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Model of Angiogenesis in Mice with Severe Combined Immunodeficiency (SCID) and Xenoengrafted with Epstein-Barr Virus–Transformed B Cells

Nina L. Woodford, DVM,[1] Douglas R. Call, PhD,[2] Daniel G. Remick, MD,[2] and Rosemary Rochford, PhD[3,*]

Xenoengraftment of human cells in mice with severe combined immunodeficiency (SCID) has been used as a model system to study the mechanisms of B-cell lymphomagenesis. In the study reported here, we determined that SCID mice can also be used as a model to study angiogenesis in B-cell lymphomas. The C.B-17 scid/scid mice were xenotransplanted with Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines (LCL), and we determined whether CD31, a marker found on endothelial cells, was detected in the human B-cell lymphomas that developed in these mice. Microvessel formation was identified by use of immunohistochemical staining for CD31. To assess possible mechanisms of angiogenic stimulus, we analyzed the expression of interleukin 8 (IL-8), a chemokine documented to promote angiogenesis, in non–small-cell lung cancer and bronchogenic carcinomas. We observed that a panel of LCL and LCL-lymphomas expressed IL-8 mRNA and protein. Neutralization of IL-8, however, did not inhibit lymphomagenesis, suggesting that IL-8 is not essential for angiogenesis in this model. To examine other parameters of angiogenesis, we identified expression of vascular endothelial growth factor in the lymphomas. These data suggest that angiogenesis accompanies EBV-associated B-cell lymphoma development, but IL-8 is not essential for this process. Thus, the SCID mouse model is amenable to testing of anti-angiogenic factors.

Angiogenesis is the development of new blood vessels from preexisting blood vessels and capillaries. Solid tumors necessarily promote angiogenesis, and this process has stimulated research into the control of neovascularization and the factors that induce blood vessel growth. The current paradigm is that tumor-related angiogenesis is driven from an imbalance of angiostatic and angiogenic factors that favors overexpression of the angiogenic factors (42). Examination of tumor-related angiogenesis has identified several angiogenic factors, including basic fibroblast growth factor, vascular endothelial growth factor (VEGF), and interleukin 8 (IL-8) (18, 29, 43).

Epstein-Barr virus (EBV) is a gammaherpesvirus with strong transformation potential in vitro (15). In vivo, EBV is associated with the immunoblastic B-cell lymphomas (IBL) and lymphoproliferative disorders (LPD) that develop in patients undergoing immunosuppressive therapy to prevent solid organ transplant rejection, in patients with acquired immune deficiency syndrome (AIDS), and in patients with primary immune deficiencies (6, 19, 32). EBV immortalizes B lymphocytes, resulting in the establishment of lymphoblastoid cell lines (LCL) and expression of the EBV latent proteins. Although LCL are useful for the molecular characterization of EBV transformation, they do not model the microenvironment that develops during lymphomagenesis. An alternative and clinically relevant model is lymphoma development in SCID mice engrafted with LCL (LCL-SCID lymphomas), or engraftment with peripheral blood lymphocytes from EBV seropositive donors (PBL-SCID lymphomas) (25, 33, 37). These lymphomas have been documented to represent a valid in vivo model system to study the mechanisms of lymphomagenesis (11, 14, 34, 35).

The EBV encodes nine latent proteins, including latent membrane protein 1 (LMP-1), which is essential for viral transformation and activates a variety of cellular genes (15). Transient transfection of HeLa cells or C33A cells with LMP-1 induces IL-8 gene expression (10, 52). Furthermore, induction of IL-8 by LMP-1 has been proposed to contribute to angiogenesis in nasopharyngeal carcinoma, an EBV-associated epithelial malignancy (52). Nevertheless, expression of IL-8 in EBV-associated IBL and LPD has not been determined. In addition, it has not been assessed whether angiogenesis accompanies EBV-driven B-cell lymphomagenesis.

The use of immunodeficient mice xenografted with human tumor cell lines has been a valuable model system to evaluate the mechanisms of solid tumor-related angiogenesis (42). In published studies, IL-8 was positively correlated with murine neovascularization in human tumor/mouse chimeras for ovarian carcinoma, gastric carcinoma, melanoma, non–small-cell lung cancer, and prostate cancer (1, 16, 22, 39, 50). The objective of the study reported here was to evaluate the usefulness of the SCID mouse model for the study of angiogenesis in B-cell lymphomas. In addition, we tested the hypothesis that neutralization of IL-8 would reduce lymphoma vascularity and growth in SCID mice xenografted with EBV-transformed B cells.

Materials and Methods

Culture of LCL. The derivation of the LCL used in this study has been described (4, 35). All lines were cultured in RPMI-1640 medium (GIBCO-BRL, Bethesda, Md.) with 10% fetal bovine se-

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[1]Unit for Laboratory Animal Medicine, [2]Department of Pathology, University of Michigan, Ann Arbor, Michigan, [3]Department of Microbiology and Immunology, SUNY Upstate Medical University, 750 East Adams Street, Syracuse, New York.
[*]Corresponding author.

rum and supplemented with 100 m*M* glutamine, penicillin, and streptomycin (GIBCO-BRL).

Mice. Four-week-old female C.B.-17/IcrHsd-*scid* mice were obtained from Harlan Sprague-Dawley (Indianapolis, Ind.). The University Committee for the Use and Care of Animals (University of Michigan) approved all experiments in mice prior to their initiation. All mice were housed in sterile Micro-Isolator™ (Lab Products Inc., Seaford, Del.) with autoclaved food, water, and bedding. Animal work was conducted, using biosafety level (BSL)-2 operating procedures and policies, in a BSL-2 facility with approval of the Institutional Biosafety Committee. Health monitoring information from the vendor indicated that the mice tested free of respiratory tract and enteric bacteria, ecto- and endoparasites, and the following serologic antigens: *Mycoplasma pulmonis*, *Encephalitozoon cuniculi*, cilia-associated respiratory bacillus, Sendai virus, pneumonia virus of mice, minute virus of mice, mouse hepatitis virus, Theiler's mouse encephalomyelitis virus, reovirus 3, hantavirus, mouse thymic virus, ectromelia virus, epizootic diarrhea of infant mice virus, mouse adenovirus, lymphocytic choriomeningitis virus, mouse cytomegalovirus, polyoma virus, and parvovirus. Mice were screened for the presence of mouse immunoglobulin (Ig) in serum by use of an enzyme-linked immunosorbent assay (ELISA) (30). Only mice with < 10 μg of mouse Ig/ml were used in subsequent studies. Five × 10^6 cells of LCL in logarithmic growth phase were injected intraperitoneally. Mice were sacrificed when clinical signs of illness were observed and were completely necropsied, then intraperitoneal lymphomas were recovered. The thoracic cavity was opened and examined for the presence of lymphomas, but none were observed.

The recovered lymphomas were designated LCL-SCID lymphomas. Portions of the recovered lymphomas were snap frozen or were fixed in 10% formalin for histologic and immunohistochemical analysis. Formalin-fixed specimens were paraffin-embedded. Sections were stained with hematoxylin and eosin and were examined for histopathologic changes by use of light microscopy. Snap-frozen specimens were used for immunohistochemical staining. The remaining lymphoma tissue was dispersed into single-cell suspensions by mincing the tissue in RPMI 1640 medium, then passing the cells through a cell strainer (Falcon, Becton Dickinson Labware, Franklin Lakes, N.J.). Dead cells and cell debris were eliminated by use of density gradient centrifugation with Ficoll-Hypaque (Pharmacia, Piscataway, N.J.). Cell preparations were then aliquoted for RNA extraction.

Enzyme-linked immunosorbent assay. The LCL and corresponding lymphoma cells were plated at a concentration of 7.5 × 10^5 cells/ml in triplicate wells of a 96-well plate and were cultured for 48 h. Supernatant was removed and analyzed for IL-8 expression by use of an ELISA as described (7). Measurement of human Ig in serum of mice was done as described (34).

Extraction of RNA and ribonuclease protection assay. The RNA from LCL and the corresponding lymphoma cells was extracted according to the one-step method of Chomczynski and Sacchi (5). A multi-probe ribonuclease protection assay (RPA) was done as described (13), using riboprobes specific for human IL-8 and human VEGF (BD-Pharmingen, San Diego, Calif.). Yeast tRNA (5 μg) was included as a negative control in each RPA, and bands were not detected. For signal quantification, data were analyzed, using the Storm Imager and ImageQuant software (Molecular Dynamics, Sunnyvale, Calif.) as described (35). For the latter, volume measurement with rectangular objects was used to generate PhosphorImager (PI) counts, which are presented as a percentage of the internal housekeeping signal (i.e., L32) present in each lane.

Immunohistochemical analysis. Cryostat sections (6 to 7 μm thick) were fixed in acetone for one minute immediately after sectioning, and were stored at –80°C. Serial lymphoma sections were rehydrated for five minutes in phosphate-buffered saline (PBS) plus 0.1% saponin (PBS-saponin), and endogenous peroxidase activity was blocked by a 30-min incubation in PBS-saponin with 1% H_2O_2. Nonspecific binding was blocked by use of the avidin-biotin blocking kit in the presence of normal horse serum (Vector Laboratories, Burlingame, Calif.). Sections were washed and overlaid with a 1:20 dilution of either isotype control or rat anti-mouse CD31 monoclonal antibody ([mAb] BD- Pharmingen). Slides were then rinsed and overlaid with secondary biotinylated rabbit anti-mouse IgG (10 μg/ml) and were incubated for 30 min at room temperature. The remainder of the immunohistochemical staining was done according to the manufacturer's protocol for the Vector ABC Elite Kit, using a peroxidase enzyme (Vector Laboratories). Three-amino-9-ethylcarbozole was used as the enzyme substrate (Vector Laboratories), and slides were counterstained with Mayer's hematoxylin. Slides were mounted by use of aqueous mounting media (Biomeda, Foster City, Calif.), and were allowed to dry 24 h before microscopic viewing.

In vivo treatment with anti-IL-8 antibody. The EW-LCL cells (5 × 10^6) were administered intraperitoneally to 24 C.B.-17 SCID mice. Rabbit serum (0.25 ml) containing polyclonal anti-human IL-8 antibody (7) diluted with 0.25 ml of sterile saline was administered subcutaneously to 12 mice every 48 h. Pre-immune rabbit serum (0.25 ml) diluted with 0.25 ml sterile saline was administered to six mice (serum controls) every 48 h, and 0.5 ml of sterile saline was administered subcutaneously to six mice (saline controls) every 48 h (1). When clinical signs of illness were observed, the mice were anesthetized with methoxyflurane (Schering-Plough Animal Health Corp., Union, N.J.), blood was drawn from the retro-orbital sinus, and mice were then sacrificed by use of cervical dislocation. Serum was collected from the mouse blood after separation by use of centrifugation. Intraperitoneal lymphomas were harvested and weighed on an analytical balance to determine total lymphoma mass. Portions of the recovered lymphomas were snap frozen and used for subsequent histologic and immunohistochemical analyses. One portion of each lymphoma was snap frozen, then was homogenized in anti-protease buffer (phosphate-buffered saline supplemented with 1 m*M* EDTA, 54 μg of trans-epoxysuccinyl-L-leucylamido-[4-guanidino]butane/ml, 0.1 mg of aprotinin/ml, and 1 μg of leupeptin/ml; Sigma Chemical Co., St. Louis, Mo.). The supernatant was used to measure the amount of IL-8 by use of the aforementioned ELISA.

Quantification for microvessel density. Intratumor microvessel density was assessed by use of light microscopy. Scanning sections at low power (200× total magnification) identified areas of high neovascularization. Within these areas, microvessel counts in a 400×-magnified field were made, with five fields counted per lymphoma. Three separate lymphomas were examined per treatment group (anti-IL-8 antibody, saline control, or serum control).

Results

Expression of IL-8 in LCL and LCL-SCID lymphomas. We analyzed expression of IL-8 in a panel of LCL that were de-

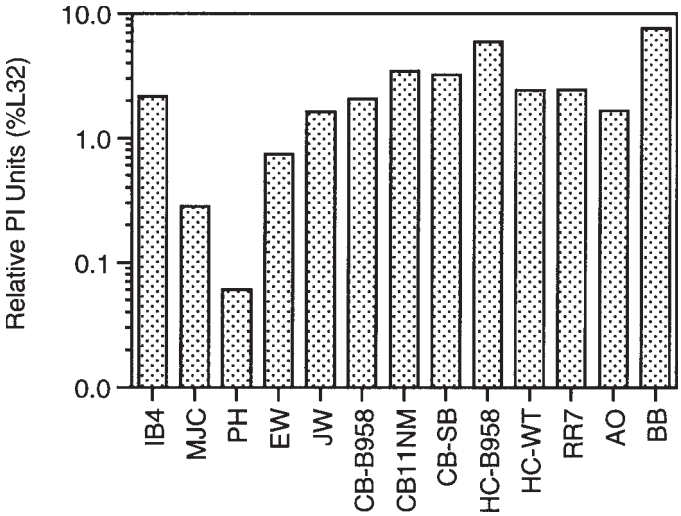

Figure 1. Interleukin 8 (IL-8) mRNA expression in lymphoblastoid cell lines (LCL). RNA was extracted from LCL during logarithmic-phase growth, and aliquots originating from 10^6 input cells were subjected to a ribonuclease protection assay (RPA) to measure IL-8 expression. The PhosphorImager (PI) counts for the IL-8-protected probe were obtained, and the data are presented as percentage of the internal housekeeping signal (i.e., L32) in each lane.

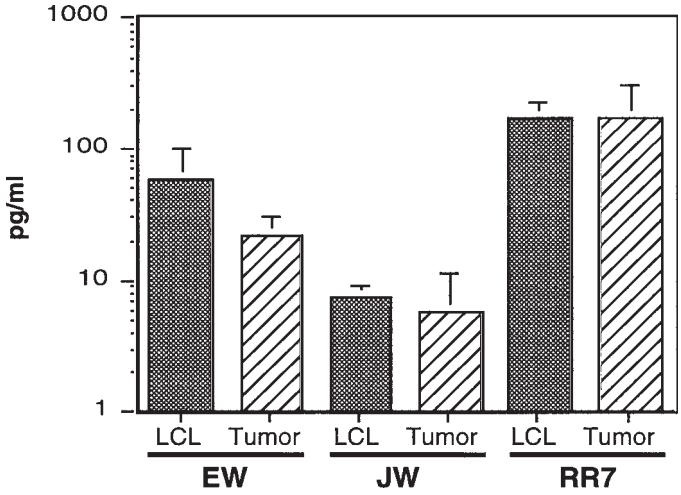

Figure 2. Interkeukin 8 protein production by LCL and LCL-lymphomas. Single cell suspensions of LCL or LCL-derived lymphomas were cultured for 48 h, and the amount of IL-8 in the supernatant was measured by use of an IL-8-specific ELISA. Standard error of the mean (SEM) for LCL was obtained by performing ELISA on three different experiments. The SEM for lymphoma was obtained from separate lymphoma samples (EW, n = 9; JW, n = 5; RR7, n = 6).

rived by transformation with the B95-8 strain of EBV (MJC, PH, EW, JW, CB-B958), by transformation with wild-type isolates of EBV (CB-NM, CB-SB, HC-WT), or by outgrowth of B-cell lymphomas (RR7, AO, BB). Although there was some variation in the amount of IL-8 mRNA, all LCL tested expressed IL-8 mRNA, as measured by use of the RPA (Fig. 1).

To determine whether IL-8 expression is maintained following lymphomagenesis, we generated B-cell lymphomas by engrafting SCID mice with the EW, JW, and RR-7 LCL. Lymphomas were harvested when mice manifested clinical signs of illness, and single-cell suspensions of the tumor cells and the parental LCL were placed in culture for 48 h. Expression of IL-8 in the supernatant of the cultured cells was measured by use of an IL-8-specific ELISA (Fig. 2). Interleukin 8 was secreted from LCL and lymphomas derived from the LCL, thereby documenting that LCL-lymphomas are capable of producing IL-8 protein and that IL-8 expression is maintained during lymphomagenesis.

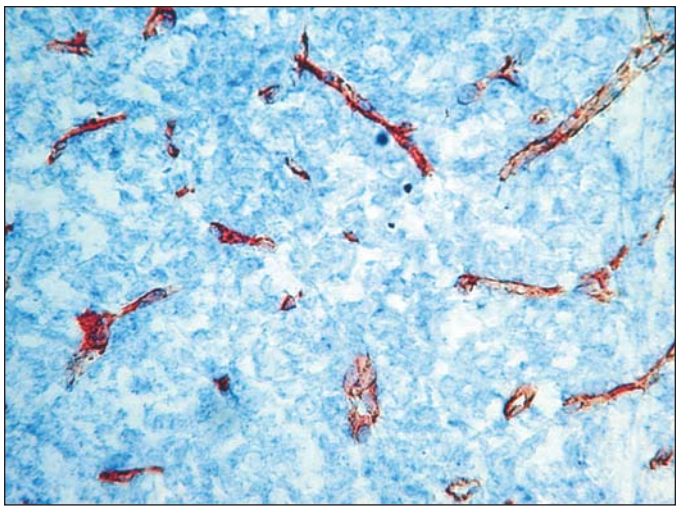

Figure 3. Photomicrograph of a section from a representative LCL-lymphoma sample to detect microvessels in LCL-SCID lymphomas. Cryostat sections from EW LCL-SCID lymphoma were incubated with the CD31-specific monocclonal antibody (mAb) to detect the presence of endothelial cells. Eight EW-LCL lymphomas were examined. Staining was visualized by use of indirect peroxidase staining, with three-amino-9-ethylcarbozole (AEC) as the substrate (red staining). Mayer's hematoxylin was used as the counterstain (blue). Magnification, 400×.

Identification of microvessels in the lymphomas. Interleukin 8 can induce angiogenesis (43), but it has not been documented that angiogenesis is occurring in the EBV-associated lymphomas. A marker of angiogenesis is the presence of endothelial cells that line the blood vessels. The CD31 (also called PECAM-1) is a marker found on endothelial cells (2, 47). To determine whether microvessels were present in the LCL-SCID lymphomas, immunohistochemical analysis was performed on eight EW-LCL-SCID lymphomas, using rat anti- mouse CD31 mAb. Examination of tissue sections stained with anti-CD31 mAb clearly revealed identifiable microvessels of murine origin within the lymphoma tissue (Fig. 3). These data indicate that lymphomagenesis in the SCID mouse model was accompanied by angiogenesis.

Neutralization of IL-8 did not inhibit lymphoma development. As IL-8 has been reported to be an angiogenic factor, and neutralization of IL-8 inhibited tumor development in a small-cell lung carcinoma transplanted into SCID mice (1), we hypothesized that neutralization of IL-8 in our LCL-SCID lymphoma model would inhibit tumor development by blocking angiogenesis. To test this hypothesis, the EW LCL cells were administered to SCID mice. Mice then received injections of neutralizing IL-8 antibodies (n = 12), serum control (n = 6), or saline alone (n = 6) every other day for 17 days. The duration from injection of LCL to tumor development is characteristic for each LCL (34). From previous experiments, mean time to tumor development following injection of EW LCL was 21.2 days, and we have not observed mice dying before 18 days after injection. Surprisingly, in this experiment, most mice treated with the IL-8 antibodies had clinical signs of illness at day 17, and two died on that

day. Blood was collected from the remaining mice, which then were sacrificed on days 18 and 19. The two mice that died before experimental termination were necropsied, and lymphomas were found in the peritoneal cavity. These particular lymphomas were not included in subsequent analysis. At the end of the experiment, all remaining mice had lymphomas in the peritoneal cavity. Lymphomas were carefully dissected to obtain all visible lymphomas present in the peritoneal cavity, and lymphoma mass was measured. Mice were also completely necropsied to verify that lymphomas had not spread to the thoracic cavity or other sites in the mouse.

We observed that the average lymphoma mass in the anti-IL8-treated mice was significantly greater than the lymphoma mass in the saline-treated control mice, whereas there was no difference in tumor mass between mice of the serum and saline control groups (Fig. 4A). Serum human Ig concentration in the anti-IL-8-treated mice was significantly higher than that in the control mice (Fig. 4B). Overall, serum human Ig concentration correlated strongly with tumor burden ($r = 0.76$, $P < 0.001$). To confirm that the dose of neutralizing anti-IL-8 antibodies was sufficient to neutralize the IL-8 produced by the lymphoma cells, IL-8 values for all tumor homogenates were determined by use of the ELISA. Amounts of IL-8 in the tumors from mice receiving the neutralizing IL-8 antibody were significantly less than those in tumors from either the serum or saline control-treated mice, though the amounts were not completely reduced (Fig. 4C). These findings plus the documented neutralizing capabilities of these antibodies (7) indicate that the anti-IL-8 antibody significantly reduced IL-8 production by the lymphoma cells.

Microvessel density of lymphomas. To determine whether intratumor microvessel density differed between lymphomas derived from mice treated with neutralizing anti-IL-8 antibody, cryostat sections from three lymphomas per treatment group were stained with anti-CD31 mAb to detect the presence of endothelial cells within the lymphoma, and microvessel counts were made from a 400×-magnified field, with five fields counted per lymphoma (Fig. 5). There was no significant difference in microvessel density between lymphomas derived from the anti-IL-8 antibody-treated mice and lymphomas derived from mice receiving serum or saline, indicating that reduction of IL-8 did not result in inhibition of angiogenesis in this tumor model.

Expression of VEGF in lymphomas. To test whether other angiogenic factors might be expressed in the lymphomas, we extracted RNA from the EW-LCL and five EW-LCL-SCID lymphomas and analyzed the expression of VEGF mRNA, using an RPA (Fig. 6). We found that all of the LCL-lymphomas expressed VEGF mRNA, although there was some variability in the amount of VEGF expression in the tumors. The VEGF also was expressed in LCL-lymphomas derived from the engraftment of the JW and RR7 LCL into SCID mice (data not shown). Although mRNA expression does not necessarily translate into subsequent changes in amounts of functional protein, it is possible that VEGF is driving angiogenesis in this model system.

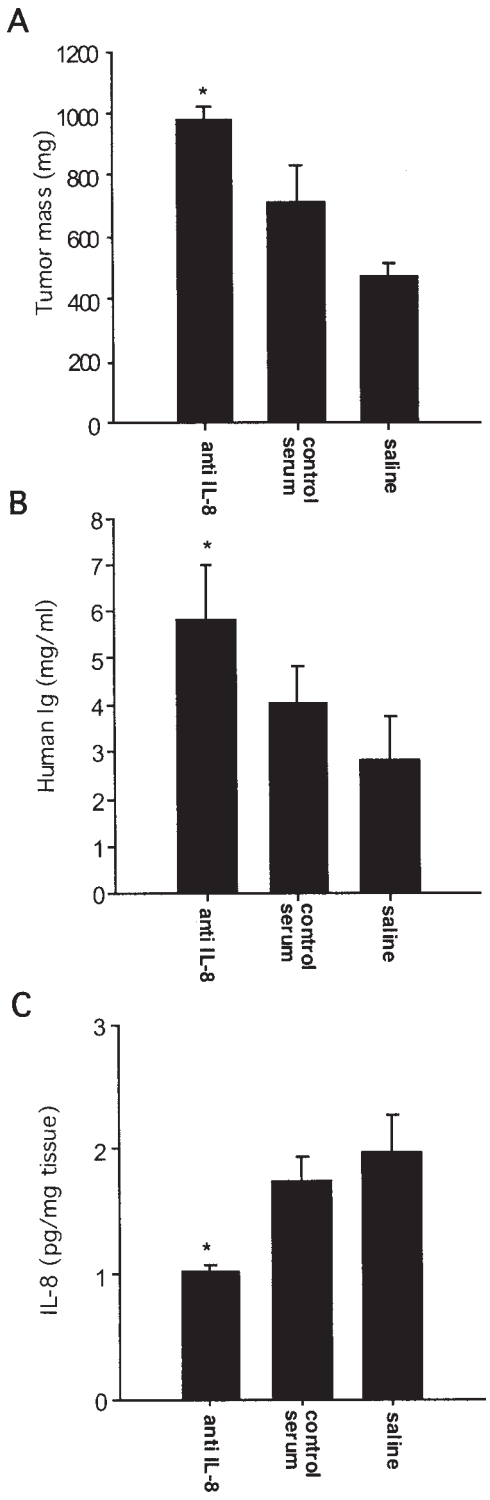

Figure 4. Effect of IL-8 inhibition on lymphomagenesis. SCID mice, inoculated intraperitoneally with EW-LCL, were treated with a neutralizing anti-IL-8 antibody (n = 12), control serum (n = 6) or saline (n = 6) subcutaneously every 48 h for 17 days. All lymphomas were harvested at 18 to 19 days after inoculation. (A) The lymphomas were weighed on an analytical scale to measure their mass. $^{*}P = 0.022$. (B) Circulating concentration of human Ig in SCID mice bearing B-cell lymphomas. Serum was collected at the time of sacrifice and was analyzed for human Ig concentration by use of an ELISA. $^{*}P < .0001$. (C) A pre-weighed portion of each lymphoma was homogenized in anti-protease buffer and was analyzed for IL-8 values by use of an ELISA. $^{*}P = 0.007$.

Discussion

We identified expression of two angiogenic factors, VEGF and IL-8, in LCL and LCL-SCID B-cell lymphomas. Furthermore, we demonstrated that microvessels were present in the lymphomas, confirming that angiogenesis occurs during lymphomagenesis. We observed the constitutive expression of IL-8 in LCL that is

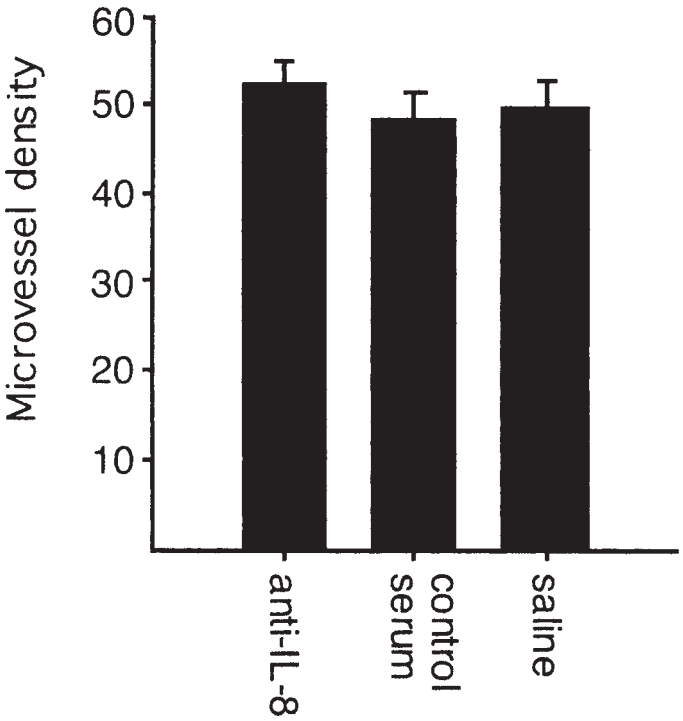

Figure 5. Microvessel density in the lymphomas. Sections were stained with anti-CD31 mAb, and intratumor microvessel density was assessed by use of light microscopy. Scanning of sections at low power (200× total magnification) identified areas of high neovascularization. Within these areas, microvessel counts were made on a 400×-magnified field, with five fields counted per lymphoma. Three separate lymphomas were examined per treatment group. Significant difference was not observed ($P > 0.5$).

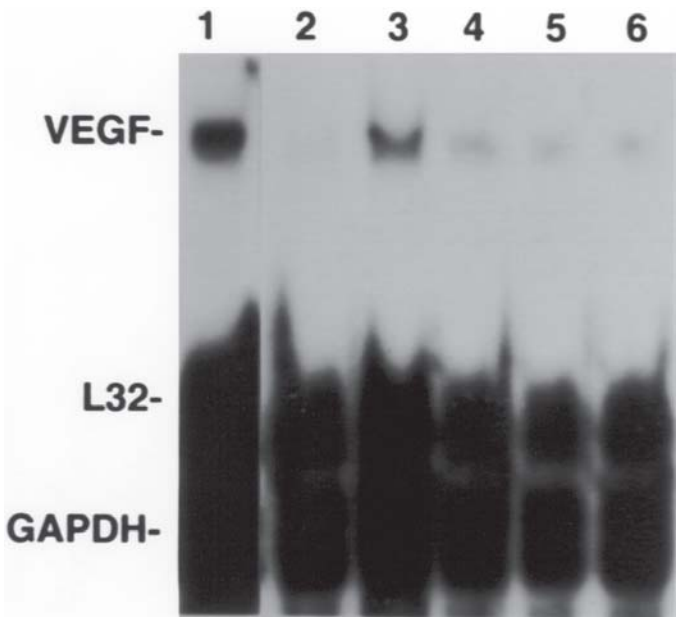

Figure 6. Vascular endothelial growth factor (VEGF) expression in LCL-lymphomas. RNA was extracted from EW-LCL (lane 1) and EW LCL-SCID lymphomas (lanes 2-6), and an aliquot originating from 10^6 input cells was subjected to RPA analysis to measure VEGF and the housekeeping genes, hL32 and GAPDH. The autoradiogram is from a 16-h film exposure on XAR film.

consistent with the observation that the EBV LMP-1 induces IL-8 expression (10, 52), but this contrasts with a report by Klein and coworkers (17), who did not observe IL-8 expression in LCL. Constitutive IL-8 production has been identified in normal human B cells and malignant human B-cell lines derived from Burkitt's lymphoma, acute myelogenous leukemia, acute lymphocytic leukemia, and chronic lymphocytic leukemia (8, 41) and constitutive expression is consistent with a functional role of IL-8 in angiogenesis.

Studies of angiogenic factors have focused principally on solid tumors, and little is known about the role of angiogenesis in B-cell non-Hodgkin's lymphomas (B-NHL), of which the EBV-IBL and LPD are a subset. The NHL are heterogeneous with respect to angiogenic features, and exhibit a range of microvasculature density that correlates with the malignancy grade of the lymphoma; benign lymphadenopathies exhibit little angiogenesis (45). Vacca and coworkers (45) proposed that angiogenesis in B-NHL is an epigenetic phenomenon possibly resulting from recruitment of inflammatory cells rather than expression of angiogenic factors by the transformed B cells. Given the potential of anti-angiogenic factors to be used in the treatment of NHL, there is need for further studies to define the mechanisms of angiogenesis in NHL and we have identified the LCL-SCID mouse model as an excellent model system for this analysis.

Our hypothesis was that IL-8 was essential for angiogenesis of B-NHL because others (23) have reported that IL-8 is a potent angiogenic factor with a direct effect on endothelial cells and IL-8 plays a critical role in mediating angiogenesis of non–small-cell lung tumors, prostate cancer, and ovarian carcinoma (1, 18, 22, 40, 50). We did not observe a significant difference between microvessel density in tumors from the anti-IL-8 antibody-treated mice, compared with that in the control groups, suggesting that reduction in IL-8 has no effect on angiogenesis in the LCL-SCID mouse model. Measuring microvessel density is one method of assessing angiogenesis, but it does not reveal the functionally of such vessels (48, 49). Tumor vessels differ from normal vessels with respect to diameter, branching, basement membranes, pericyte coat, and leakiness (12). Further studies to determine the role of IL-8 on angiogenesis should investigate the functionality of tumor vessels through assessment of oxygenation, blood flow, extravasation of soluble tracers, or scanning electron microscopy (12, 28).

Apparently, the role of IL-8 in B-cell lymphomagenesis is complicated and may involve a secondary effect consequent to inflammatory cell recruitment or inhibition of alternative angiogenic growth factors. Nevertheless, in our model system, neutralization of IL-8 failed to inhibit tumor development and, indeed, tumor mass was significantly greater when IL-8 was neutralized. The anti-IL-8 polyclonal antibody used in the assay has been documented to effectively neutralize IL-8-induced in vitro neutrophil chemotaxis (7), suggesting that lack of effect of the neutralizing antibody on LCL growth was not due to failure of the neutralizing antibody.

Moore and coworkers (22) found that neutralizing antisera to growth-related gene-α (*GRO*-α), but not IL-8, reduced tumor growth in vivo, suggesting that prostate cancer cell lines use distinct CXC chemokines to mediate tumorigenicity. Similarly, alternative factors must mediate lymphoma-associated angiogenesis in the EW cell line. One candidate could be VEGF, which has been reported to influence murine neovascularization in other human tumor/mouse chimeras (3, 51). We have documented that VEGF is expressed in our cell lines, and Vacca and coworkers (46) docu-

mented that B-NHL secrete VEGF. In addition, the EBV latent membrane protein 1 (LMP-1) was reported to increase production of VEGF in cell lines derived from nasopharyngeal carcinoma (26). The LMP-1 also is expressed in LCL (33) and, thus, could also be inducing VEGF expression in the EBV-transformed B cells lines. It is worthwhile to note that IL-8 has also been reported to inhibit fibroblastic growth factor-induced endothelial cell proliferation in vitro and inhibit neovascularization of murine subcutaneous sponge implants (31), implying that there is a complex balance between angiogenic growth factors and chemokines. In sum, the role of IL-8 secreted by tumor cells goes beyond simply a direct effect on adjacent endothelial cells and, instead, is part of a complex interplay between the tumor cell and the host immune response. Interleukin 8 appears to have alternative functions in various tumor types, further complicating its role in tumor growth and angiogenesis.

One perplexing question is why did neutralization of IL-8 enhance tumor growth? One likely explanation is that IL-8 can recruit natural killer (NK) cells to the sites of tumor growth. Natural killer cells still exist in SCID mice, and are considered to be the major effector cells that prevent survival and growth of hemopoietic xenografts in recipient SCID mice (9, 27). Impairment of NK cells by increased tumor production of IL-6 has been documented to increase lymphoblastoid tumorigenicity in athymic mice (44), indicating that NK-cell dysfunction can be a mechanism of tumor cell escape from immune surveillance. Interleukin 8 has been reported to induce chemotaxis of activated NK cells (38), and NK cells have IL-8 receptors (24). Epstein-Barr virus-transformed B cells are susceptible to murine NK cells in vitro and in nude mice (20, 21). Thus, inhibition of IL-8 in the LCL-SCID mouse model may have resulted in dysfunction of NK-cell migration or activation, resulting in more favorable tumor growth conditions relative to those in control mice.

Finally, the strong positive correlation between tumor burden and circulating serum human Ig concentration suggests a method to track the size of an intraperitoneal lymphoma as it develops in a live mouse. Unlike many solid tumor cell lines that can be administered subcutaneously in the flank and growth be assessed by use of calipers, the LCL-SCID lymphomas do not develop a distinct solid mass when administered subcutaneously, but rather the cells invade into the retroperitoneal space (unpublished observations), making it difficult to assess tumor burden without opening the abdominal cavity. Periodic measurement of circulating human Ig would allow investigators to follow lymphoma development in the LCL-SCID chimera.

Thorough investigation of angiogenic factors is required for individual tumor types before treatment with anti-angiogenic therapy is pursued. Results of our study indicate that there is microvessel formation within the LCL-SCID lymphomas that is consistent with angiogenesis, despite reduction in IL-8 expression. The LCL-SCID model is an appropriate model to further study the unique biology of tumor development, angiogenesis, and possible therapies for preventing growth of EBV-associated B-cell lymphomas.

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