Apoptosis of Burkitt’s Lymphoma Cells Induced by Specific Interaction of Surface IgM With a Self-Antigen: Implications for Lymphomagenesis in Acquired Immunodeficiency Syndrome

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In a previous study, we described a cell line (BRG-P) derived from a woman with Burkitt's lymphoma (BL) and acquired immunodeficiency syndrome that shared the same characteristic cytogenetic abnormalities as the patient’s malignant cells. This cell line contained subclones that displayed an isotype switch from IgM to IgA1 and an accumulation of point mutations in the Vγ region genes. Because these two features suggested an antigen-driven process, we began a search for the antigen responsible for the stimulation of the malignant B cells. Specifically, we hypothesized that, because the patient’s tumor had presented as a lymphomatous infiltration of the breast, the malignant B cells were recruited to this site because of the reactivity of their surface Ig with breast tissue. A hybridoma (BRG-H) was obtained by fusing BRG-M cells (an IgM producing subclone of the BRG-P cell) with an appropriate cellular partner. The monoclonal antibody (BRG MoAb) produced by this hybridoma reacted strongly with two of five breast cancer cell lines and stained normal and malignant ductal epithelial cells on breast tissue sections. The antigen recognized by the BRG MoAb consisted of a single, minimally glycosylated polypeptide chain of 45 kD (p45). The BRG MoAb failed to react with a panel of human cell lines from different tissues, except for one cell line from a uterine cervical carcinoma. No reactivity was detected for a panel of exogenous antigens from various pathogens, including human immunodeficiency virus and self-antigens frequently recognized by polyspecific antibodies. Experiments were performed to investigate the functional consequences of the interaction of surface IgM with its specific ligand. Coculture of BRG-M cells with p45+, but not with p45−, breast cells caused apoptosis of BRG-M cells. The specificity of the interaction was shown by the observation that apoptosis was prevented by pretreatment of BRG-M cells with a monovalent Fab(‘ fragment of rabbit IgG antibody to human μ chains. Moreover, only BRG-M cells, but not other BL cells, underwent apoptosis after exposure to p45+ breast cells. The interaction between the CD40 molecule expressed by BRG-M cells and its specific ligand (CD40L) prevented p45-induced cell apoptosis. Because this interaction mimics that occurring in vivo between T and B cells during immune responses, our data suggest that various events contributed to the emergence of the BL, in this particular patient, including antigenic stimulation possibly assisted by T-cell help.

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antigen responsible for the stimulation of BRG cells and (2) to determine the effects of the interaction between this specific antigen and the membrane Ig of the malignant cells.

The patient’s clinical course showed lymphomatous infiltration of the breast. Thus, we postulated that malignant B cells could react with an antigen expressed by breast tissue. We show here that the IgM produced by the lymphoma cells had specificity for an antigen of the breast ductal epithelium and that interaction between surface Ig and its specific antigen resulted in apoptosis of the malignant BRG cells in vitro. This apoptosis was prevented by exposure of BRG cells to CD40 ligand (CD40L), a molecular structure expressed by activated T cells and certain other cells that is important for their interaction with B cells. Our findings suggest a model for lymphomagenesis in which different events, including autoreactivity, antigen selection, and the help of CD40L-expressing cells, contribute to the expansion of the malignant clone.

**MATERIALS AND METHODS**

*Description of the patient and derivation of lymphoma cell lines and of hybridomas.* The case of patient BRG, a 30-year-old woman who was seropositive for HIV, was reported previously.11 The patient developed a mammary nodule that was diagnosed as a high-grade B-cell lymphoma. Peripheral blood (PB) smears and bone marrow biopsy at diagnosis showed the presence of a small number (2% to 5%) of Burkitt’s-type (L3) leukemic blasts. Surface marker analyses of PB mononuclear cells (PBMC) showed a small population of cells that coexpressed IgM, CD10, and CD38. The number of circulating neoplastic B cells increased during the course of the disease and the patient died of disseminated lymphoma. Chromosomal analyses showed a t(8; 14) translocation.

A B-cell line (BRG-P) was derived spontaneously from the patient’s PBMC, as previously described.11 The majority of BRG-P cells expressed IgM and a minority were positive for surface IgA. Subclones that expressed only IgM or IgA1 and that shared the same c-myc and Ig H chain gene rearrangements as BRG-P cells were obtained by limiting dilution.

Hybridomas were obtained by fusing the cells of one IgM-producing subclone (BRG-M) with those of the SHM D-33 heteromyeloma cell line.20,21 Hybridomas were selected in hypoxanthine, aminopterin, thymidine (HAT) medium (Seromed-Biochrom KG, Berlin, Germany) and ouabain (Sigma Aldrich, Milano, Italy) and supplemented with SFM (Promocell). The supernatants of stably cultured cells were analyzed by flow cytometry (FACScan; Becton Dickinson). In selected experiments, immunofluorescence was performed on cells preincubated in RPMI-1640 medium (Seromed) containing trypsin (100 μg/mL) (Sigma) or promase (100 μg/mL; protease VI; Sigma) for 1 hour at 37°C.

An anti-CD40 MoAb was kindly donated by Dr S.M. Fu (University of Virginia, Charlottesville, VA) and an anti–Apo-I/Fas/C9D5 MoAb was a generous gift of Dr P. Krammer (DKFZ, Heidelberg, Germany). Both MoAbs were used in indirect immunofluorescence as described.11

**Reactivity of BRG MoAb with antigens from pathogens or self-antigens.** The BRG MoAb was analyzed for its reactivity with double-stranded RNA (dsRNA), single-stranded DNA (ssDNA), or rabbit γ-globulin (RGG) by ELISA, as described.12 Reactivity against EBV viral capsid antigen (VCA; DuPont-DeNemours, Bad Hamburg, Germany), rubella virus (Clone Systems, Bologna, Italy), cytomegalovirus (CMV; Clone System), toxoplasma (Clone System), and chlamydia (Sorin Biomedica, Vercelli, Italy) was assayed by ELISA. Reactivity for antigens from HIV-1, HIV-2, human T-lymphotropic virus-1 (HTLV-1), and HTLV-2 was determined by ELISA (Sanofi Diagnostics, Pasteur SA, Marnes la Coquette, France) and Western blot assays. Binding to poliovirus type 1, 2, or 3 was analyzed using neutralization tests. Reactivity to /i/blood group cold agglutinins was tested by hemagglutination using group O* umbilical cord or adult human erythrocytes.

**Cell lines and cell cultures.** The following human cell lines were used: MDA-MB-468, MDA-MB-231, MDA-MB-157, MCF-7, and EVSA-T (from breast adenocarcinomas); HELA and A431/6 from cervical carcinomas; NEVO from skin carcinoma; and SKMEL28 from a melanoma. CALU1, CALU6, LC89, and A549 were derived from lung carcinoma; N592 from a lung small cell cancer; Y79 from a retinoblastoma; HT1080 from acutabulum fibrosarcoma; and KS from spindle cell Kaposi sarcoma. All these cell lines were obtained from the American Type Culture Collection (ATCC; Rockville, MD), except for EVSA-T and LC89, which were raised as detailed in Paganuzzi et al.24 MCF-7 and EVSA T-cell lines were maintained in Dulbecco’s modified Eagle’s medium (DMEM; Seromed), whereas the others were cultured in RPMI-1640 medium. Media were supplemented with 10% fetal calf serum (FCS; Seromed), 1% glutamine, 1% Na pyruvate, and 2% penicillin-streptomycin. One BL cell line (LAM) was originated from an AIDS patient, as previously reported,25 whereas the BJAB BL cell line was obtained from ATCC.

MDA-MB-468 cells were fractionated according to the expression of the antigen recognized by BRG MoAb, using an immunorosetting method described previously.26 Briefly, cells were sensitized with BRG MoAb, followed by a mouse IgG1 antihuman IgM MoAb as a second-step reagent (Becton Dickinson). The cells were subsequently resorted with ox erythrocytes coated with a purified goat antiguine Ig antibody (Southern Biotechnology Associates) and rosettes were separated from nonrossetting cells by Ficoll-Hypaque density gradients.

**Immunohistochemical staining.** Seven samples of breast tumors were studied. Four of these were invasive ductal carcinomas, one was an intraductal carcinoma (micropapillary variant), and two were invasive lobular carcinomas. Cryostat sections were air-dried and fixed with cold acetone for 5 minutes. After rinsing with Tris-buffered saline (TBS), sections were incubated for 1 hour at 4°C with a goat antihuman Ig antiserum (Southern Biotechnology Associates) to block human Ig infiltrating the tissue. Rinsing with TBS was followed by overnight incubation at 4°C with BRG MoAb. After
another rinsing with TBS, sections were incubated for 45 minutes at 4°C with a 1:1 mixture of a mouse IgG1 anti-human IgM MoAb (Becton Dickinson; 1:10 in TBS) and of purified human IgG (1 mg/mL in TBS). This step was followed by routine processing for alkaline phosphatase antialkaline phosphatase (APAAP), ie, sections were incubated for 30 minutes at 4°C with a rabbit antirabbit IgG antiserum (Dako, Glostrup, Denmark) followed by APAAP for 30 minutes at 4°C. The alkaline phosphatase reaction was shown using a standard chromogen as the substrate (Naphthol AS-MX phosphate and Fast Red TR salt; Sigma). Controls for the above-mentioned reactions included the incubation of sections with all of the reagents, either without the BRG MoAb or with a supernatant of the IgM CLL 35.22 hybridoma (see above).

Biochemical characterization of the antigen recognized by BRG MoAb. Cells (10 × 10⁶) from MDA-MB-468 or MCF-7 cell lines were lysed using RIPA (Trition 1%, 150 mmol/L NaCl, 100 mmol/L Tris, pH 8, 2 mmol/L EDTA). Cell lysates were subsequently reacted with BRG MoAb (10 μg) or with control IgM (10 μg). These were monoclonal IgM purified from the sera of two patients with Waldenström macroglobulinemia. A goat anti-human IgM (Southern Biotechnology Associates) and Sepharose-bound protein A (Pharmacia) were subsequently added. The immunocomplexes were pelleted and resuspended in 50 mmol/L Tris HCl, pH 6.8, 2% sodium dodecyl sulfate (SDS), 10% glycerol, and 0.01% bromophenol blue (with or without 4% 2-mercaptoethanol [2-ME]), sonicated, boiled, electrophoresed through 5% to 10% acrylamide gels, and electroblotted onto Hybond-C extra nitrocellulose membranes (Amersham International, Amersham, Bucks, UK). The membrane was probed with purified BRG MoAb, followed by a goat IgG anti-human-IgM (Cappel Organon Teknik Corp, West Chester, PA) and by 125I-protein A (Amersham International). In control tests, purified monoclonal IgM were used instead of the BRG MoAb. The gels were exposed to Hyper-Film MP (Amersham International). The films were scanned with a Cannon CLC700 scanner (Cannon Inc, Costa Mesa, CA) and elaborated with an Adobe Photoshop 3.0 program (Adobe System Inc, Mountain View, CA).

Endogenous labeling was performed as previously described. Briefly, 10 × 10⁶ cells were cultured with 20 μCi/mL 35S-methionine-cysteine (Tran 35S-label; ICN Pharmaceuticals Inc, Costa Mesa, CA) for 18 hours, harvested, lysed with Nonidet-P40 (NP40, Sigma), and analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). In selected experiments, cells were labeled in the presence of tunicamycin (25 μg/mL; Sigma).

Apoptosis assays. BRG-M cells were resuspended at a concentration of 3 × 10⁶/mL in RPMI 1640 supplemented with 10% FCS (RPMI-FCS) and cocultured with MDA-MB-468 at the optimal BRG-M:MDA-MB-468 cell ratio of 4:1. In parallel experiments, apoptosis was induced by exposing BRG-M cells to F(ab') fragment of rabbit IgG antibody to human μ chains [F(ab')2-μ-Ab], either without the BRG MoAb or with a supernatant of the IgM CLL 35.22 hybridoma (see above). F(ab') fragments were prepared by digesting rabbit IgG with 1% pepsin (Sigma). The resulting F(ab') fragments, were purified on a Sephadex G150 column (Pharmacia) and subsequently reduced with 10 mmol/L dithiothreitol (DTT) followed by alkylation with 30 mmol/L iodoacetamide (both from Sigma). Based upon the results of preliminary experiments, F(ab')2-μ-Ab was used at a concentration of 50 μg/mL.

In other experiments, apoptosis was inhibited with CD40L. The supernatant of mCD40L-CD8- α hybridoma cell line, kindly provided by Dr. P. Lane (Basel Institute for Immunology, Basel, Switzerland) was used as a source of CD40L. This hybridoma produces a fusion protein composed of CD40-L plus CD8-α. BRG-M cells at the concentration of 3 × 10⁶ in RPMI-FCS were cocultured with MDA-MB-468 cell line or with F(ab')2-μ-Ab, with or without CD40L, statically added to the final dilution of 1:20. This dilution was selected based on the results of the preliminary titration tests. Cells were analyzed at several time intervals for the presence of apoptosis.

RESULTS

Reactivity of IgM produced by BRG BL cells with breast cell lines. Immunofluorescence experiments were performed to test the reactivity of the IgM produced by BRG lymphoma cells with breast epithelial cell lines. To obtain soluble IgM antibody, a hybridoma (BRG-H) was made by fusing an IgM producing subclone of BRG-P cells (BRG-M) with the SHM-D33 heteromyeloma cell line. The IgM antibody produced, BRG MoAb, reacted strongly with cells from two breast cell lines (MDA-MB-468 and EVSA-T), but failed to stain cells of three others (MDA-MB-231, MDA-MB-157, and MCF-7). The IgM containing supernatants of a control hybridoma (CLL35-22) consistently failed to react with cells of any of the above cell lines (Fig 1). The reactivity of the BRG MoAb was abrogated by pretreatment of MDA-MB-468 cells with pronase or trypsin, indicating that the surface antigen recognized by BRG MoAb was a protein (Fig 1).

BRG MoAb failed consistently to react with cells of four human lung carcinoma (CALU1, CALU6, LC89, and A549), one small-cell lung cancer (N592), one skin carcinoma (NEVO), one retinoblastoma (Y79), one melanoma (SKMEL28), one fibrosarcoma (HT1080), and one spindle cell Kaposi sarcoma (KS). A weak fluorescence staining with a small, but significant shift of the flow cytometry profile was observed with cells from one uterine cervical carcinoma (A4316); another uterine cervical carcinoma cell line (HeLa) was negative (data not shown).

ELISA tests were also performed to determine the possible reactivity of BRG MoAb with a panel of self-antigens and exogenous antigens of common pathogens. These included dsDNA; ssDNA; RGG; and recombinant proteins of HIV-1, HIV-2, HTLV-1, HTLV-2, EBV, rubella virus, CMV, toxoplasma, and chlamydia. The results of all of these tests were consistently negative. Reactivity with HIV-1 and HIV-2 antigens, also tested by Western blotting, was negative.
Fig 1. Reactivity of BRG MoAb with certain epithelial breast cell lines. (A) IgM purified from the supernatant of BRG-H cell line (BRG MoAb) was used to stain the cells of MDA-MB-468 and MCF-7 epithelial breast cell lines (■) by indirect immunofluorescence. The negative control was performed using IgM from CLL 35.22 hybridoma (□). (B) Cells of MDA-MB-468 cell line were stained with BRG MoAb after trypsin or pronase treatment (□) or no treatment (■). Stained cells were analyzed by flow cytometry.

Agglutination tests for human red blood cell i and I antigens were negative as were the neutralization assays for poliovirus 1, 2, and 3.

Molecular characterization of the antigen detected by BRG MoAb. Initial attempts to characterize the molecule(s) in cell lysates reactive with BRG MoAb by standard Western blotting proved unsuccessful. To overcome these difficulties, the antigen(s) reactive with BRG MoAb were first immunoprecipitated from cell lysates and subsequently analyzed by immunoblotting on 10% acrylamide gels. A band of an apparent molecular mass of 45 kD was detected with this method in cell lysates of MDA-MB-468 cells. This band was present both in the gels run under reducing (data not shown) as well as under nonreducing conditions (Fig 2A). This 45-kD band was never detected in the immunoprecipitates of MCF-7 cell lysates (Fig 2A), thus confirming the immunofluorescence data. Several bands in the 50- to 60-kD region were observed in the immunoprecipitates of both MDA-MB-468 and MCF-7 cell lysates obtained with both BRG MoAb and with the control monoclonal IgM, possibly indicating nonspecific binding of human IgM to certain cellular structures (Fig 2A). The immunoprecipitates of cell lysates obtained with BRG MoAb were analyzed by immunoblotting using 5% gels. These analyses consistently failed to show molecules of a molecular mass greater than the 45-kD band described above. Thus, the 45-kD band was the only antigen recognized specifically by BRG MoAb.

In another set of experiments, MDA-MB-468 cells were cultured for 18 hours in the presence of a mixture of 35S-labeled methionine and cysteine. Cell lysates were subsequently immunoprecipitated with BRG MoAb or with two control human monoclonal IgM and analyzed by SDS-PAGE. Three or four bands were consistently observed in the 50- to 60-kD region in all of the immunoprecipitates, irrespective of the IgM reagent used for immunoprecipitation. However, a band corresponding to a molecular mass of 45 kD was detected only in the lysates immunoprecipitated with BRG MoAb, confirming its specificity for this antigen.
molecular structure (Fig 2B). The mobility of the specific band did not change when SDS-PAGE was run under reducing conditions (data not shown), thus indicating that the protein antigen detected by BRG MoAb consisted of a single polypeptide chain. Moreover, the SDS-PAGE mobility of the 45-kD band did not change when cells were endogenously labeled in the presence of tunicamycin, which inhibits N-linked glycosylation. This indicates that the 45-kD protein was minimally N-glycosylated. Because of these findings and for the sake of brevity, from this point the antigen detected by BRG MoAb will be referred to as p45.

Reactivity of BRG MoAb with breast tissue. We next tested the capacity of BRG MoAb to stain tissue sections of breast carcinomas (Fig 3). A moderate to strong reactivity of BRG-M cells. Because it has been reported that ligation of CD40 with its ligand (CD40L) also prevented the delivery of the apoptotic signal. Therefore, it appears that F(ab')-ap-Ab that induced apoptosis of the majority of BRG-M cells (Fig 5), F(ab')-ap-Ab failed to induce apoptosis of BRG-M cells (Fig 5).

This is consistent with the inability of this reagent to cross-link the cell surface IgM. However, the presence of F(ab')-ap-Ab, by inhibiting the binding of surface IgM to the p45 molecules, also prevented the delivery of the apoptotic signal.

BRG-M cells are rescued from apoptosis by CD40L. Upon binding to its complementary structure (CD40) on the B-cell surface, CD40L prevents apoptosis of certain B-cell subsets such as germinal center cells.12 In these experiments, we tested whether apoptosis of BRG-M cells induced by exposure to MDA-p45+ cells could be inhibited by CD40L. Immunofluorescent studies showed that BRG-M cells expressed abundant surface CD40, as is typical for BL cells (data not shown and Holder el al13). BRG-M cells were exposed to MDA-p45+ cells in the presence or absence of CD40L. Cells were cultured for 24 hours, harvested, PI-stained, and analyzed for apoptosis by flow cytometry (Fig 6). Whereas exposure to MDA-p45+ cells caused apoptosis of a large fraction of cells (50% to 70%), the presence of CD40L resulted in almost complete inhibition of apoptosis. CD40L also inhibited apoptosis of BRG-M cells induced by F(ab')2-αμ-Ab (Fig 6).

DISCUSSION

This study shows that the malignant B cells from a patient with BL and AIDS reacted specifically through their surface-
Fig 3. Staining of breast tumor sections with BRG MoAb or control CLL 35.22 monoclonal IgM. (A and B) A ductal carcinoma invading the breast skin is strongly stained by BRG MoAb (A) and not by control CLL35.22 MoAb (B). Note that keratinocytes surrounding the tumor infiltrate are negative. (C and D) A neoplastic duct with intraductal papilliferous growth stains with the BRG MoAb (C) and not with control CLL35.22 MoAb (D). Alveoli adjacent to the duct are unstained (arrows). (E and F) An apparently normal mammary lobule in the periphery of a ductal carcinoma; ducts stain with the BRG MoAb (E) and not with control CLL35.22 MoAb (F). (Original magnification × 250.)
Fig 4. Apoptosis of BRG-M cells exposed to MDA-p45+ cells. BRG-M cells were cocultured with MDA-p45+ or MDA-p45- cells (at a 4:1 ratio) for 24 hours, harvested, and analyzed for apoptosis by four different methods. (A) Flow cytometry analysis of PI-stained cells. BRG-M cells incubated with MDA-p45- cells are shown on the left, whereas those exposed to MDA-p45+ cells are on the right. (B) Electrophoresis of DNA to detect DNA laddering. DNA fragmentation is observed only in BRG-M cells exposed to MDA-p45+ cells. (C) Giemsa staining of cytospin preparations of BRG-M cells exposed to MDA-p45+ cells. Note the nuclear fragmentation of apoptotic cells (original magnification × 1,100). (D) Ultrastructural appearance of an apoptotic BRG-M cells. Note the peripheral heterochromatin condensation in crescents and the prominent blebbing of the plasma membrane (original magnification × 7,000).

IgM receptor with a self-antigen (p45) expressed on human breast ductal cells. This interaction caused apoptosis of malignant BL cells that could be prevented by exposure to CD40L.

A monoclonal IgM (BRG MoAb), produced by a hybridoma obtained from BRG-M cells, reacted specifically with p45 antigen and failed consistently to bind antigens from several pathogens, including HIV. Moreover, the BRG MoAb did not react with molecules such as RGG, dsDNA, and ssDNA that represent common targets of polyreactive antibodies, thus indicating that the BRG MoAb had a restricted range of specificity. Finally, the BRG MoAb failed to react with cells from a panel of human cell lines mainly of epithelial origin, with the exception of those from human cervical carcinomas.

Immunohistochemical observations of breast sections showed that p45 was expressed selectively by ductal cells, both malignant and normal. Although normal tissue samples were not available, this conclusion was derived from analyses of normal alveolar tissue that surrounded ductal tumors and of uninvolved ductal epithelium present in lobular carcinomas. Because histopathologic analyses indicated that the patient did not have breast cancer, the native p45 autoantigen expressed by her normal breast cells was likely to be the target for the BL cells. This would have, in turn, favored

Fig 5. Inhibition of apoptosis of BRG-M cells by a monovalent F(ab')2-µ-Ab. BRG-M cells were incubated with F(ab')2-µ-Ab (10 µg/mL) or F(ab')2-µ-Ab (50 µg/mL) for 1 hour at 4°C and placed in culture. Cells exposed to F(ab')2-µ-Ab were cultured alone or with MDA-p45+ cells as indicated. After 24 hours in culture, the cells were stained with PI and analyzed for apoptosis by flow cytometry.

Fig 6. Exposure to CD40L prevents apoptosis of BRG-M cells. BRG-M cells were cultured with MDA-p45+ cells or F(ab')2-µ-Ab in the presence or absence of CD40L (supernatant of CD40L-CD8α hybridoma, at the final dilution of 1:20). The cells were harvested after 24 hours, stained with PI, and analyzed for apoptosis by flow cytometry.
the recruitment of BL cells into the breast, a feature that characterized the clinical course of the patient.

Although the pathogenetic relevance of stimulation by exogenous or self-antigens in lymphoproliferative disorders was postulated more than 30 years ago on theoretical grounds, only recently has it received experimental support. Patients have been described in whom the malignant lymphoid cells produced antibodies or autoantibodies of defined antigenic specificity. Some of these cases were AIDS patients who developed BL or other lymphoproliferative disorders. Moreover, in a number of patients, indirect evidence of antigenic stimulation was provided by the finding that these malignant cells had features characteristic of memory cells. Based on these findings, there has been increasing recognition that stimulation by exogenous or self-antigens may act as a promoting event in the expansion of malignant clones. Moreover, observations both in humans and in experimental animal models have emphasized the role of T cells in supporting the clonal expansion of malignant B cells.

Our observations on patient BRG suggest a complex role for the stimulation of malignant cells by antigen. Although specific antigen may prolong cell survival or induce cell proliferation in other lymphoproliferative disorders, in the BL studied here (and perhaps in other similar cases), the specific interaction between surface IgM and p45 caused cell apoptosis, as supported by several lines of evidence. Thus, upon coculture with MDA p45 cells, but not with MDA p45 cells or other cells not expressing p45, BRG-M cells underwent apoptosis. This apoptosis could be blocked by a monovalent F(ab')-ap-Ab. Moreover, BL cells other than BRG were not induced into apoptosis by p45. Because ligation of surface IgM by divalent anti-IgM antibody caused apoptosis of BL cells, it is likely that p45 expressed on the breast cells also induced apoptosis by cross-linking surface IgM. In connection with this, it is perhaps worth noting that, like most BL cells, BRG-M cells do not express Apo1/Fas/CD95 surface structures (data not shown), thus ruling out that apoptosis could be induced through a non-BCR mediated signal such as the Apo1-receptor pathway.

The outcome of the process initiated in vitro by the encounter between p45 and its specific receptor on the BRG-M cell surface (ie, cell death or cell rescue) appeared to be dictated by the availability of CD40L. Because CD40L is expressed on the surface of activated T cells as well as of other cells such as basophils or mast cells, the collected evidence suggests that such CD40L-expressing cells may have been involved in the pathogenesis of BRG lymphoma in vivo. Unfortunately, the paucity of circulating T cells and the rapidly deteriorating clinical conditions of the patient prevented studies on the T-cell response to p45 peptides or to other antigens from breast cells. However, the aforementioned observations of accumulation of somatic mutations in the Vh gene segments encoding the antibody CDR and the isotype switch from IgM to IgA provide an indirect support for T-cell participation in the maturation of these cells and possibly in lymphomagenesis. In this regard, it is of note that IgA-producing clones of BRG-P cell line maintained their specificity for p45 and were induced into apoptosis upon coculture with p45 expressing cells (data not shown). Thus, the process of intraclonal maturation detected in the lymphoma cells did not significantly alter their antigenic specificity, despite the apparent disadvantage imposed by the expression of a receptor capable of delivering apoptotic signals. These disadvantages were possibly counterbalanced by the advantages provided by CD40L. These advantages may have also prevented any additional form of receptor editing by the lymphoma cells.

Analyses of the c-myc rearrangement showed that the breakpoint on chromosome 8 involved the c-myc gene rather than flanking sequences, whereas the breakpoint on chromosome 14 involved the JH locus. This rearrangement likely resulted from recombinase errors before membrane Ig receptor expression. Thus, this rearrangement was an early event in the process of transformation, a finding that is also consistent with the observation that the same c-myc translocation characterized both the IgM- and IgA1-producing clones. Therefore, it appears that antigenic stimulation occurred after the major cytogenetic changes characteristic of all BL. Whether and how the presence of the rearranged c-myc facilitated the onset of an autoimmune reaction directed to p45 remains to be seen.

The present findings offer a number of clues to explain some of the phenomena occurring in AIDS patients with BL. For example, these observations support the notion that antigenic stimulation is a relevant predisposing factor for BL pathogenesis. Moreover, the requirement for the help of CD40L-expressing cells is consistent with the observation that BL may arise in HIV-seropositive patients before impairment of the immune system becomes obvious. Moreover, because CD40L-expressing cells other than T cells presumably are not deleted by HIV, there is a large window of rescue. The antigenic stimulation supporting the pathogenesis of lymphomas may involve HIV-related antigens or antigens of opportunistic microorganisms, or even self-antigens, thus reinforcing the concept that immune dysregulation caused by HIV may concomitantly induce autoimmunity and neoplasia.

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