Injection of Human Primary Effusion Lymphoma Cells or Associated Macrophages into Severe Combined Immunodeficient Mice Causes Murine Lymphomas1

Elisabeth Zenger, Nancy W. Abbey, Mark D. Weinstein, Leon Kapp, Jeremy Reis, Inessa Gofman, Carl Millward, Ron Gascon, Ahmed Elbaggari, Brian G. Herndier, and Michael S. McGrath2

Departments of Laboratory Medicine [E. Z., M. S. M.], Medicine [R. G., A. E., M. S. M.], and Pathology [M. D. W., M. S. M.], University of California, San Francisco, California 94110; Histology Laboratory, M. D. Anderson Cancer Center, Science Park, Smithville, Texas 78957 [N. W. A.]; SLRI, Biomedical Corp., Menlo Park, California 94025 [L. K., J. R.]; School of Medicine, University of California, Davis, California 95616 [I. G.]; Department of Soft Tissue Pathology, Armed Forces Institute of Pathology, Washington, DC 20306 [C. M.]; and Department of Pathology, University of California, San Diego, California 92093 [B. G. H.]

ABSTRACT

The pathogenesis of immunodeficiency-associated lymphoma is poorly understood. During the past several years, numerous lines of evidence implicating a multistep process of malignant transformation, also known as sequential pathogenesis, have emerged. Tumor-associated macrophage production of specific lymphostimulatory products has been demonstrated and hypothesized to be central to this process. While attempting to establish primary effusion lymphoma in severe combined immunodeficient (SCID) mice, we discovered a potential model of murine lymphomagenesis consistent with the sequential pathogenesis model. This pathogenesis-based model of lymphoma could significantly impact the current thinking about postransplantation and other immunodeficiency-related lymphoproliferative disorders. Human primary effusion lymphoma-derived CD14+ cell-injected SCID mice developed aggressive murine large cell lymphomas. Tumor cell preparations containing CD14 cells or isolated CD14 cells induced lymphoma/lymphoproliferative diseases in 74% (20 of 27) of injected SCID mice. No tumors were induced by tumor-associated CD3 cells (0 of 4), normal human macrophages (0 of 13), or a murine macrophage cell line (0 of 10). Human macrophages were detected in tumor-bearing animals up to 6 months postinjection in association with the murine T-cell tumors but were not detected in controls or unaffected animals. These observations are consistent with the macrophage-initiated sequential pathogenesis model of disease (M. S. McGrath et al., Acquir. Immune Defic. Syndr., 8: 379–385, 1995; M. S. McGrath et al., Infectious Causes of Cancer: Targets for Intervention, pp. 231–242, Totowa, NJ: Humana Press, 2000).

INTRODUCTION

There is an increased incidence of neoplasia in general, and lymphoproliferative disorders in particular, in patients who are immunodeficient by way of immunosuppressive therapy or disease (1, 2). These lymphoproliferations may appear as solitary or multiple tumors, diffuse infiltration of a parenchymal organ, enlargement of native lymphoid tissue, or as lymphomatous effusions of body cavities and are often polyclonal when critically evaluated. Although the mechanisms leading to these disorders are poorly understood, there is evidence implicating antigen-initiated events, which may then lead to outgrowth of an independent tumor (3–6). The normal immune response is a balance between T-cell activation by antigen-presenting cells such as macrophages and inhibitory influences on further antigen presentation and T-cell activation. In the case of immunodeficiency-associated lymphoproliferations, a multistep sequential pathogenesis model has been proposed in which the normal balance of lymphocyte proliferation and suppression is disrupted (3, 4, 7). The events responsible for this disruption are unknown, but antigen-presenting cells such as macrophages or follicular dendritic cells are likely players during the early stages of these disorders (1).

Direct and indirect evidence of a role for macrophages in multistep malignant transformation known as sequential pathogenesis is increasing (7, 8). The largest epidemiology study of non-Hodgkin’s lymphoma to date showed that events that decreased antigen presentation/macrophage inflammation, such as nonsteroidal anti-inflammatory drug use, were significantly protective for lymphoma development (9). Nonsteroidal anti-inflammatory drugs block macrophage production of IL-6 and other inflammatory mediators implicated in tumorigenesis in experimental models (10). Malignant transformation in mice given i.p. injection with mineral oil was associated with high-level IL-6 production from macrophages, and indomethacin treatment was protective (10). Although IL-6 has traditionally been considered a differentiation factor for normal B cells, it has been implicated as both an autocrine and a paracrine growth factor in multiple myeloma and lymphoma (11, 12). Molecular studies have identified a significant subset of large cell lymphomas with prominent fes-expressing macrophage populations (7, 13). fes is a tyrosine protein kinase associated with malignant transformation in animal tumor systems (14). It is also an intracellular signal molecule communicating transmembrane signals in macrophages initiated by macrophage colony-stimulating factor, granulocyte macrophage colony-stimulating factor, and IL-3. Increased levels of the TH1 cytokine IFN-γ from tumor-associated macrophages have been reported in a series of macrophage-rich lymphomas (15). Abundant IFN-γ-induced monokine (mig) production by tumor-associated macrophages suggested an important role for that chemokine in interacting with CXCRI3 present on lymphoma cells in another study (16).

One subtype of lymphoma that contains easily identifiable macrophages is PEL or BCBL (17–19). These are a rare type of non-Hodgkin’s lymphoma that grow in the pleural, pericardial, or peritoneal cavities as lymphomatous effusions occurring predominantly, but not exclusively, in AIDS patients. Cytokine production from these tumors has been characterized. Macrophages from these tumors express high levels of inflammatory cytokines, such as IL-6 and IL-10, which have been implicated in lymphomagenesis (12, 20). While attempting to establish PEL in SCID mice, we discovered a potential model of murine lymphomagenesis consistent with the sequential pathogenesis model. This communication describes a series of experiments in which PEL material from different patients induced solid lymphomas in SCID mice. Although PELs represent a rare form of lymphoma, they were chosen for use in this study for a number of reasons. They are generally a rapidly fatal neoplasm that occurs almost exclusively in immunosuppressed individuals. An association with both EBV and KSHV infection has been established (17), although herpes virus-negative PELs have been described (21). Although the clinical presentation is unique, it is debatable whether the basic

Received 2/5/01; accepted 7/31/02.

3 The abbreviations used are: IL, interleukin; SCID, severe combined immunodeficient; PEL, primary effusion lymphoma; UCSF, University of California San Francisco; BCBL, body cavity-based lymphoma; KSHV, Kaposi’s sarcoma-associated herpes virus; PCNA, proliferating cell nuclear antigen; DBPS, Dubelcco’s PBS; IMDM, Iscove’s modified Dulbecco’s medium; PI, postinjection; FACS, fluorescence-activated cell-sorting.

1 Supported by Grants R01-CA67381 (to M.S.M.), the AIDS and Cancer Specimen Resource (NCI) Grant U01-CA65529 (to M.S.M.), MH59037 (BH), and (NIAID) Grant K11 AI01221 (to E.Z.) from the NIH.
2 To whom requests for reprints should be addressed, at San Francisco General Hospital, Building 80, Ward 84, 1001 Potrero Avenue, San Francisco, CA 94110. Phone: (415) 206-5268; Fax: (415) 206-3765; E-mail: MMCraith@php.ucsf.edu.

5536

Downloaded from cancerres.aacrjournals.org on April 20, 2017. © 2002 American Association for Cancer Research.
mechanisms underlying development of PEL and other forms of lymphoma are likely to be different. If mechanisms of lymphomagenesis are similar among all types of lymphomas, it is possible that because of multiple identifiable associated factors, PEL merely represents an accelerated form of lymphoma.

MATERIALS AND METHODS

**Immunophenotyping of PEL Cells.** PEL effusions from two different HIV-positive patients were used to induce lymphoma in mice. The first patient had lymphomatous ascites, and the second had lymphomatous pleural effusion. Ascites or pleural fluid collected from these patients was characterized via standard techniques used in our laboratory (22) with antibodies against CD3, CD5, CD14, PCNA, CD20, and CD45 (all from Becton Dickinson, San Jose, CA). Cultured or frozen cells were analyzed similarly before injection into mice. All specimens were obtained in accordance with the UCSF Committee on Human Research guidelines through the UCSF AIDS and Cancer Specimen Resource.

**PEL Cell Culture.** Effusions were obtained from the abdominal or pleural cavity of two different HIV-infected patients with PEL by paracentesis. The effusions were centrifuged, the supernatant was collected, and the cells were percoll-separated. After washing, cells were either cultured overnight in RPMI 1640 + 10% fetal bovine serum and 10% cell-free effusion fluid as described previously (23, 24) or frozen in 10% DMSO for future use. Frozen cells were rapidly thawed, resuspended, and injected directly without being cultured. To dislodge cultured cells from plastic flasks, media were removed and replaced with cold calcium and magnesium-free PBS (PBS). The flask was placed on ice for 5 min, and the cells were scraped off with a tissue culture scraper. In the first experiments, plastic-adherent cells were compared with nonadherent cells in the same flask. In the second experiment, PEL cells were cultured in either Teflon vessels (23) or plastic as described previously. In the second experiment, the adherent and nonadherent cell populations from the plastic culture flasks were combined for injection.

**Control Macrophage Cultures.** To test whether normal human macrophages are associated with lymphomagenesis in SCID mice, primary human macrophage cultures were prepared from three different donors as described previously (24).

** Buffy coat preparations were obtained from the Stanford Blood Bank (Stanford, CA) and used within 24 h of being drawn. The 30-ml preparations were diluted with an equal volume of DPBS (Mediatech, Herndon, VA). Thirty ml of the diluted preparations were then placed into 50-ml conical tubes, and 14 ml of a percoll solution (1.087 g/cc) were pipetted underneath. The tubes were centrifuged at 1,800 rpm for 30 min. After centrifugation, the upper clear plasma layer was discarded. The buffy cell layer was collected with a pipette and transferred to a clean centrifuge tube, washed with DPBS, and centrifuged at 1,400 rpm for 10 min. The DPBS was aspirated off, and the cells were resuspended in medium and transferred into 150-mm glass Petri plates and incubated for 3–4 h at 37°C in a humidified CO2 incubator. The Petri plates were then washed two times with DPBS. The attached cells were scraped off the plates, centrifuged, resuspended, placed into T75 tissue culture flasks, and grown in a humidified CO2 incubator. The Petri plates were washed two times with DPBS. The attached cells were scraped off the plates, centrifuged, resuspended, placed into T75 tissue culture flasks, and grown in a humidified CO2 incubator. The Petri plates were washed two times with DPBS.

**Murine Lymphoma Induction via Human Tumor Cell Inoculation.** For all mouse studies, 8-week-old SCID mice were obtained from Taconic (Germantown, NY) and housed at UCSF. All studies were performed with the approval of the UCSC Committee on Animal Research. Mice were acclimated for at least 1 week before starting the experiment. Because we had initially intended to transplant human tumors, all mice, including control animals, received i.p. injection with 0.1 ml of anti-ialsi-GM1 antiseraum (Wako Pure Chemical Industries, Ltd.) at least 24 h before i.p. inoculation with PEL cells. This method of antibody pretreatment has been demonstrated to increase tumor engraftment in other systems (26). Throughout the three tumor induction experiments, control animals received antibody only and did not receive cells or other injections beyond the antibody. In the first experiment, 105 cells cultured in plastic were injected into seven C.B-17-SCID mice. Three mice received cell-rich culture supernatant, and four mice received macrophage-enriched adherent material scraped off the cell culture flasks.

In the second experiment, four ICR SCID mice received 102 cells cultured in plastic, four received 104 cells cultured in plastic, four received 105 cells cultured in Teflon, and three received 106 unseparated frozen cells. In the second experiment, there was no attempt to enrich the injected material for macrophages by adherence or by FACS-sorted and cultured cells were combined for injection into the mice. In the third experiment, frozen PEL cells were thawed and stained with anti-CD3 or anti-CD14 antibodies, and FACS-separated cells were injected into mice as in the first two experiments. Four C.B-17-SCID mice received 175,000 CD13+ cells, four mice received 175,000 CD14+ cells, and four mice received 6 × 107 cells prepared similarly and stained with CD3 and CD14, but not sorted. Individual mice died acutely without overt evidence of disease or were euthanized at the first sign of illness or at 6 months (experiments 1 and 2) or 4 months (experiment 3). Postmortem exam was performed, and the following tissues were collected for histology and immunohistochemistry if available: liver, spleen, brain, bone, and any grossly abnormal tissue including thymic lymphomas. In mice that died acutely, autolysis was often precluded or gross and histological examination of spleens. Additionally, some splenic tissue was misinterpreted by the histology technician and discarded. One mouse was partially cannibalized by its cagemates, and only the liver was available for examination.

To test whether normal macrophages were associated with lymphomagenesis, human macrophages from the three different donors described above were injected into C.B-17-SCID mice (200,000 cells/mouse; 5 mice for donor 1 and 4 mice each for donors 2 and 3). Additionally, to determine whether syngeneic proliferating macrophages were associated with lymphomagenesis in SCID mice, 10 mice received 200,000 IC21 cells i.p. As was done for the mice in experiment 3, mice in the normal macrophage control experiment were observed for 4 months after cell injection and euthanized, and tissues were collected. Also included in this experiment were nine mice treated with antibody only as described above.

** Cell Sorting.** CD14+ monocyte and CD3+ T-cell fractions were isolated from frozen pleural effusion PEL material via cell sorting. Cells were rapidly thawed, washed with calcium- and magnesium-free PBS, and stained for CD3 and CD14 and CD14 markers following the standard protocols from package inserts specific for each antibody (Becton Dickinson). The antibody-labeled cells were then sorted on FACS Vantage (Becton Dickinson). A fraction of the sorted material was then phenotypically analyzed as described above before injection into mice. Mice received injection with antibody-labeled but not sorted PEL cells (starting material), CD3+ T-cell fraction, and CD14+ monocyte cell fraction.

**Histology and Immunohistochemistry.** Tissues were fixed in neutral buffered formalin for paraffin embedding and histological examination after H&E staining. Sections from sternum and lumbar vertebral were decalcified postfixation and then processed routinely for histological examination of bone marrow. Immunophenotyping with human- and murine-specific reagents was done by standard three-step immunohistochemical techniques (27) using antibodies against CD20, CD3, CD45RO, and lysozyme (DAKO Corp). The CD20, CD45RO, and lysozyme antibodies are human specific and do not cross-react with mouse. The CD3 antibody cross-reacts with both human and mouse CD3.
GATCA-3; and (j) 3’ mouse c-jun, 3’-GCAACTGCTGCGTTAGCATGAGT-5’. The PCR conditions were as follows: 1 cycle of 95°C for 3 min; 35 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min; and 1 cycle of 72°C for 10 min. We performed dilutional analysis studies that showed that the sensitivity of HLA PCR with these primers was 1:100 for detecting human DNA in murine tissue. Sensitivities for the viral primers were at least 1:1000 (17), wherein 1 infected cell would be detected within 1000 uninfected cells.

RESULTS

Immunophenotyping of PEL Cells. As is typical of PEL, the predominant cell populations in the effusions used in these experiments were large immunoblastic cells. Phenotypic analysis results are presented in Table 1. Within the tumor cells were 1–3% CD14+ macrophages. PCNA staining revealed 8–39% of the CD14+ cells as proliferating in the initial percoll-separated material. Overnight culture of ascites tumor cells increased CD14/PCNA expression from 8% to 58%. In the first experiment, post overnight adherence (tissue culture flask attachment) caused a 3–5-fold enrichment of CD14 cells. In the second experiment, the fresh effusion and frozen/thawed effusion had the same percentage of CD14 cells; however, the frozen CD14 cells were less positive for the PCNA antigen. The third experiment used the same frozen effusion cells as the second experiment.

Murine Lymphoma Induction. The studies shown in Table 2 represent a sequential set of SCID mouse injections with various PEL-derived cell populations attempting to test whether PEL macrophages influence lymphomagenesis. Results for the lymphoma induction experiments presented in Table 2 list each mouse, its treatment, time to death PI with antibody and/or cells, and its pathology. In the pilot first experiment in this series, ascites cells were cultured overnight and injected into C.B-17 SCID mice as described. The cells were cultured in plastic flasks, and either nonadherent cells or cells scraped from the plastic flask were injected. The nonadherent cells were primarily lymphoid tumor cells with macrophages in similar numbers to the original effusion. The cell population scraped from the flask was enriched for proliferating macrophages over 5-fold. Overall, five of seven animals developed systemic high-grade lymphoma or lymphoproliferative disease by 14 weeks after injection of PEL, whereas the two control animals were well and showed no evidence of gross disease (Table 2). Upon euthanasia at 6 months PI, one of the control animals had a low-grade thymic lymphoma but showed no evidence of extrathymic involvement. Animals receiving injection with PEL-derived cells had a wide spectrum of pathological change, including hyperplastic lymphoproliferative disease as well as large cell high-grade lymphoma (Fig. 1). Phenotyping showed that the tumors were murine large cell lymphomas expressing CD3 but not CD20 or human CD45RO.

The pilot study described above yielded surprising results that encouraged further study and development of this model. Minimally, animals receiving injection with human large cell lymphoma ascites developed murine high-grade large cell lymphomas. The next experiment (Table 2) was performed to answer several questions about the developing animal model: (a) is it reproducible with a different PEL effusion? (b) Can fewer cells be used? (c) Can cells cultured in Teflon, a tissue culture substrate in which the macrophage activation state may be different from that of cells cultured and scraped off plastic, be used? (d) Can frozen material be used? The answer to all these questions was yes. As described in “Materials and Methods,” there were four different treatment groups addressing these questions. Overall, in the second experiment, 10 of 15 treated mice again developed aggressive murine large T-cell lymphomas and lymphoproliferative disease affecting the spleen, liver, and bone marrow. Cancer incidence among treated groups was not significantly different. The tumors appeared histologically similar, except that the Teflon group developed systemic disease without thymic involvement. Six of 10 affected mice also had thymic lymphoma in addition to aggressive systemic disease. None of three control mice had thymic lymphoma or other abnormalities detected.

Identification of Human Macrophages in SCID Mouse Tumors. Although the SCID mouse tumor cells consistently expressed CD3, the histological pattern was reminiscent of the “starry sky” pattern present in human Burkett’s lymphoma, where the tumors have significant numbers of macrophages. Tumors that arose in animals in these experiments were stained with human-specific anti-lysozyme antibody. Control tissues were stained as well. Fig. 2 shows examples of the immunohistological study.

Human macrophages were consistently observed in association with murine lymphomatous T cells, but not in uninvolved areas (Fig. 2). Fig. 2E shows very clearly the human lysozyme-positive macrophages in these tumors 4 months after mice received i.p. injection of CD14+ cells (experiment 3 shown; tissue staining results were similar in the first two experiments). The tumor-associated macrophages appeared identical in the human (Fig. 2C) and the murine (Fig. 2E) tumors. Tissues from non-tumor-bearing animals and one with a spontaneous tumor (Fig. 2D) were uniformly negative for lysozyme staining, confirming the specificity of the antibody as identifying human and not murine macrophages.

Human Tumor-associated Macrophages but not T Cells Are Associated with SCID Mouse Tumor Induction. The first two experiments demonstrated lymphoma induction in PEL cell-injected SCID mice. The induced tumors contained murine T cells and human macrophages. To test whether PEL macrophages would induce these SCID tumors, FACS-separated CD14 cells or CD3 cells were injected into SCID mice (Table 2). Cells previously frozen from the pleural effusion of the patient in experiment 2 were used. These cells were thawed, stained, sorted, and injected as described in “Materials and Methods.” Flow cytometric analysis is displayed in Table 1 and Fig. 3. The original frozen material contained 1.6% CD14+ cells and 45% CD3+ cells. The material sorted for CD3 contained 0.05% CD14+ cells and 94% CD3+ cells. The fraction sorted for CD14 contained 69% CD14+ cells and 6% CD3+ cells. Mice receiving human CD14+ cells, either alone or in mixed suspension, developed high-grade lymphoma. As shown in Table 2, three of four mice receiving injection with the CD14+ fraction developed gross lymphomas. One of these mice was severely ill with widespread multicentric lymphoma involving peripheral lymph nodes in addition to liver, spleen, and bone marrow. None of the mice receiving injection with CD3 cells developed gross or histological evidence of lymphoma, although they did develop bilaterally symmetrical alopecia. Histopathology of affected skin was normal except for lack of hair in follicles.

To test whether other sources of human CD14 cells would induce
lymphoma, peripheral blood mononuclear cell-derived macrophages from three donors were injected into SCID mice as in the previous three experiments. As a proliferating macrophage control, IC21 murine macrophage cells were also injected into 10 SCID mice. Table 3 shows that neither normal human macrophages nor the murine macrophage cell line was associated with lymphomagenesis in SCID mice. Results are shown in Table 3. None of the mice receiving injection with either normal human macrophages or IC21 cells developed lymphoma, although two of the mice that received antibody alone developed low grade thymic lymphoma.

**PCR of Human and Murine Lymphomas.** Because of the strong association among herpes viruses, HIV, and PEL, PCR was done to try to detect the presence of KSHV, EBV, and HIV in the original and sorted PEL material and in the murine tumors that developed. PCR for HLA was also done. The PEL material from both patients was positive for EBV, KSHV, and HIV. Both CD14+ and CD3+ fractions were positive for HIV. PCs for EBV and KSHV were not done on sorted material. PCs for HLA, EBV, KSHV, and HIV were uniformly negative on murine tumor and normal tissue. All mouse DNA was tested with mouse-specific c-jun primers to insure PCR compatibility of the cellular DNA. Specimens were easily compatible. The HLA-negative status underscores the murine nature of the tumors. A rare human macrophage in upwards of 1000 murine cells, based on crude cell counting estimates, would not be expected to cause a positive HLA PCR signal, which in our hands has a sensitivity of only 1–5%. Similarly, the presence of KSHV and EBV cannot be discounted exclusively by PCR. Sensitivities for both sets of PCR primers showed detection rates capable of detecting of 1 virus-positive cell within 1000 cells (17). However, it is unlikely that either EBV or KSHV is involved in the lymphomagenesis of these murine tumors.

**DISCUSSION**

The SCID mouse has been a useful tool to study the human immune response and human tumors, primarily through adoptive transfer studies (26). One of the pitfalls of this model has been the occurrence of
naturally occurring tumors in these mice (principally thymic lymphomas), enhanced susceptibility to the induction of thymic lymphomas by irradiation or drug administration, and production of human or murine B-cell tumors after inoculation of EBV-infected human peripheral blood lymphocytes (28, 29). A KSHV-containing BCBL cell line injected into SCID mice produces tumors derived from the BCBL cells rather than from murine cells (30). Our model differs in that tumors of murine origin are developed after injection of human PEL.
CD14, and two-color analysis was performed as described in "Materials and Methods." For several days in tumor-enhancing media or tumor cell lines and involved injection of fresh tissue; use of tissue that has been cultured killer cell activity (26). In general, previous adoptive transfer studies and/or the use of antibody pretreatment directed at lowering natural adopted human tumors is uncertain but may relate to cell handling into SCID mice. Why our mice develop murine tumors rather than adopted human tumors may be key to our understanding of the biology of neoplasia. Whether these cells play a role in actual tumor induction, accelerate or support a preexisting propensity for neoplasia, or simply come in secondarily is uncertain. It is not surprising that macrophages were observed in the tumors that developed in these mice. However, the strong association between human macrophages and these tumors is notable and supports a direct or indirect inductive or supportive role for macrophages in these tumors. The role may be a simple activation of endogenous retroviral elements, complicated dysregulated immunopathology, novel viral infection, or any of a number of other possibilities. Although some of these lysozyme-positive cells could represent murine macrophages that had engulfed human lysozyme, especially in the spleens, the pervasive presence of human lysozyme-positive cells in anaplastic thymic lymphomas 4 months after i.p. injection supports the idea of long-lived, tumor-promoting, human macrophages.

Immunodeficiency-associated lymphoproliferations are often polyclonal when critically evaluated. This polyclonality was defined by multiple studies as a process having fewer than 5% of a clonal B cell present within a histologically defined lymphoma section (14, 39). Molecular studies on HIV-associated polyclonal lymphomas using an inverse PCR technique identified clonal HIV in a subset of these lesions (14), and cell sorter analysis demonstrated that the clonal form of HIV was present in tumor-associated macrophages (7, 13). Additional observations including the experiments described here have lead to and supported the "sequential neoplasia" model wherein a clonal macrophage would drive early stages of tumorigenesis, with sequential evolution of an independent tumor (40). This model could hold great promise in changing the current approach to treatment of AIDS-associated lymphoma and potentially lymphoma in general.

In the most general form, the "sequential pathogenesis" model of disease is initiated by macrophages that provide an environment for evolution of a specific disease (7). In this study, animals receiving

Table 3 Summary of normal macrophage control experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>Lymphoma incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody only</td>
<td>2/9</td>
</tr>
<tr>
<td>Donor 1 macrophages</td>
<td>0/5</td>
</tr>
<tr>
<td>Donor 2 macrophages</td>
<td>0/4</td>
</tr>
<tr>
<td>Donor 3 macrophages</td>
<td>0/4</td>
</tr>
<tr>
<td>IC21 cells</td>
<td>0/10</td>
</tr>
</tbody>
</table>

Fig. 3. Flow cytometric analysis of CD3 and CD14 expression on frozen PEL cells before and after cell sorting. A, isotype control. B, unsorted material. C, CD3+ fraction. D, CD14+ fraction. Cells in B–D were stained with antibodies directed against CD3 and CD14, and two-color analysis was performed as described in "Materials and Methods."
injection with human cells developed murine lymphomas, indicating that tumors were induced rather than transplanted. These data and our previous work demonstrating clonal HIV in a subset of lymphoma-associated macrophages suggest that macrophages are likely candidates for stimulation of lymphomagenesis. The process of sequential pathogenesis is believed to be initiated by events causing macrophage proliferation. In the case of HIV, that event could be the clonal integration of HIV near a gene that promotes cellular proliferation (7, 13, 14). In an immunosuppressed state, normal immune function is disrupted, and the complex network of soluble factors may be misregulated. Through inappropriate or uncontrolled elaboration of growth factors, secondary cell proliferation occurs, and secondary events occurring in these cells can lead to an outgrowth of frank malignant neoplasm. The current study provides experimental support of the sequential pathogenesis theory. We and others have demonstrated lymphomastimulatory products produced by tumor-associated macrophages, including PEL-associated macrophages, that provide lymphomagenetic products produced by tumor-associated macrophages, including PEL-associated macrophages, that provide lymphomagenetic

REFERENCES

Injection of Human Primary Effusion Lymphoma Cells or Associated Macrophages into Severe Combined Immunodeficient Mice Causes Murine Lymphomas

Elisabeth Zenger, Nancy W. Abbey, Mark D. Weinstein, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/62/19/5536

Cited articles
This article cites 35 articles, 11 of which you can access for free at:
http://cancerres.aacrjournals.org/content/62/19/5536.full.html#ref-list-1

Citing articles
This article has been cited by 5 HighWire-hosted articles. Access the articles at:
/content/62/19/5536.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.