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

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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 posttransplantation and other immunodeficiency-related lymphoproliferative disorders. Human primary effusion lymphoma cells, murine macrophages, and IFN-γ have been identified as significant lymphostimulatory products. In this article, we provide evidence that injection of PEL material into SCID mice causes murine lymphomagenesis consistent with the sequential pathogenesis model. This communication describes a series of experiments in which PEL material from different patients caused 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, although herpes virus-negative PEL have been described. Although the clinical presentation is unique, it is debatable whether the basic

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 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, 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 macrophages, and IL-3. Increased levels of the TH1 cytokine IFN-γ from tumor-associated macrophages have been identified as a significant subset of large cell lymphomas with prominent fes-expressing macrophages, 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 CXC/33 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 PEL have been described (21). Although the clinical presentation is unique, it is debatable whether the basic
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. Asciates 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 processed similarly before injection into mice. The flasks were placed on a slow-rotating platform at 37°C and 5% carbon dioxide. After washing, the adherent cells were harvested with a pipette, 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 flasks were 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. Theuffy 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 twice 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. Cells were cultured in a medium composed of 50% Myelocult medium (Stem Cell Technologies, Vancouver, British Columbia, Canada), 25% IMDM (Biowhittaker, Walkersville, MD) + 10% FCS supplemented with antibiotics and glutamine. 25% IMDM conditioned medium from HS27 human diploid fibroblasts (UCSF Cell Culture Facility, San Francisco, CA), 1 mg/ml IL-3, and 1 ng/ml macrophage colony-stimulating factor. For experiments, 1-month-old macrophages were scraped off the flasks in DPBS, and the cells were diluted to a concentration of 10,000 cells/ml.

Additionally, IC21, a mouse macrophage cell line (25), was used to determine whether syngeneic proliferating macrophages were associated with lymphomagenesis in SCID mice. IC21 cells were obtained from Dr. Lonnie Kapp (SLIL Biomedical Corp., Menlo Park, CA) and grown in IMDM (Stem Cell Technologies, Vancouver, British Columbia, Canada), 25% IMDM (Biowhittaker, Walkersville, MD) + 10% FCS supplemented with antibiotics and glutamine. For experiments, cells were trypsinized to remove them from the flasks, centrifuged, and resuspended in DPBS to a concentration of 1,000,000 cells/ml.

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 UCSF 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-asialo-GM1 antisera (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, 107 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 IRC SCID mice received 107 cells cultured in plastic, four received 106 cells cultured in plastic, four received 107 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 to plastic. Supernatant and cells were resuspended, and plastic flasks 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 CD3+ cells, four mice received 175,000 CD14+ cells, and five mice received 6 × 108 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) PI. 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 often precluded gross or 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 markers following the standard protocols from package inserts specific for each antibody (Becton Dickinson). The antibody-labeled cells were then sorted on a 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 and 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 vertebrae 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.

PCR of Human and Murine Lymphomas. DNA was extracted from PEL material and murine tissue using DNeasy tissue kit (Qiagen) for use in PCR in the determination of KSHV, EBV, HLA, and HIV gag sequences. Oligonucleotide primer sequences were as follows: (a) EBV 5′ primer, AGAAAGGGGCGGTTGGTTG; (b) EBV 3′ primer, GGCTCCTTGTGGTGC; (c) HLA 5′ primer, CAGGTGCCACTGTACACAG; (c) HLA 3′ primer, CCGTAGCAGCGCTAGAGTTG; (e) HHV8 5′ primer (K5 cDNA, Biosource International), AGCCGAAAGCATTCCACCT; (f) HHV8 3′ primer (K5 cDNA, Biosource International), TCCCTGAGTGAAAGGAAGAG; (g) HIV gag 5′ primer (SK38, Biosource International), TAAATCACCCTATCCAGTAGGAAAAA; (h) HIV gag 3′ primer (SK39, Biosource International), TTTGTTCTTGCTTATGTCGCAAAGTG; (i) mouse c-jun, 5′-CATGGAGCTCTAGGAGC-
were transplanted into mice. Antibody negated any possibility that these were human T-cell tumors that uniformly negative staining of tumor cells by the antihuman CD45RO large cell lymphomas expressing CD3 but not CD20 or human CD45RO. Phenotyping showed that the tumors were murine hyperplasic lymphoproliferative disease as well as large cell high-grade PEL-derived cells had a wide spectrum of pathological change, including evidence of extrathymic involvement. Animals receiving injection with the control animals had a low-grade thymic lymphoma but showed no evidence of gross disease (Table 2). Upon euthanasia at 6 months PI, one of the control animals developed a 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.

**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 normal and showed no evidence of 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. Although the anti-CD3 antibody used reacted with mouse CD3, the uniformly negative staining of tumor cells by the antihuman CD45RO antibody negated any possibility that these were human T-cell tumors that were transplanted into mice.

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 non-tumor-bearing tissues (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. 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

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**Table 1** Phenotypic analysis of PEL effusion material

<table>
<thead>
<tr>
<th>Experiment/material</th>
<th>% CD14+</th>
<th>% CD14+/PCNA*</th>
<th>% CD45+</th>
<th>% CD20+</th>
<th>% CD3+</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Case 1: Ascites</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>3</td>
<td>8</td>
<td>30</td>
<td>0.2</td>
<td>37</td>
</tr>
<tr>
<td>Postculture supernatant</td>
<td>2</td>
<td>58</td>
<td>ND*</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Postculture adherent</td>
<td>10</td>
<td>52</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2) Case 2: pleural effusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>1.4</td>
<td>39</td>
<td>73</td>
<td>13</td>
<td>20</td>
</tr>
<tr>
<td>Frozen</td>
<td>2</td>
<td>16</td>
<td>ND</td>
<td>ND</td>
<td>30</td>
</tr>
<tr>
<td>3) Sorted pleural effusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presort CD14 fraction</td>
<td>1.6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>45</td>
</tr>
<tr>
<td>Postsort CD3 fraction</td>
<td>0.05</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>94</td>
</tr>
</tbody>
</table>

* The number of CD14 positive cells that were also positive for PCNA.
  * ND, not done.
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 21 was associated with lymphomagenesis in SCID mice. Results are shown in Table 4–8.

**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...
into SCID mice. Why our mice develop murine tumors rather than adopted human tumors is uncertain but may relate to cell handling and/or the use of antibody pretreatment directed at lowering natural killer cell activity (26). In general, previous adoptive transfer studies involved injection of fresh tissue; use of tissue that has been cultured and/or the use of antibody pretreatment directed at lowering natural killer cell activity (26). In general, previous adoptive transfer studies involved injection of fresh tissue; use of tissue that has been cultured for several days in tumor-enhancing media or tumor cell lines and antibody pretreatment has not commonly been reported.

Spontaneous thymic lymphomas occur in 3–15% of SCID mice (28, 29). These low-grade tumors originate in the thymus, disseminate rarely (approximately 5%), and are generally incidental findings at necropsy (28). Leukemic bone marrow has not been described with spontaneous SCID mouse thymic lymphoma. In contrast to the spontaneous tumors observed in SCID mice, the human tumor-cell-induced murine lymphomas were unique. The incidence of murine T-cell lymphomas in our mice was >50% in mice receiving injection with material containing human PEL-associated CD14+ cells. Thymic involvement as part of aggressive systemic disease was observed in only 11 of 20 tumor-bearing animals, suggesting that the tumors did not always originate in the thymus. Most of the tumor-bearing mice were overtly ill and died from lymphomatous complications or were euthanized for humane reasons. One mouse had evidence of disseminated intravascular coagulopathy, another had lymphomatous meningitis, and another had multicentric lymphoma with involvement of peripheral as well as central lymphoid tissue. Ten of 20 of the affected mice had leukemic pattern of bone marrow infiltration (Fig. 1A). Nineteen of 20 mice were <6 months of age at the identification of tumors. Control mice (3 of 44) developed infrequent lymphomas that were incidental findings at necropsy 4–6 months PI. Clearly, there is strong circumstantial evidence that inoculation of human macrophage-containing PEL played a crucial role in the fatal lymphomas that developed in these mice.

Macrophages are classically thought of as terminally differentiated cells without the ability to proliferate. There is a growing body of evidence that challenges this dogma. The concept of “proliferating macrophages” deserves discussion. Macrophages positive for PCNA were present in the original, cultured, and frozen PEL material used in these experiments. We have demonstrated abnormally high PCNA macrophage expression in peripheral blood of patients with refractory HIV-associated lymphoma (31). In vitro sensitivity to macrophage-targeted therapy is associated with in vivo response in these patients. Abnormal activated and/or proliferating macrophages may be important in other disease states as well. For example, we have described abnormal macrophages in patients with AIDS dementia (32). A series of very elegant experiments involving various human and animal nephropathies has very clearly demonstrated a role for proliferating macrophages in the progression of those diseases (33–36). Langerhans’ cell histiocytosis is a disease characterized by the accumulation of a locally proliferating clonal population of macrophage-derived cells (37). The importance of recognizing and studying this cell lies in the potential to directly affect and potentially reverse a wide variety of chronically progressive diseases.

Most tumors in animals contain substantial numbers of mononuclear phagocytes. Determining what role these cells have in these 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 (38, 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 the evolution of a specific disease (7). In this study, animals receiving...
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 neoplasia. The current study provides experimental support of the sequential pathogenesis theory. We and others have demonstrated lymphostimulatory products produced by tumor-associated macrophages, including PEL-associated macrophages, that provide one possible explanation of how macrophages may support lymphoma development (10, 12, 13, 15, 16). Work is under way to more clearly define how these abnormal macrophages support lymphoma development in these mice and humans.

REFERENCES


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