing lytic replication of HHV-8

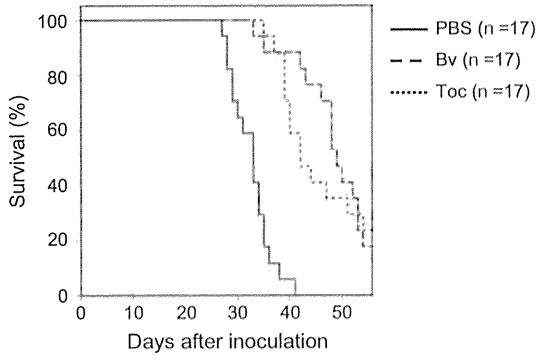

Fig. 6 Overall survival curve. Treatment with bevacizumab (Bv) or tocilizumab (Toc) prolonged survival in vivo

concomitantly with blocking virus production (Klass et al. 2005). These strategies are considered to be effective, but there is no proven standard therapy targeting specific molecules that are related to PEL pathogenesis. PEL has a unique clinical presentation with malignant effusion, causing treatment difficulty; therefore, targeting malignant effusion is a reasonable strategy in the treatment of PEL. We evaluated the efficacy of bevacizumab and tocilizumab in terms of controlling fluid retention. Although a direct anti-proliferative effect of bevacizumab or tocilizumab on PEL cells was not observed in vitro (Fig. 2), both mAbs significantly suppressed in vivo ascites formation in a PEL mouse model. Treatment with mouse anti-human VEGF mAb has been reported to inhibit the development of ascites in SCID/beige mice inoculated intraperitoneally with PEL cells (Aoki and Tosato 1999). Our study evaluated the therapeutic effect of a humanized anti-VEGF mAb, bevacizumab, on PEL xenograft NRJ mice by not only the volume of ascites, but also the efficacy for organ invasion and overall survival. Furthermore, we assessed the effect of anti-IL-6 receptor mAb, tocilizumab, on PEL in vitro and in vivo for the first time.

PEL cells produce VEGF and IL-6, and express IL-6Rα (Fig. 1); however, the direct anti-proliferative effect of bevacizumab or tocilizumab on PEL cells was not observed in vitro (Fig. 2). These results demonstrated that VEGF and IL-6 are not critical growth factors but other pathogenic factors in PEL cells. In vivo efficacy of bevacizumab and tocilizumab indicates the potential role of VEGF and IL-6 for fluid retention.

IL-6 signaling is characterized by the binding of mammalian forms of IL-6 to membrane-bound IL-6R. The IL-6/IL-6R complex binds and activates gp130, leading to downstream activation of signaling pathways such as the JAK/STAT and MAPK/ERK pathways. Unlike normal cells, constitutively activated Stat3 is detected in a wide variety of human cancer cells, including PEL (Aoki et al. 2003; Al Zaid Siddiquee and Turkson 2008). As depicted in Fig. 3, tocilizumab decreased VEGF mRNA and IL-6-induced VEGF production. Although Jak2 inhibitor AG490 directly suppressed Stat3 phosphorylation and induced apoptosis in PEL cells (Aoki et al. 2003), tocilizumab inhibited IL-6-mediated Stat3 phosphorylation (Fig. 4a) and Stat3 binding to VEGF promoter (Fig. 4b), inducing no growth inhibition (Fig. 2). We showed that IL-6 increased VEGF via additional Stat3 phosphorylation and Stat3 binding to VEGF promoter in PEL cells. The mechanism of tumor development in AIDS patients is a multistep and multifactorial process. Although the HIV-induced immunocompromised status is obviously involved in the development of PEL, cytokines may also contribute to the pathogenesis. Since the production of IL-6 is induced by HIV (Nakajima et al. 1989; Birx et al. 1990; Scala et al. 1994) and IL-6 increases in the plasma of HIV patients (Breen et al. 1990; Rieckmann et al. 1991), co-infection with HIV is considered to contribute to the pathogenesis of PEL, at least via the production of IL-6.

HHV-8-infected cells secrete not only human IL-6 (hIL-6) but also viral IL-6 (vIL-6). In contrast to hIL-6, vIL-6 does not require hIL-6R for receptor complex formation and signaling initiation (Molden et al. 1997; Osborne et al. 1999; Mullberg et al. 2000). vIL-6 has been also reported to promote VEGF secretion (Aoki et al. 1999); however, vIL-6 is mainly expressed not in latently infected cells but in the lytic lifecycle of HHV-8 infection (Nicholas et al. 1998), and the affinity of vIL-6 to gp130 is one thousand times lower than that of human IL-6 (Aoki et al. 2001). In addition, vIL-6 has been shown to cause the pathogenesis by inducing endogenous IL-6 expression in cell lines from patients with multicentric Castleman's disease (MCD) (Mori et al. 2000) and in transgenic (Tg) mice that constitutively express vIL-6 under control of the MHC class promoter (Suthaus et al. 2012). The production of endogenous IL-6 but not vIL-6 is largely required for the development of the MCD-like phenotype in Tg mice (Suthaus et al. 2012). Taken together, endogenous IL-6 plays an important role in HHV-8-associated diseases and is considered to be a promising therapeutic target, even in the presence of vIL-6.

In conclusion, we have shown the potent efficacy of bevacizumab and tocilizumab against PEL. Although inhibitory effects of tocilizumab on a PEL mouse model other than suppressing VEGF are expected because inhibition of VEGF was not complete in vitro, inhibition of IL-6R could be a promising therapeutic strategy for PEL from its in vivo effectiveness. Our data provide new insights into controlling fluid retention in PEL and the rationale for a clinical study in a single agent or in combination with conventional chemotherapy.

Acknowledgments We thank Ms. I. Suzu and Ms. S. Fujikawa for technical assistance and Ms. Y. Endo for secretarial assistance.

This work was supported by a Health and Labour Sciences Research Grant from the Ministry of Health, Labour, and Welfare of Japan (H25-AIDS-I-002), the Global COE program, "Global Education and Research Center Aiming at the Control of AIDS," and Grants-in-Aid for Science Research (No. 25114711) from the Ministry of Education, Science, Sports, and Culture of Japan.

Conflict of interest The authors have declared that no conflict of interest exists.

References

Akira S, Isshiki H, Sugita T, Tanabe O, Kinoshita S, Nishio Y, Nakajima T, Hirano T, Kishimoto T (1990) A nuclear factor for IL-6 expression (NF-IL6) is a member of a C/EBP family. EMBO J 9(6):1897–1906

Akira S, Nishio Y, Inoue M, Wang XJ, Wei S, Matsusaka T, Yoshida K, Sudo T, Naruto M, Kishimoto T (1994) Molecular cloning of APRF, a novel IFN-stimulated gene factor 3 p91-related transcription factor involved in the gp130-mediated signaling pathway. Cell 77(1):63–71

Al Zaid Siddiquee K, Turkson J (2008) STAT3 as a target for inducing apoptosis in solid and hematological tumors. Cell Res 18(2):254–267. doi:10.1038/cr.2008.18

Aoki Y, Tosato G (1999) Role of vascular endothelial growth factor/vascular permeability factor in the pathogenesis of Kaposi's sarcoma-associated herpesvirus-infected primary effusion lymphomas. Blood 94(12):4247–4254

Aoki Y, Jaffe ES, Chang Y, Jones K, Teruya-Feldstein J, Moore PS, Tosato G (1999) Angiogenesis and hematopoiesis induced by Kaposi's sarcoma-associated herpesvirus-encoded interleukin-6. Blood 93(12):4034–4043

Aoki Y, Narazaki M, Kishimoto T, Tosato G (2001) Receptor engagement by viral interleukin-6 encoded by Kaposi sarcoma-associated herpesvirus. Blood 98(10):3042–3049

Aoki Y, Feldman GM, Tosato G (2003) Inhibition of STAT3 signaling induces apoptosis and decreases survivin expression in primary effusion lymphoma. Blood 101(4):1535–1542. doi:10.1182/blood-2002-07-2130

Birx DL, Redfield RR, Tencer K, Fowler A, Burke DS, Tosato G (1990) Induction of interleukin-6 during human immunodeficiency virus infection. Blood 76(11):2303–2310

Boulanger E, Gerard L, Gabarre J, Molina JM, Rapp C, Abino JF, Cadranel J, Chevret S, Oksenhendler E (2005) Prognostic factors and outcome of human herpesvirus 8-associated primary effusion lymphoma in patients with AIDS. J Clin Oncol 23(19):4372–4380. doi:10.1200/JCO.2005.07.084

Breen EC, Rezai AR, Nakajima K, Beall GN, Mitsuyasu RT, Hirano T, Kishimoto T, Martinez-Maza O (1990) Infection with HIV is associated with elevated IL-6 levels and production. J Immunol 144(2):480–484

Castillo JJ, Shum H, Lahijani M, Winer ES, Butera JN (2012) Prognosis in primary effusion lymphoma is associated with the number of body cavities involved. Leuk Lymphoma 53(12):2378–2382. doi:10.3109/10428194.2012.694075

Cesarman E, Chang Y, Moore PS, Said JW, Knowles DM (1995) Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas. N Engl J Med 332(18):1186–1191. doi:10.1056/NEJM199505043321802

Cheranov SY, Karpurapu M, Wang D, Zhang B, Venema RC, Rao GN (2008) An essential role for SRC-activated STAT-3 in 14, 15-EET-induced VEGF expression and angiogenesis. Blood 111(12):5581–5591. doi:10.1182/blood-2007-11-126680

Drexler HG, Meyer C, Gaidano G, Carbone A (1999) Constitutive cytokine production by primary effusion (body cavity-based) lymphoma-derived cell lines. Leukemia 13(4):634–640

Ferrara N (2002) VEGF and the quest for tumour angiogenesis factors. Nat Rev Cancer 2(10):795–803. doi:10.1038/nrc909

Goto H, Kariya R, Shimamoto M, Kudo E, Taura M, Katano H, Okada S (2012) Antitumor effect of berberine against primary effusion lymphoma via inhibition of NF-kappaB pathway. Cancer Sci 103(4):775–781. doi:10.1111/j.1349-7006.2012.02212.x

Greene W, Kuhne K, Ye F, Chen J, Zhou F, Lei X, Gao SJ (2007) Molecular biology of KSHV in relation to AIDS-associated oncogenesis. Cancer Treat Res 133:69–127

Heinrich PC, Behrmann I, Haan S, Hermanns HM, Muller-Newen G, Schaper F (2003) Principles of interleukin (IL)-6-type cytokine signalling and its regulation. Biochem J 374(Pt 1):1–20. doi:10.1042/BJ20030407

Hong DS, Angelo LS, Kurzrock R (2007) Interleukin-6 and its receptor in cancer: implications for translational therapeutics. Cancer 110(9):1911–1928. doi:10.1002/cncr.22999

Keller SA, Schattner EJ, Cesarman E (2000) Inhibition of NF-kappaB induces apoptosis of KSHV-infected primary effusion lymphoma cells. Blood 96(7):2537–2542

Kishimoto T (2005) Interleukin-6: from basic science to medicine–40 years in immunology. Annu Rev Immunol 23:1–21. doi:10.1146/annurev.immunol.23.021704.115806

Klass CM, Krug LT, Pozharskaya VP, Offermann MK (2005) The targeting of primary effusion lymphoma cells for apoptosis by inducing lytic replication of human herpesvirus 8 while blocking virus production. Blood 105(10):4028–4034. doi:10.1182/blood-2004-09-3569

Molden J, Chang Y, You Y, Moore PS, Goldsmith MA (1997) A Kaposi's sarcoma-associated herpesvirus-encoded cytokine homolog (vIL-6) activates signaling through the shared gp130 receptor subunit. J Biol Chem 272(31):19625–19631

Mori Y, Nishimoto N, Ohno M, Inagi R, Dhepakson P, Amou K, Yoshizaki K, Yamanishi K (2000) Human herpesvirus 8-encoded interleukin-6 homologue (viral IL-6) induces endogenous human IL-6 secretion. J Med Virol 61(3):332–335

Mullberg J, Geib T, Jostock T, Hoischen SH, Vollmer P, Voltz N, Heinz D, Galle PR, Klouche M, Rose-John S (2000) IL-6 receptor independent stimulation of human gp130 by viral IL-6. J Immunol 164(9):4672–4677

Nagy JA, Meyers MS, Masse EM, Herzberg KT, Dvorak HF (1995) Pathogenesis of ascites tumor growth: fibrinogen influx and fibrin accumulation in tissues lining the peritoneal cavity. Cancer Res 55(2):369–375

Nakajima K, Martinez-Maza O, Hirano T, Breen EC, Nishanian PG, Salazar-Gonzalez JF, Fahey JL, Kishimoto T (1989) Induction of IL-6 (B cell stimulatory factor-2/IFN-beta 2) production by HIV. J Immunol 142(2):531–536

Nicholas J, Zong JC, Alcendor DJ, Ciufo DM, Poole LJ, Sarisky RT, Chiou CJ, Zhang X, Wan X, Guo HG, Reitz MS, Hayward GS (1998) Novel organizational features, captured cellular genes, and strain variability within the genome of KSHV/HHV8. J Natl Cancer Inst Monogr 23:79–88

Osborne J, Moore PS, Chang Y (1999) KSHV-encoded viral IL-6 activates multiple human IL-6 signaling pathways. Hum Immunol 60(10):921–927

Rieckmann P, Poli G, Kehrl JH, Fauci AS (1991) Activated B lymphocytes from human immunodeficiency virus-infected individuals induce virus expression in infected T cells and a promonocytic cell line, U1. J Exp Med 173(1):1–5

Scala G, Ruocco MR, Ambrosino C, Mallardo M, Giordano V, Baldassarre F, Dragonetti E, Quinto I, Venuta S (1994) The expression of the interleukin 6 gene is induced by the human immunodeficiency virus 1 TAT protein. J Exp Med 179(3):961–971

Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF (1983) Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science 219(4587):983–985

Shih T, Lindley C (2006) Bevacizumab: an angiogenesis inhibitor for the treatment of solid malignancies. Clin Ther 28(11):1779–1802. doi:10.1016/j.clinthera.2006.11.015

Suthaus J, Stuhlmann-Laeisz C, Tompkins VS, Rosean TR, Klapper W, Tosato G, Janz S, Scheller J, Rose-John S (2012) HHV-8-encoded viral IL-6 collaborates with mouse IL-6 in the development of multicentric Castleman disease in mice. Blood 119(22):5173–5181. doi:10.1182/blood-2011-09-377705

Tanaka T, Narazaki M, Kishimoto T (2012) Therapeutic targeting of the interleukin-6 receptor. Annu Rev Pharmacol Toxicol 52:199–219. doi:10.1146/annurev-pharmtox-010611-134715

Toomey NL, Deyev VV, Wood C, Boise LH, Scott D, Liu LH, Cabral L, Podack ER, Barber GN, Harrington WJ Jr (2001) Induction of a TRAIL-mediated suicide program by interferon alpha in primary effusion lymphoma. Oncogene 20(48):7029–7040. doi:10.1038/sj.onc.1204895

Trikha M, Corringham R, Klein B, Rossi JF (2003) Targeted anti-interleukin-6 monoclonal antibody therapy for cancer: a review of the rationale and clinical evidence. Clin Cancer Res 9(13):4653–4665

Wu W, Rochford R, Toomey L, Harrington W Jr, Feuer G (2005) Inhibition of HHV-8/KSHV infected primary effusion lymphomas in NOD/SCID mice by azidothymidine and interferon-alpha. Leuk Res 29(5):545–555. doi:10.1016/j.leukres.2004.11.010