REGULATION OF B CELL LYMPHOMAGENESIS BY A MALIGNANT Qa1+ INDUCER T CELL CLONE

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Understanding of the immunobiology of leukemias and lymphomas has been considerably aided by two concomitant developments—the generation of a series of monoclonal antibodies that define specific surface antigens on tumor cells and the identification and characterization of sets of lymphocytes with highly specific immunologic functions.

Immune regulation of chronic B cell lymphomagenesis has been particularly difficult to approach experimentally because few animal models exist. In the BCL 1 system (1–3), rapidly proliferating id+ (idiotype)[1] B cells appear to depress the total number of both Ly T subsets in the spleen and LN as the tumor progresses (4). In the MOPC 315 myeloma model, id specific helper and suppressor T cells control the differentiation and maturation of MOPC 315 myeloma cells, both by cell contact and by factors secreted by both cell subsets (5–10). Both the BCL 1 and MOPC systems resemble the more rapidly proliferating B cell dyscrasias, rather than the chronic B cell lymphomas.

To define the immunoregulatory cellular interactions that govern B cell lymphomagenesis, we have developed an experimental model of chronic B cell lymphoma induced by the retrovirus murine sarcoma-murine leukemia-Moloney (MSV-MuLV-M). The immunopathology of these B cell lymphomas is similar to Waldenstrom's macroglobulinemia in the human since the tumor-bearing C57Bl/6 mice have elevated serum IgM, a high proportion of mu+ B cells in the mesenteric lymph node and develop slowly proliferating plasmacytoid-lymphocytic lymphomas two years after injection with retrovirus. Repeated attempts by

[1] Abbreviations used in this paper: BCGF, B cell growth factor; BM, bone marrow; BSS, buffered salt solution; CFU-C, granulocyte-monocyte colony-forming units; CFU-E, erythroid colony-forming units; Con A, concanavalin A; CSF, colony stimulating factor; CTL, cytotoxic T lymphocyte; epo, erythropoietin; FACS, fluorescence-activated cell sorter; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; id, idiotype; Ig, immunoglobulin; IL-1, interleukin 1; IL-2, interleukin 2; i.p., intraperitoneally; i.v., intravenously; LPS, lipopolysaccharide; MLN, mesenteric lymph node; MSV-APS, Murine Sarcoma Virus-Amy P. Sing; MSV-MuLV-M, MSV-murine sarcoma Moloney; PBS, phosphate-buffered saline; PFC, plaque-forming cells; SRBC, sheep erythrocyte; TRF, T cell replacing factor.

our laboratory to clone malignant mu^+ B cells from the primary lymphoma have not resulted in the establishment of B cell clones, but rather, in a series of malignant $Thy-1.2^+$ $Ly-1^+$ $Ly-2,3^-$ T cell clones. These T cell clones, when incubated with purified B cells in vitro, induced the spontaneous secretion of Ig (11), suggesting that the malignant B cell might be stimulated by the T cell or immunoregulatory molecules that it secretes.

We have now extensively analyzed the immunobiological function of B lymphoma–associated T cell clones in vivo and in vitro. In the work to be presented, we demonstrate that the same subset of malignant $Ly-1^+$ $Qa-1^+$ T cells is repeatedly associated with mu^+ B cell lymphomas. This subset of T cells has primitive antigen-independent inducer activity that promotes both hematopoietic and lymphocytic differentiation and function.

Materials and Methods

Mice. Female C57Bl/6 (B6) mice were obtained from The Jackson Laboratory, Bar Harbor, ME.

Virus. Murine sarcoma virus B-75, which contains MuLV-Moloney as a helper leukemia virus (MSV-MuLV-M), was kindly supplied by Dr. Jack Gruber, National Cancer Institute, Bethesda, MD, and was stored at $-70°C$. Each 3 wk-old B6 mouse was injected intramuscularly with 0.3 ml of 2×10^4 focus-forming units virus per milliliter into the right quadriceps muscle.

T Cell Clones. T cell clones were isolated (*a*) from the mesenteric lymph node (MLN) of B6 mice injected two or more years previously with MSV-MuLV-M or (*b*) from the spleens of B6 mice injected 2 wk previously with MSV-MuLV-M. The cells were plated at 0.3 cells/well on (2,000 rad irradiated) B6 splenocytes in Eagle's minimal essential medium (containing 10% heat-inactivated FCS, 100 U/ml penicillin-streptomycin, 1 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol, 1% vitamins, and 1% nonessential amino acids) in a total volume of 0.1 ml/well. Those cells which proliferated as a result of the cloning procedure were analyzed using the fluorescence-activated cell sorter (FACS), recloned and injected i.p. at log dilutions into normal B6 mice.

Antisera. Ly-1, Ly-2, or Thy-1.2 rat monoclonal antibodies were concentrated from culture supernatants of hybridoma cell lines 53-7.313 (anti–Ly-1), 53-6.72 (anti–Ly-2), and 30 H12 (anti–Thy-1.2) provided by Dr. N. Warner. Specific binding of these reagents to cell surface antigens was determined using rabbit anti-rat $F(ab')_2$ serum conjugated to fluorescein isothiocyanate (FITC) (N. L. Cappel Laboratories, Cochranville, PA). Anti–Qa-1 alloantiserum was provided by Dr. L. Flaherty. Binding of the anti–Qa-1 to the cell surface was determined both by using rabbit anti-mouse $F(ab')_2$ serum conjugated to FITC and protein-A FITC.

Preparation of Cells for Cell Sorter Analysis. $3-5 \times 10^6$ cells were centrifuged at 300 g, the pellets incubated with 0.1 ml monoclonal antibody for 30 min at $4°C$, washed $3\times$ in cold PBS and centrifuged into a pellet. Following a second 30-min incubation at $4°C$ with the appropriate fluorescein-conjugated antibody, the cells were washed $3\times$ in cold PBS and analyzed using the Becton Dickinson FACS IV (Mountain View, CA). All sera used were ultracentrifuged at 100,000 g for 20 min immediately before use to remove aggregates.

Erythroid Colony-forming Unit Assay (CFU-E). Bone marrow (BM) and spleen cell suspensions were cultured in plasma clots as described by Axelrod et al (12). 5×10^4 bone marrow or spleen cells were grown in microtiter wells in the presence of 0.25 U/ml of Erythropoietin Step III (Connaught Laboratories, Willowdale, Ontario), beef embryo extract (Gibco Laboratories, Grand Island, NY), L-asparagine (Sigma Chemical Co., St. Louis, MO), 1% bovine serum albumin (BSA), Fraction V (Sigma), NCTC-109 (Microbiological Associates, Bethesda, MD), 20% heat-inactivated fetal calf serum (FCS; Irvine Scientific Co., Irvine, CA), modified Eagle's Medium with Hanks' balanced salt solution

(Gibco) and 10% bovine citrated plasma (Gibco). The cultures were incubated for 48 h at 37°C in 5% CO_2. The clots were removed from the microtiter wells, placed on slides, fixed with 5% glutaraldehyde, stained with 1% benzidine (Eastman Kodak Co., Rochester, NY) and counterstained with hematoxylin (Fisher, Medford, MA). The slides were examined microscopically. Aggregates of eight or more nucleated benzidine-positive cells were scored as a colony.

Granulocyte-Monocyte Colony-forming Unit Assay (CFU-C). 10^5 BM cells in a total volume of 3 ml were cultured in 35 × 10-mm Falcon petri dishes in McCoy's 5a medium (Gibco) containing 0.8% methyl cellulose (Fisher Scientific Co., Pittsburgh, PA), 10% heat-inactivated FCS, supplemented with sodium bicarbonate, sodium pyruvate, Eagle's MEM vitamins, Eagle's MEM essential amino acids, nonessential amino acids, L-glutamine, L-serine, and L-asparagine. Before plating, 0.2 ml lung conditioned media or endosera were pipetted per dish as a source of CSF. Cells were grown for 7 d at 37°C in 7.5% CO_2. Cell growth was evaluated using an inverted microscope; a colony consisted of 20 or more cells.

Infection of BM Cultures with MSV-MuLV-M.   Bone marrow cells were obtained from the femurs of C57Bl/6 mice as previously described (13). The cells were washed extensively, and resuspended at a concentration of 10 × 10^6 cells/ml. 1 ml of MSV-MuLV-M (2 × 10^4 FFU/ml) was then added per culture, and the cells incubated overnight at 37°C. 24 h later, the cells were washed 3×, and incubated in a CFU-E assay (as detailed above).

Cytolytic Assay.   LSTRA tumor cells (H-2^d) were treated with 150 μCi sodium chromate-51, washed three times with cold PBS containing 5% heat-inactivated FCS, and incubated 4 h with various numbers of lymphocytes obtained from either in vitro cultures or directly from experimental animals. The percent cytotoxicity was calculated as follows: experimental release-spontaneous release/freeze-thaw-spontaneous release.

In each experiment, normal lymphocytes were incubated with radiolabeled target cells to assess normal cell activity. Tumor cells were also incubated in medium to ascertain the spontaneous release value.

Assessment of Plaque-forming Cell Activity.   Spleen cells at 10 × 10^6/ml and sheep erythrocytes at 10% were resuspended in Ca^{++} free buffered salt solution. 50 λ aliquots of each were combined with 50 λ BSS and 50 λ absorbed guinea pig complement (diluted 1:33 in BSS) in Cunningham chambers. Following a 30-min incubation at 37°C, the plaques were enumerated microscopically.

Factor Assays.   All cultures were performed in 96-well flat-bottom culture plates (Falcon) using RPMI medium supplemented with 10% FCS, L-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 U/ml), and 2-mercaptoethanol (5 × 10^{-5} M).

IL-2.   The presence of IL-2 in test supernatants was determined using IL-2–dependent T cell blasts as described (14–16). In brief, Con A–stimulated T cell blasts (isolated on ficoll-hypaque, o = 1.078) were cultured at 2 × 10^4/well for 3 d with dilutions of supernatants. Proliferation was measured by incorporation of [^{3}H]thymidine following a 6-h pulse with 0.5 μCi (specific activity 2 Ci/mM).

IL-1.   The presence of IL-1 was measured using the costimulator assay (17). 5 × 10^5 BALB/c thymocytes were cultured ± limiting doses (1.25–2.5 μg/ml) of Con A and test supernatants. (Those supernatants that supported proliferation in the presence of Con A contained IL-1.)

TRF.   TRF activity (18) was measured on T cell–depleted (19) spleen cells from C57Bl/6 mice, (modified from reference 20). 5 × 10^5 T-depleted spleen cells were cultured with 5 × 10^5 SRBC ± supernatants for 5 d and direct anti-SRBC/PFC/well determined in a plaque assay

BCGF.   "B cell growth factor (BCGF)" activity was measured using a modification of a co-stimulator assay, in which suboptimal doses (5 μg/ml) of LPS were cultured with T-depleted spleen cells (3 × 10^5/well). Those supernatants that promoted cell growth (measured by ^{3}HTdR incorporation after 3 d) contained BCGF.

Results

Isolation of Cell Surface Analysis of Retrovirus-stimulated T Cell Clones. In our earlier studies, we showed that C57Bl/6 mice injected with MSV-MuLV-M developed Mu^+ B cell lymphomas two or more years after injection with virus. It was from these mice that the "Clone A" T cell clones were established (12). The following set of experiments was designed to determine whether phenotypically similar T cell clones could be isolated from B6 mice injected with MSV-MuLV-M before the development of B cell lymphomas.

2–3-wk old B6 mice were injected intramuscularly with 5×10^4 FFU MSV-MuLV-M. 14 d later, when the sarcomas induced at the site of inoculation had regressed, the mice were killed, the spleens removed, and splenocytes plated directly on irradiated feeder cells. Simultaneously, an aliquot of splenocytes was analyzed using the FACS. One month later, the clones that proliferated were analyzed using monoclonal antisera. As seen in Fig. 1, all the clones (designated
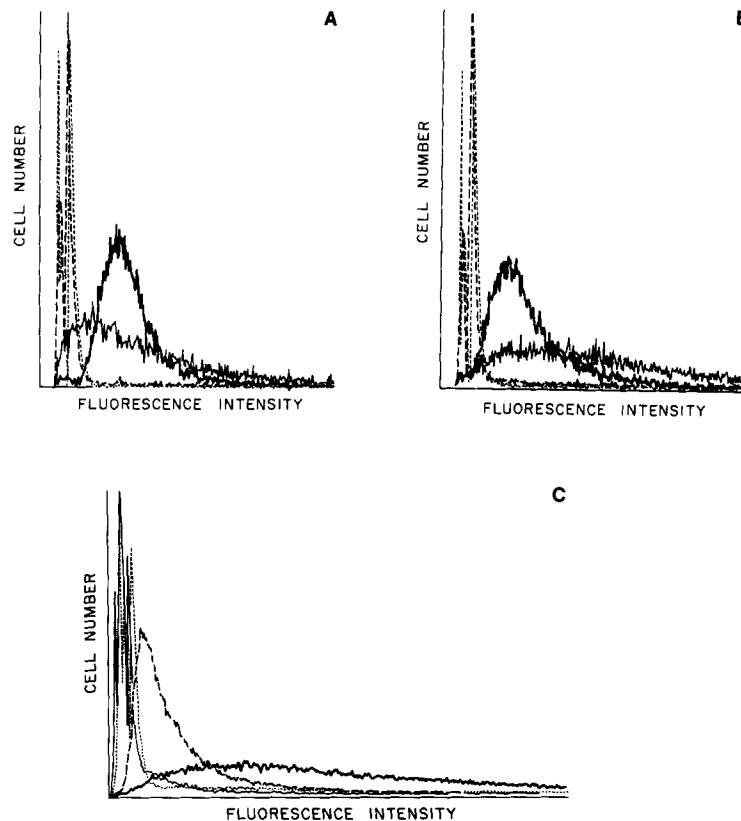

FIGURE 1. T cell clones were isolated from splenocyte populations of C57Bl/6 mice injected 14 d previously with MSV-MuLV-M. The clones were first incubated with a 1:50 dilution of normal rat serum (-----), anti-Thy-1.2 (▬▬), anti-Ly-1 (———), or anti-Ly-2 (· · · ·) monoclonal antibodies. The cells were then washed, and incubated with a 1:50 dilution of goat anti-rat FITC, washed 3× and analyzed on a FACS. *A* and *B* represent two independently isolated T cell clones called the MSV-APS series. *C* represents Clone A incubated with the identical dilutions of normal rat serum (· · · ·), anti-Thy-1.2 (-----) anti-Ly-1 (▬▬) or anti-Ly-2 (———) monoclonal antibodies.

the MSV-APS series) expressed the Ly-1 and Thy-1.2 lymphocyte differentiation antigens. The cells did not express Ly-2,3 nor Ig. This phenotype is identical to the clone A series previously reported (11) demonstrating that the same subpopulation of Ly-1$^+$ T cells was recovered from retrovirus-infected animals before the onset of lymphomagenesis. The MSV-APS series was then compared with clone A to determine the Qa-1 phenotype. As seen in Fig. 2, all the clones were Qa-1$^+$, substantiating the hypothesis that T cell clones isolated from tumor-bearing mice or from mice injected with MSV-MuLV-M were phenotypically identical.

Graded doses of the MSV-APS T cell clones were injected intraperitoneally or intravenously into normal B6 mice. At a dose of 10 × 10^6 cells, the recipients died of immunoblastic T cell sarcomas ~7 d after injection, proving that the MSV-APS clones, similar to the clone A series, were extremely malignant.

From these experiments, we concluded that the injection of C57Bl/6 mice with MSV-MuLV led to the expansion of an Ly-1$^+$ Qa-1$^+$ subset of T cells. These cells could be cloned in vitro, had the identical phenotype as T cell clones isolated
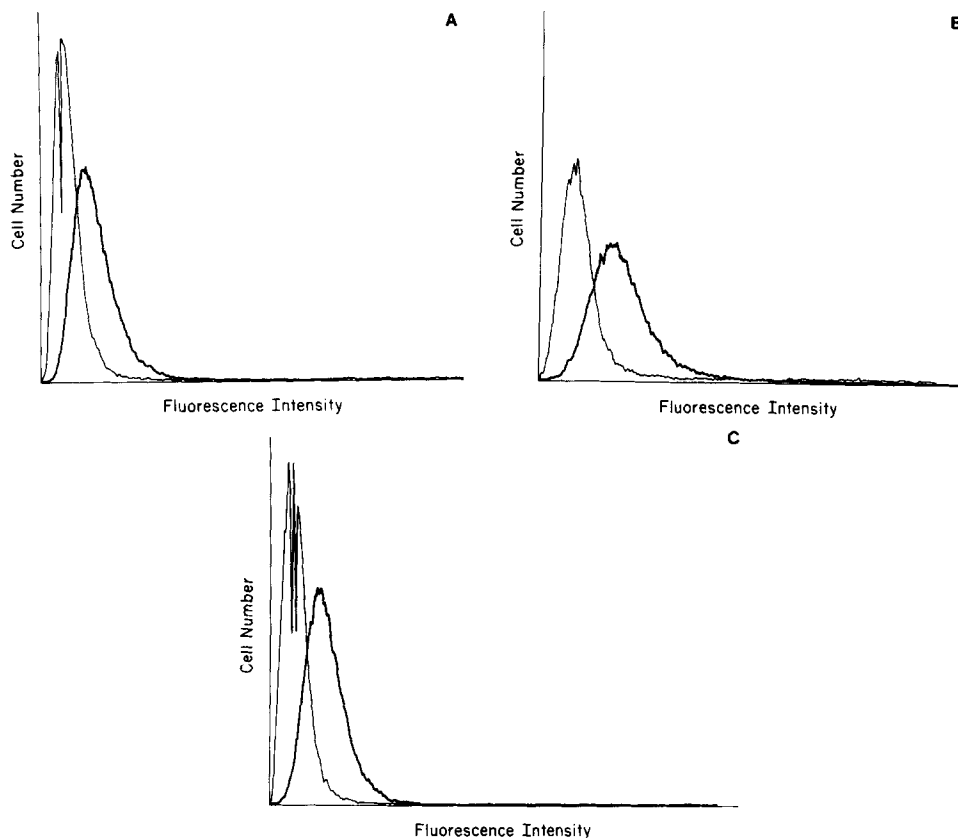

Figure 2. MSV-APS 1 and 2 and clone A were incubated with a 1:10 dilution of normal mouse serum (———) or anti–Qa-1 serum (━━━) and subsequently with a 1:50 dilution of rabbit anti-mouse FITC.

from MSV-induced B cell tumors, and were malignant in syngeneic recipient mice.

Modulation of Granulopoiesis During Lymphomagenesis. During MSV-MuLV-M–induced B cell lymphomagenesis in B6 mice, several immunopathological changes occurred. The mice developed hyperviscosity syndrome, lymphadenopathy, and megakaryocytosis. We next analyzed levels of granulopoiesis in lymphoma-bearing B6 mice to determine whether a possible interaction might occur between the Qa-1$^+$ subset of inducer T cells and granulocyte precursors.

1 × 10^5 bone marrow or spleen cells derived from normal B6 mice or lymphoma-bearing B6 mice were cultured for 7 d in semi-solid media containing 0.8% methyl cellulose in the presence of CSF to determine CFU-C levels. As seen in Table I, both bone marrow and splenic populations from lymphoma-bearing B6 mice had significantly elevated numbers of CFU-C, demonstrating that granulopoiesis increased during MSV-induced lymphomagenesis.

Modulation of Hematopoietic Function by T Cell Clones. We next analyzed the effect that the T cell clones might have on either CFU-C or CFU-E differentiation. B6 BM cells were mixed with either in vivo or in vitro passaged clone A cells and the number of CFU-C ascertained 7 d later. As seen in Table II, the addition of either in vivo or in vitro passaged clone A cells resulted in significantly

TABLE I

Analysis of CFU-C Activity in the Bone Marrow and Spleen During MSV-MuLV-M–induced Lymphomagenesis

Normal BM	MSV BM	Normal spleen	MSV spleen
45 ±6.9	94.7±10.1	0	6.0±2.5
52±10.5	98.3±6.3	0	5.3±0.7

Numbers of CFU-C ± SEM per 10^5 bone marrow or spleen cells plated are shown. Cells isolated from B6 mice with MSV-MuLV-M induced B cell lymphomas or from age- and sex-matched normal B6 mice were individually assessed for precursor cell activity.

TABLE II

Effect of Clone A on Granulopoiesis

BM	Percent added clone A cells		
	+10	+100	+300
46.7±7.2*	50.3±7.3 (107)	106±3.4 (225)	84±5.7 (178)
52.5±8.5‡	73.5±8.6 (140)	90.5±10.3 (172)	123±15.3 (232)

CFU-C ± SEM (percent control in parenthesis) per 10^5 bone marrow cells plated.

* Normal bone marrow cells were mixed with in vitro passaged clone A cells. Clone A cells were irradiated with 10,000 rad before coculture with bone marrow cells.

‡ Normal bone marrow cells were cocultured with clone A cells that had been maintained in vivo. Clone A cells were irradiated with 10,000 rad before coculture with bone marrow cells.

elevated numbers of CFU-C. Further experiments showed that the clone could not substitute for colony-stimulating factor (CSF), nor did it release factors that promoted CFU-C differentiation.

To determine whether the inducer T cell clones would influence erythropoiesis, the following experiments were performed. 5×10^4 BM cells were obtained from normal B6 mice and were incubated with 2.5×10^3, 5×10^3, 2.5×10^4, or 8×10^4 (10,000 rad) irradiated clone A cells. As seen in Fig. 3, the addition of clone A cells to syngeneic BM cells resulted in enhanced erythropoietin-dependent erythropoiesis. Further experiments showed that the clone could not substitute for erythropoietin nor did it release factors that promoted CFU-E differentiation. In addition, infection of B6 BM cells with virus did not enhance CFU-E differentiation, leading us to conclude that the clone per se, and not factors or virus, promoted erythroid differentiation.

These two sets of experiments showed that clone A stimulated the differentiation of both erythroid and granulocytic precursors in the bone marrow. More importantly, these data showed that this immunobiological function of clone A closely paralleled the in vivo situation since tumor-bearing mice had dramatically elevated levels of CFU-C precursors. Coupled with earlier results showing that the T cell clones enhanced spontaneous Ig secretion by B cells (11), it appeared that we had isolated a series of antigen-independent inducer T cell clones.

Enhancement of Antigen-stimulated Systems by Inducer T Cell Clones. To test whether clone A could enhance the immune response to antigen, B6 mice were immunized intraperitoneally with SRBC plus/minus clone A. 3, 5, or 7 d later, the mice were killed and the splenocytes analyzed for mu and gamma secreting
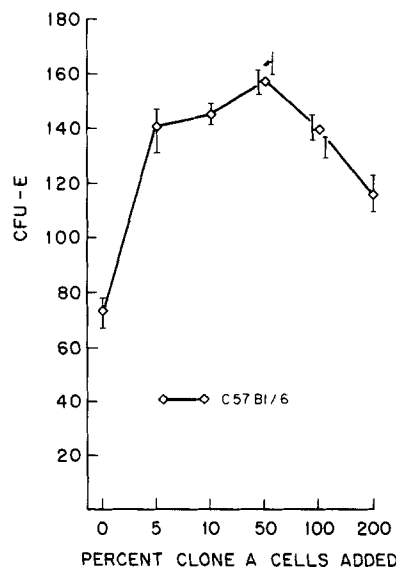

FIGURE 3. The effect of clone A on erythropoiesis. 5×10^4 C57Bl/6 bone marrow cells were incubated with increasing numbers of (10,000 rad irradiated) clone A cells in a CFU-E assay. 48 h later, the clots were removed, fixed, stained with 1% benzidine, and counter-stained with hematoxylin.

PFC response to SRBC in vivo. As seen in Figs. 4 and 5, clone A significantly enhanced the gamma PFC response to SRBC in vivo.

To determine whether inducer function could be detected during the generation of alloreactive cytotoxic cells, B6 mice were injected intraperitoneally with a suboptimal dose of LSTRA tumor cells (1×10^6) and varying doses of lethally
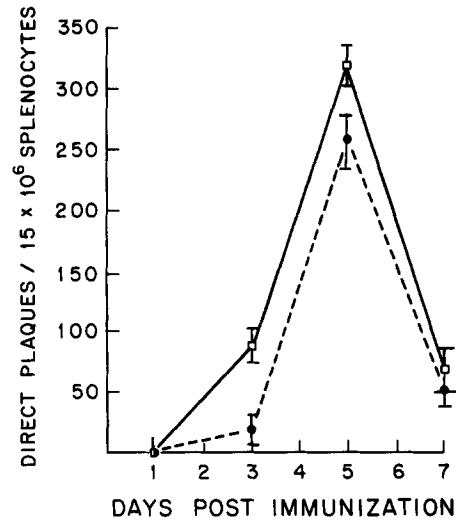

FIGURE 4. Enhancement of IgM response by clone A. Splenocytes from C57Bl/6 mice immunized with SRBC (●) or SRBC plus 5×10^5 (10,000 rad irradiated) clone A (□) were harvested 1, 3, 5, or 7 d following immunization and assayed for PFC using the Jerne plaque technique. Results represent the number of PFC/15 $\times 10^6$ splenocytes plated.
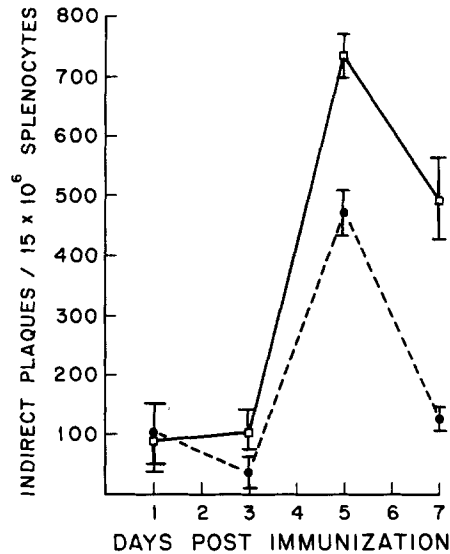

FIGURE 5. Enhancement of IgG response by clone A. Splenocytes from C57Bl/6 mice immunized with SRBC (●) or SRBC plus 5×10^5 (10,000 rad irradiated) clone A (□) were harvested 1, 3, 5, or 7 d later and assayed for indirect PFC using the Jerne plaque technique. Results represent the number of PFC/15 $\times 10^6$ splenocytes plated.

irradiated clone A cells i.p. As seen in Fig. 6, alloreactivity was significantly enhanced when the mice were injected with clone A cells plus LSTRA. Moreover, the enhancing effect was dose dependent. Repeated experiments confirmed that as few as 10^4 clone A inducer cells enhanced alloreactive CTL function in vivo. However, the injection of clone A did not enhance syngeneic CTL activity even when B6 mice were challenged with TNBS-modified EL4 cells ± (10,000 rad irradiated) clone A.

Immunoregulatory Factors Released by Clone A.   To determine whether factors were released from clone A cells that might mimic the activity of the clone, supernatants were obtained from clone A cultures grown for 48 h in RPMI 1640, 10% heat-inactivated FCS, penicillin-streptomycin, and 1 mM L-glutamine. Various doses of supernatant were added at the initiation of an in vitro sensitization and the generation of alloreactive CTL assessed at the end of a 5-d in vitro sensitization. As seen in Fig. 7, the addition of as little as 0.25 ml (10%) supernatant at the initiation of an in vitro sensitization resulted in significantly elevated CTL function.

It should be emphasized that clone A was isolated from an animal bearing an MSV-induced B cell lymphoma and that B6 mice injected with MSV-MuLV-M as weanling mice develop indolent B cell lymphomas only two or more years following virus injection. We suspected that the repeated association of the malignant $Ly1^+$ T cells with the mu^+ B cell meant that the T cells could support B cell proliferation. To test this hypothesis directly, supernatants derived from clone A were extensively analyzed for IL-1, IL-2, TRF, or BCGF activities. Repeated experiments showed that the clone did not secrete IL-1, IL-2 or TRF.
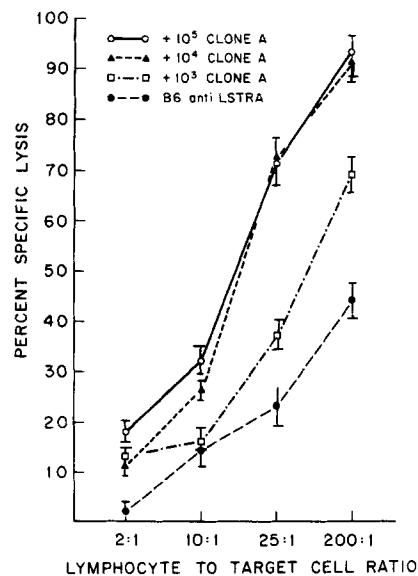

FIGURE 6.   Enhancement of alloreactive CTL function in vivo by clone A. C57Bl/6 mice were immunized with 1 × 10^6 LSTRA tumor cells ± (10,000 rad irradiated) clone A cells. The mice were killed 10 d later, and their splenocytes assessed for CTL reactivity and Cr^{51}-labeled LSTRA cells.
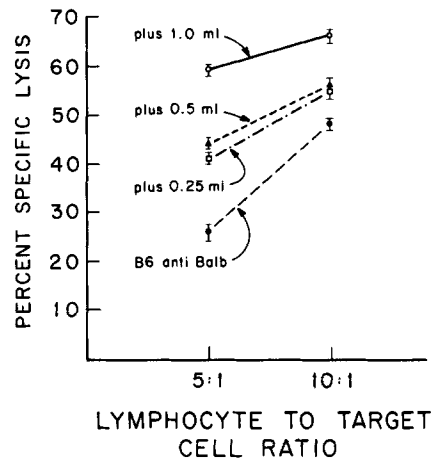
FIGURE 7. Enhancement of alloreactive CTL function by the addition of clone A supernatants. C57Bl/6 splenocytes were incubated in vitro with (2,000 rad irradiated) BALB/c splenocytes at a 2:3 ratio. Graded doses of clone A supernatant were added at the initiation of the in vitro sensitization. 5 d later, the flasks were harvested, and the CTL activity in each group assessed on Cr^{51}-labeled LSTRA cells.
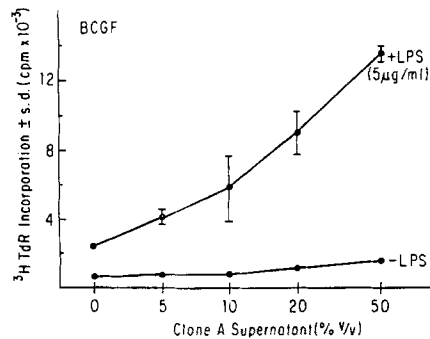

FIGURE 8. 3×10^5 anti–Thy-1.2, anti–Ly^+-1.2 + C′ treated C57Bl/6 splenocytes were incubated ± 5 μg/ml LPS/well. 0–50% vol/vol (v/v) clone A supernatants were added, and ^3H TdR uptake measured 3 d later.

However, as seen in Fig. 8 and Table II, clone A did secrete a factor promoting LPS-dependent B cell proliferation.

Discussion

These results demonstrate that a series of malignant Ly-1$^+$ Qa-1$^+$ inducer T cell clones can repeatedly be isolated from B6 mice injected with MSV-MuLV-M. The prototype clone, clone A, isolated from a B6 mouse bearing an MSV-MuLV-M–induced B cell lymphoma, expresses the identical phenotype and is also malignant in vivo. Extensive functional analyses of this clone show that: (a) erythropoiesis and granulopoiesis are enhanced; (b) humoral and cellular responses in vivo are amplified; and (c) factors are secreted that enhance LPS-dependent B cell proliferation and CTL function. Conversely, factors prepared identically from the clones have no effect on erythroid or granulocytic matura-

tion, or B cell secretion of immunoglobulin. The factors do not contain IL-1, IL-2, or TRF.

Several phenomena remain unresolved in this model. Although there is a continual association of $Thy-1.2^+$ $Ly-1^+$ inducer T cells with malignant B cells, the T cell compartment appears to be a minor component of the tumor cell population when the cells are "immunophenotyped." In vitro, only the T cells proliferate probably because the B cells are deprived of BCGF. The fact that clone A cells are extremely malignant when injected following cloning raises the important question of what regulates their proliferation during lymphomagenesis. Extensive experiments in our laboratory have shown that incubation of tumor-derived B cells with clone A or unselected $Ly-1^+$ T cells obtained from MSV-MuLV-M–infected B6 mice did not suppress the proliferation of T cells in vivo or in vitro. We are now testing the hypothesis that Fc receptors on the T cell clones may be mu specific or that $Ly-1^+$ $Qa-1^+$ T cell proliferation in this tumor model may be directly suppressed by antigen-stimulated B cells (21).

A separate question is whether or not mu^+ B cell lymphomas require the functional interaction of the $Ly-1^+$ $Qa-1^+$ T cell subset for their sustained growth. The BCGF data support the view that the clones make a factor that stimulates B cells, although whether B cell growth is restricted solely to the mu^+ subset is not entirely clear. In fact, the possibility exists that this subset of $Ly-1^+$ T cells (of which clone A is a prototype) may be idiotypically restricted in its help and can function only via an id^+ B cell. What factor(s) promotes the differentiation of CTL in vitro is also puzzling since we know that the cells do not make interleukins.

The point should additionally be made that supernatants collected from clone A cultures contain MuLV-M. Evaluation of the clone's high molecular weight DNA as a transfectant revealed that it lacked significant transforming activity in contrast to other B or T cell tumors. In fact, when digested with a variety of restriction endonucleases, the transforming activity of clone A DNA remained extraordinarily low (22), perhaps reflecting the fact that weakly oncogenic viruses such as MSV-MuLV-M require long latent periods or multiple oncogenic events for oncogenesis to occur.

Relative to the last point, our results raise the fundamental question of what constitutes a malignant cell. In a separate animal system, we have shown that BALB/cBy mice infected with MSV-MuLV-M develop massive splenic immunoblastic ($Ly-1^+$) T cell sarcomas (23). Upon repeated cloning, however, only immature malignant monocytes can be recovered, all of which anomalously express the class I antigen Qa-2 (24, 25). Since these monocytes present protein antigen that promotes $Ly-1^+$ T cell proliferation (26), we believe that the monocyte, and not the T cell, is malignant. In this newly discovered model we have just presented, the $Thy-1.2^+$ $Ly-1^+$ $Qa-1^+$ T cell subset may be initially transformed by retrovirus. This subset, in turn, promotes the proliferation of B cells via the constitutive production of BCGF and other, as yet unrecognized, signals. Thus, excessive B cell proliferation may be due to a minor subset of malignant T cells that continually stimulate their normal growth. Studies with B6 nude mice should resolve whether or not the B cell in this retrovirus model, requires the function of mature $Ly-1^+$ $Qa-1^+$ T cells for its sustained growth.

The fact that $Qa-1^+$ but not $Qa-1^-$ T cells are repeatedly cloned from the B

lymphoma cell population (or from MSV-infected mice) is striking. In detailed FACS analyses the pattern of T cell compartment expansion during lymphomagenesis is invariant. The Thy-1.2$^+$ Ly-1$^+$ Qa-$^+$1 subset expands while, simultaneously, the Ly-23$^+$ subset becomes virtually undetectable. This observation suggests that the differentiation of Ly-23$^+$ T cells is actively suppressed during lymphomagenesis and/or that the Ly1 Qa-1$^+$ subset proliferates in response to viral antigen "chronically" presented by the macrophage and/or B cell. Earlier data showing that clone A promotes the secretion of Ig by highly purified normal B cells (11) substantiates reports that this Qa-1$^+$ subset of Ly-1$^+$ cells is involved in delivering helper signals to B cells resulting in secretory differentiation (27, 28). How the complex interaction between the Thy-1.2$^+$ Ly-1$^+$ Qa-1$^+$ T cell subset and Qa-1$^-$ subset (29) shifts during lymphomagenesis can only be resolved when the individual Qa-1$^+$ and Qa-1$^-$ subsets are positively selected from Ly-1$^+$-enriched T cell populations, and evaluated in vitro using defined antigen systems. Moreover, it is now evident that the expansion of Ly-1$^+$ Qa-1$^+$ or Qa-1$^-$ subsets depends upon underlying cellular pathology. In MRL/1pr mice, for example, the Ly-1$^+$ Qa-1$^-$ subset proliferates during autoimmune lymphomagenesis[2] in contrast to the model just presented.

Another emerging fact is that the lineage of B cells in B cell tumors is clonal (31–33), while the associated T cell sets remain quite heterogeneous (34–36). Although we expected to recover a mu$^+$ B cell from a Waldenström's-like lesion, we did not expect to clone a restricted subset of malignant Ly-1$^+$ Qa-1$^+$ T cells. The repetitive B-Ly-1$^+$ Qa-1$^+$ T cell association may explain why patients with Waldenström's macroglobulinemia may develop immunoblastic cell sarcomas following aggressive therapy (37, 38). In fact, we would predict, in this latter circumstance, that T cell IBL sarcomas would express the T3 T4 phenotype. Data in the human system further show that patients with cutaneous T cell lymphomas may simultaneously develop a plasmacytoma (39) and that patients with "smoldering" adult T cell leukemias which are OKT-1$^+$, OKT-3$^+$, OKT-4$^+$, and OKT-710$^+$ have hypergammaglobulinemia (40). This observation suggests that excess helper function, in the presence of a second signal, may "drive" a subset of B cells toward malignancy.

One final point should be emphasized. The pathologic classification of non-Hodgkins lymphomas has been and clearly remains controversial (40, 41). No matter which classification scheme used, the fact remains that malignant lymphomas are comprised of immunologically active subpopulations of cells that interact during lymphomagenesis. Our work and that of others supports the view that extensive functional characterization of subsets of cells residing within the tumor is essential in understanding the immunobiology of the NHL.

Taken together, our data show that this animal model of plasmacytoid-lymphocytic lymphoma consists of two cells: mu-bearing B cells and a subset of malignant inducer T cells that express Qa-1. The fact that the clone secretes BCGF suggests that the interaction between the two cell subsets may be obligatory in maintaining B lymphoma cells.

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[2] Cronin, P., A. Sing, V. Kelley, L. Glimcher, and C. L. Reinisch. 1984. Expansion of an Ly1$^+$Qa1$^-$ subset of T cells during autoimmune disease: the result of inappropriate Ia expression. Submitted for publication.

Summary

A series of Thy-1.2$^+$ Ly-1$^+$ Qa-1$^+$ malignant T cell clones have been isolated from murine sarcoma virus-murine leukemia-Moloney (MSV-MuLV-M)–induced B cell lymphomas or from MSV-MuLV-M–infected B6 mice. These T cell clones enhance both antigen-independent and -dependent lymphocyte differentiation and function. They also induce the differentiation of granulocytes and erythrocytes in the stem cell compartment, a function that parallels the immunopathology of the disease in vivo. The malignant T cell appears to sustain B lymphoma growth in vivo by releasing a factor (BCGF) that promotes B cell proliferation.

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