Reproducible Obtaining of Human Myeloma Cell Lines as a Model for Tumor Stem Cell Study in Human Multiple Myeloma

By Xue-guang Zhang, Jean Philippe Gaillard, Nelly Robillard, Zhao-Yang Lu, Zong-Jiang Gu, Michel Jourdan, Jean Michel Boiron, Régis Bataille, and Bernard Klein

We report a novel, reproducible methodology which enabled 10 human myeloma cell lines (HMCL) to be obtained from each of 10 tumor samples harvested from 9 patients with extramedullary proliferation. Fresh samples were cultured with interleukin 6 (IL-6) and granulocyte macrophage-colony stimulating factor (GM-CSF) at a high cell density and resulting HMCL growth became progressively dependent on IL-6 alone, no longer requiring GM-CSF. These HMCL, which had the same immunoglobulin gene rearrangements as the patients' original myeloma cells, were designated XG-1 to XG-9. XG HMCL had a plasma cell morphology, expressed plasma cell antigen (Ag), namely cytoplasmic immunoglobulins, CD38, B-B4 Ag, and CD77, and lacked the usual B-cell antigens. They also expressed activation antigens such as CD28 with coexpression of CD28 and its ligand, B7 Ag, in four HMCL. Six HMCL expressed CD40, 4 CD23, and 5 its ligand, CD21. The XG HMCL bore adhesion molecules VLA-4 and CD44 (all 10 HMCL), VLA-5 (7 HMCL), and CD56 (4 HMCL). Finally, cytogenetic study of 8 HMCL indicated a 14+ chromosome, and t(11;14) translocation was found in 6 of 8 and 5 of 8 HMCL, respectively. The possibility of obtaining malignant plasma cell lines reproducibly from each patient with extramedullary proliferation offers a unique tool for studying the phenotype and abnormalities of the still unidentified tumor stem cell in this disease.

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MULTIPLE MYELOMA (MM) is a B-cell neoplasia affecting terminal cells of B-cell differentiation (ie, plasma cells). The course of this disease can be schematically classified into three phases: 1) an inactive phase in which tumor cells are nonproliferating mature plasma cells; 2) an active phase in which a small percentage of proliferating plasmablastic cells (>1%) is detected among a great majority of nonproliferating plasma cells; and 3) a fulminant phase, with possible extramedullary proliferation, characterized by an increase in the plasmablastic compartment. Several groups have shown that interleukin-6 (IL-6) is a major growth factor for these malignant plasmablastic cells in vitro and probably in vivo since anti-IL-6 antibodies block plasmablastic cell proliferation in patients with terminal disease. The lack or small quantity of proliferating tumor plasma cells in the great majority of patients with MM has made it rather difficult to identify the phenotype and the abnormalities of the malignant stem cell, which is capable of self-renewal and can feed the compartment of nonproliferating malignant plasma cells. The presence of non-B-cell antigens on plasma cells has suggested an immature origin for the malignant stem cell. However, such non-B-cell antigens have also been identified on normal plasma cells, which relativizes this hypothesis. Immunoglobulin (Ig) genes of tumor plasma cells have multiple somatic mutations, which implies that the tumor stem cell is a memory B cell or a plasmablastic cell that has undergone the process of antigen selection and Ig gene mutation in the germinal centers of lymphoid follicles. The recent demonstration of the existence of a tiny population of memory pre-switch B cells expressing tumor-specific VDJ genes together with cμ genes may suggest that the malignant stem cell is a memory pre-switch B cell. Again, the difficulty in answering this question relates to the tiny size of the proliferating malignant compartment in this disease. One means of investigating this question is to study the acute phase of the disease. In hematopoietic neoplasia affecting differentiated cells, these acute phases are probably due to additional oncogenic mutations occurring in highly proliferating malignant stem cells and leading to increased proliferating potential and progressive blockade of their differentiation into more mature tumor cells. In MM, the acute phases are always characterized by the expansion of proliferating plasmablastic cells. In the present study, we report a methodology that enabled us to obtain human myeloma cell lines (HMCL) from each of 10 tumor samples studied in 9 patients with extramedullary proliferation. All these HMCL were early plasmablastic cell lines secreting low amounts of Ig, and their growth was completely dependent on addition of exogenous IL-6.

MATERIALS AND METHODS

Patients. Nine patients with fulminant MM and extramedullary proliferation were studied (Table 1). One patient (No. 3) was studied both at diagnosis of a primary plasma cell leukemia (PCL) and during relapse with pleural effusion (PE). Seven patients had secondary PCL and one PE. Eleven patients with active disease and only medullary involvement were also studied.

Isolation and proliferation assays of freshly explanted myeloma cells. Peripheral blood, pleural effusion, and bone marrow samples were obtained from patients after informed consent. Mononuclear cells were obtained by centrifugation of heparinized samples over Ficoll-Hypaque gradient. Mononuclear cells contained 60% to 95% of myeloma cells according to the different tumor samples. The short-term culture assay used was previously described in detail.
HUMAN MYELOMA CELL LINES

Table 1. Patients' Clinical Characteristics

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<tr>
<th>Cell Line</th>
<th>Patient's Sex</th>
<th>Clinical Stage</th>
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<td>XG-9</td>
<td>M</td>
<td>PCL (secondary)</td>
<td>IgA</td>
<td>Bone marrow</td>
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</table>

and the percentages of myeloma cells in S-phase were determined by a double immunofluorescence technique.²

**Cell culture.** Freshly explanted mononuclear cells (10⁶ cells/mL), containing 60% to 95% of myeloma cells, were cultured in RPMI 1640 medium supplemented with 5 × 10⁻³ mol/L 2-ML, 10% fetal calf serum, and with either rIL-6 (1 ng/mL), rIL-6 (1 ng/mL) and rGM-CSF (10 ng/mL), or no cytokine. Every 3 days, half-culture medium was replaced with fresh culture medium supplemented for each culture group with the initial cytokine combination. After 6, 9, 15, and 18 days of culturing, dead cells were removed using cell centrifugation over Ficoll-Hypaque gradient, and viable cells were recultured at a 10⁵ cell/mL concentration in fresh culture medium as described above.

**Phenotypic analysis.** The phenotype of freshly explanted myeloma cells and XG lines was studied using monoclonal antibodies (MoAb) to various human antigens (Ag) and (Fab)² fragments of a goat anti-mouse lg antibody coupled with fluorescein isothiocyanate (Immunotech, Marseilles, France). Fluorescence analysis was performed with an ATT 3000/MCA 3000 flow cytometer (Bürker, Wissembourg, France) according to the methodology we previously described.¹³ The fluorescence intensity obtained with isotype-matched control MoAb was <2.5. Cytoplasmic Ig heavy and light chains were determined by specific antibodies coupled with fluorescein (Kallestad, Austin, TX) as reported elsewhere.²

**Morphologic and cytogenetic studies.** Air-dried cytopsins were stained with Wright- Giemsa for microscopic observation. For cytogenetic analysis, routine methods were used for culture harvest, slide preparation and RQ-band staining; 1 hour before harvesting, colchicine was added to block mitosis (final concentration: 0.1 μg/mL). Hypotonic treatment (0.075 mol/L KCl) was limited to 35 minutes. Cells were fixed with methanol:acetic acid (3:1). Karyotypic designations were assigned according to the International System for Human Cytogenetic Nomenclature.

**Southern and Northern blot analysis.** XG cells were cultured with 1 ng/mL of IL-6, harvested during the exponential growth phase and frozen in liquid nitrogen for DNA and RNA studies. Digestion of 10 μg of DNA samples from myeloma or XG cells was performed with restriction enzymes HindIII and EcoRI (Boehringer Mannheim, Meylan, France). Hybridization, using a 3²P-dCTP-labeled IGH probe, and the autoradiographic procedure are reported elsewhere.¹² The IGH probe was kindly provided by MP Lefranc (Montpellier, France). For Northern blot, cDNA encoding for GAPDH or human IL-6 was labeled with 3²P-dCTP using a random primer method according to the manufacturer's recommendations (Amer sham, Buckinghamshire, England). Hybridization was carried out as reported elsewhere.¹³ The cDNA for human IL-6 (pP64-huIL-6) was generously provided by L Aarden (Amsterdam, The Netherlands), and the cDNA for GAPDH was a gift of M Piekaczysk (Montpellier, France).

**Cytokine production by XG cells.** XG cells were washed three times with culture medium and cultured for 1 or 5 days with or without rIL-6 (1 ng/mL) at a 10⁵ cell/mL concentration. At the end of the culture period, supernatants were collected and stored at −20°C until further use.

**Cytokine responsiveness of XG cells.** To investigate their responsiveness to various cytokines, XG cells were washed once with culture medium, incubated for 5 hours at 37°C in culture medium alone and washed again twice. The cells were then cultured at various concentrations in 96-well flat-bottomed microplates for 2 or 5 days with either culture medium alone or graded concentrations of various cytokines. 0.5 μCl of tritiated thymidine (25 Ci/mmol; CEA, Saclay, France) was added for the last 8 hours of culture, and tritiated-thymidine incorporation was determined as reported elsewhere.² IL-6 bioassay. The biologic activity of IL-6 was evaluated using the B9 hybridoma bioassay as previously described.¹⁴ One unit of IL-6 was defined as the amount yielding half-maximal proliferation and corresponding to 1 pg of rIL-6. By using the B9 bioassay, 10 μg of B-E8 anti-IL-6 MoAb were found to inhibit 5,000 U rIL-6.

**Antibodies and cytokines.** The following MoAb were used: CD2, CD3, CD10, CD11a, CD11b, CD11c, CD13, CD14, CD15, CD16, CD18, CD19, CD21, CD23, CD24, CD25, CD28, CD30, CD32, CD33, CD37, CD38, CD40, CD44, CD49h, CD49d, CD49f, CD54, CD56, CD57, CD65, CD67, CD71, CD72, anti-b2m; HLA-1, HLA-2, IgG, IgA, IgM, and IgD were purchased from Immunotech (Marseillels, France); CD5, Leu1, and CD34 from Beckton Dickinson (Mountain View, CA); CD20, B1 from Coulter (Hialeah, FL); and PNA from Sigma (St Louis, MO). The CD-77 (BLA) MoAb was provided by Dr J. Wiel (URA, Paris, France); the B7 MoAb (104 MoAb) by Dr J. Banchereau (Schering Plough, Dardilly, France); the anti-plasma cell B-B4 MoAb by Dr J. Wijdenes (Innotestherapy, Besançon, France); the GPX7 and GPX3 anti-human gp130 IL-6 transducer and MT18 anti-IL-6R by Dr K. Yasukawa (Tosoh, Kanagawa-ken, Japan); human recombinant (hr) IL-3 and GM-CSF by Dr J. Banchereau (Schering Plough, Dardilly, France) and hr-IL-6 by Dr N. Viti (Sanofi Elf Biorecherche, Labègue, France). Control marine IgG1 MoAb used in the short-term cultures of tumor samples was purchased from Immunotech.

**RESULTS**

**Response of freshly explanted myeloma cells to cytokines.** Proliferation of freshly explanted myeloma cells was studied in 7 of the 10 tumor samples used to generate HMCL. As outlined in Fig 1, the cells which engendered the cell lines spontaneously proliferated when cultured for 5 days in vitro. This spontaneous myeloma cell proliferation was inhibited by 60% to 100% in 6 patients by B-E8 anti-IL-6 MoAb (Fig 1). In patient 9, the very low spontaneous proliferation was unaffected by anti-IL-6 MoAb. Recombinant GM-CSF or IL-6 further increased spontaneous proliferation, and this stimulating effect was inhibited by anti-IL-6 MoAb (Fig 1).

**Establishment of IL-6-dependent HMCL (XG-1 to XG-9).** Figure 2 shows a representative example of the process for establishing HMCL. Freshly explanted myeloma cells cultured without addition of exogenous cytokines rapidly ceased to proliferate and died within 6 days. With rIL-6 (1 ng/mL), the absolute number of myeloma cells increased slightly during the first 12 days of culture and decreased thereafter. When rIL-6 (1 ng/mL) and rGM-CSF (10 ng/mL) were added together...
at the onset of culturing, the absolute number of myeloma cells rose after 6 days of culturing. An IL-6-dependent cell line was then obtained. The combined use of rIL-6 and rGM-CSF enabled 10 IL-6-dependent HMCL to be obtained reproducibly from each of the 10 different tumor samples cultured. These cell lines were termed XG-1 to XG-9. Two HMCL were obtained from the same patient: one from peripheral blood at diagnosis (XG-3P) and the other from pleural effusion during relapse (XG-3E). With this methodology, no cell lines could be obtained from bone marrow myeloma cells of 11 patients with medullary involvement alone.

Cytology of XG cells and Ig gene rearrangements. For each XG HMCL, XG cells had a plasma cell cytology. Five HMCL (XG-1, XG-2, XG-5, XG-7, XG-9) were plasmablastic cell lines containing more than 70% plasmablasts characterized by the presence of nucleoli, non-condensed chromatin and little cytoplasm. Five HMCL (XG3P, XG-3E, XG-4, XG-6, XG-8) had a more differentiated phenotype, with >50% plasmocytes and proplasmocytes (lack of nucleoli, more abundant cytoplasm and condensed chromatin). In plasmacytic cell lines, mitoses were found mainly in plasmablastic cells (results not shown).

HindIII and ECORI restriction enzymes were used to locate Ig heavy chain gene rearrangements in each XG HMCL (Figs 3 and 4). Original myeloma cells (available for 7 patients) had Ig heavy chain gene rearrangements similar to those of the corresponding XG HMCL (Figs 3 and 4).

Cytogenetic analysis. Eight XG HMCL were studied, all presenting complex karyotypes with numerous gains and losses of chromosomes and structural abnormalities. Four HMCL were hypodiploid (XG-1, XG-5, XG-6, XG-7) and 4 hyperdiploid (XG-2, XG-3, XG-4, XG-8). Various structural abnormalities involving chromosome 1 were found for the 8 HMCL. Four had partial loss of chromosome 13 (13q-, XG-6 and XG-8) or monosomy 13 (XG-1, XG-7). The most striking feature was the presence of structural abnormalities involving chromosome 14 in 6 of the 8 HMCL: t(11;14) (q13;q32) in XG-5 cells, complex t(11;14; ?)(q13;q32; ?) in XG-1 and XG-2 cells, der14 t(11;14)(q13;q32) in XG-6 and XG-8 cells, and der 14 t(14; ?)(q32; ?) in XG-3 cells. For 4 HMCL (XG-1, XG-3, XG-4, XG-5), structural abnormalities were found involving the 8q24 band. In particular, XG-5 cells shared both t(11;14) and (t8;14)(q24;q32).

IL-6-dependent proliferation of XG lines. After 1 to 3 years of culturing, XG HMCL have retained their depen-
HUMAN MYELOMA CELL LINES

Fig 3. Immunoglobulin gene rearrangements of XG cells.

dence on exogenous IL-6 for in vitro growth. In short-term proliferation assays, stimulation indexes ranged from 5- to 60-fold as a function of the different HMCL. Half-maximal proliferation was induced by 50 to 100 pg/mL of rIL-6 and maximal proliferation by 400 to 1000 pg/mL (Fig 5). rGM-CSF or rIL-3 alone was unable to support XG cell growth but both increased proliferation 2- to 3-fold in the presence of low IL-6 concentrations (100 pg/mL) (Table 2). However, no increase was noted with high IL-6 concentrations (1 ng/mL) (results not shown).

Finally, XG cells, like purified freshly explanted myeloma cells, produced no detectable IL-6 activity, and IL-6 gene was not expressed spontaneously or after stimulation with rIL-6 and rGM-CSF (results not shown).

Phenotype of XG HMCL. Detailed phenotypic analysis of XG HMCL was performed by searching for the expression...
of 57 surface membrane Ag. Percentages of labeled cells and mean fluorescence intensities are indicated in Table 3. Briefly, XG HMCL expressed plasma cell Ag, namely cytoplasmic immunoglobulins, CD38, B-B4 Ag and CD77, and lacked the usual B-cell Ag. They also expressed activation antigens such as CD28, with coexpression of CD28 and its ligand, B7 Ag, in 4 HMCL. Six HMCL expressed CD40, 4 CD23, and 5 its ligand, CD21. XG HMCL bore the adhesion molecules VLA-4 and CD44 (10 HMCL), VLA-5 (7 HMCL), and CD56 (4 HMCL).

DISCUSSION

In this study, we showed that the combined use of IL-6 and GM-CSF enabled IL-6-dependent HMCL to be obtained from each of 10 tumor samples from 9 patients with extramedullary proliferation. No HMCL were obtained from tumor samples cultured with IL-6 alone. This absolute need for GM-CSF is partly attributable to its ability to act directly on myeloma cells by increasing their response to IL-6. This was previously demonstrated, recently confirmed, and shown here in these cell lines. We found that IL-3, which shares the same KH97 transducer chain with granulocyte macrophage-colony stimulating factor (GM-CSF), had similar properties (results not shown). Moreover, it has been demonstrated that IL-5 also stimulates myeloma cell growth. The explanation as to why these cytokines, all using the same KH97 transducer chain, have a synergistic action with IL-6 may possibly be found in a recent study demonstrating that IL-3 produced a 6-fold increase in IL-6 high affinity receptors on a myeloma cell line. In addition, it should be noted that freshly explanted myeloma cells were not purified and that the early cultures also contained cells from the tumoral environment. Thus, GM-CSF-stimulated accessory cells and/or alternative cytokine might also be critical in preventing the early death of freshly explanted myeloma precursors.

Using this methodology, no HMCL were obtained from patients with medullary involvement alone. In such patients, myeloma cell contact with bone marrow stromal cells in these latter patients may be essential to promote cell growth, as previously emphasized by Caligaris-Cappio et al. A similar hypothesis was recently demonstrated for mineral oil-induced murine peritoneal plasmacytoma. Indeed, stromal-cell-dependent plasma cell lines, whose proliferation is IL-6-dependent, have been obtained from these plasmacytoma. In patients with PCL, additional mutations could have occurred, enabling tumor cells to escape from stromal-cell dependency and proliferate in extramedullary sites.

The possibility of obtaining myeloma cell lines reproducibly from every patient with extramedullary proliferation offers a unique tool for study of the phenotype and abnormalities of the malignant stem cell population in this disease. The first major result is that all XG HMCL had a plasma cell cytology and phenotype. XG cells expressed cyto-

![Fig 5. Proliferative response of XG cells to IL-6. 10^5 XG cells were cultured for 5 days in 200 μL of culture medium containing various concentrations of rIL-6. Proliferation was assayed using tritiated thymidine incorporation. Results are the mean values of tritiated thymidine incorporation in counts per minute (cpm) determined on sextuplet culture wells.](image-url)

Table 2. GM-CSF or IL-3 Increased the Proliferative Response of XG Cells to IL-6

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<th>Reagent Added</th>
<th>Tritiated Thymidine Incorporation (cpm)</th>
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<td>Control</td>
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XG cells were cultured for 5 days with either no cytokine (control), rGM-CSF, rIL-3, rIL-6, rGM-CSF + rIL-6, or rIL-3 + rIL-6. Proliferation was measured by tritiated thymidine incorporation. Results are the mean values of tritiated thymidine incorporation in counts per minute determined in sextuplet culture wells.
## Table 3. Phenotype Analysis of XG Cells

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Results are the percentages of labeled cells and in parentheses the mean fluorescence intensity.
plasmic heavy and/or light Ig chains, had a large density of
CD38 and B-B4 plasma cell Ag and lacked CD19 and
CD20 B cell Ag. They also expressed CD77 or Burkitt's
lymphoma antigen present on germinal center B cells and
early plasma cells, but not on circulating B cells, and bound
peanut agglutinin similarly to freshly explanted myeloma
cells. All these characteristics clearly demonstrate the
plasma cell phenotype of the 10 XG HMCL. This finding is
in agreement with reports in the literature that all HMCL,
proven by their Ig rearrangements to belong to the original
patient's malignant clone, have a plasma cell phenotype.
Only IM-9, ARH77, and GM1500 cell lines, previously re-
ported as HMCL, have a B-cell phenotype. However,
these cell lines are infected with Epstein Barr Virus (EBV)
and have never been proven to belong to the tumor clone
by their Ig rearrangements. According to our previous ex-
prience in culturing bone marrow cells of MM patients, these
cell lines are probably lymphoblastoid ones spontaneously
growing from latently EBV-infected nonmalignant B cells.

As mentioned in the introduction, the presence of somatic
mutations in myeloma cell Ig genes implies that the mali-
gnant stem cell, defined as being capable of self-renewal and
of feeding the malignant plasma cell compartment, is a mem-
ory B cell or a plasmablastic cell that has undergone Ig gene
mutations through antigen selection in germinal centers of
lymphoid follicles. The possibility, in every patient with
terminal disease and extramedul lary proliferation, of ob-
taining tumor cell lines with a plasma cell but never a B-
cell phenotype strongly suggests that the malignant myeloma
stem cell is a plasmablastic cell and not a pre-switch memory
B cell. This is true at least for patients with extramedul-
ary proliferation, although the debate remains open for those
with medullary involvement alone. There is presently no
proof that the recently evidenced clonal pre-switch B cells
in patients with MM are truly malignant (Fig 6). These
cells might be the progeny of ancestral nonmalignant mem-
ory B cells from which the tumor clone has emerged (Fig 6,
hypothesis 2). Indeed, memory B cells have a long life in
vivo, so that it is not surprising to detect them as a tiny
cell population in patients with MM in vivo.

The possibility of obtaining HMCL reproducibly will also
improve our understanding of some biologic mechanisms of
this disease. First of all, exogenous IL-6 is essential for the
growth of all XG HMCL, which is in agreement with the
concept of IL-6 as a major growth factor for myeloma cells
in vitro and in vivo. This finding probably reflects the
growth factor activity of IL-6 for polyclonal plasma cells,
as shown by the IL-6 transgenic mouse model and in pa-
ients with cardiac myxoma. The complete dependence of
the 10 XG HMCL on exogeneous IL-6 also emphasizes that
IL-6 is a paracrine myeloma cell growth factor, ie, produced
by the bone marrow environment as we previously dem-
onstrated. Actually, the autocrine hypothesis is justified pres-
ently only for some HMCL that have been grown for long
periods in vitro. For the U266 HMCL, autocrine IL-6
production is due to in vitro mutations since the original
U266 HMCL obtained in 1970 did not produce IL-6 and
required exogenous IL-6 for in vitro growth. In addition,
we previously showed that cytokines such as IFN-α or
TNF-α (unpublished results) can induce such autocrine pro-
duction of IL-6 in exogeneous IL-6-dependent XG HMCL
and rapidly select for autonomously growing subclones. Thus,
when HMCL were freshly and reproducibly obtained from
each patient tumor sample studied, their growth was
always dependent on addition of exogenous IL-6. Again, in
the hypothesis of a myeloma stem cell producing autocrine
IL-6 as a growth factor, it would be difficult to explain why
a process of autization always targets other myeloma stem
cells that do not produce their own IL-6.

Several antigens could be important for activation of XG
HMCL and perhaps of the tumor stem cell in vivo. First, all
XG HMCL expressed CD28, which is in agreement with a
previous report mentioning the presence of this potent T-
cell activation Ag on myeloma cells. None expressed
CTLA4, and 4 expressed B7 Ag. These IL-6-dependent
HMCLs are a good model for exploring the function of
CD28 on myeloma cells and determining whether an auto-
ocrine activation loop is operational in the case of coex-
pression of CD28 and B7 Ag on the 4 HMCL. Six HMCLs bore
CD40 and 4 CD30. These Ag belong to the same protein
family including NGF and TNF receptors. CD40 is a
potent B-cell-activation Ag which, in conjunction with IL-
16, induces the massive proliferation and differentiation of
B cells into plasma cells. Again, it needs to be determined
whether this activation pathway is operational in XG cells
and, more generally, in the pathology of MM. Finally, 4
HMCL expressed CD23 and 5 a weak density of CD22.
CD21, which is lacking on myeloma cells, was recently
shown to be the ligand for sCD23. Interestingly, KPM8226
myeloma cells were found to bind the largest amount of
sCD23, whereas only weak labeling of these cells was
found with anti-CD21 MoAb. This suggests that a specific
conformation of CD21 is present on myeloma cells that is
poorly recognized by anti-CD21 MoAb but that binds sCD23
efficiently. These considerations are of importance, given
the role of sCD23 in conjunction with IL-1 alpha in inducing
the differentiation of centrocytic cells into plasmablastic
cells.

Concerning adhesion molecules, all XG HMCL strongly
expressed CD54, and 4 of 10 a low density of its ligand
LFA-1 Ag, which is in agreement with previous studies. All
XG HMCL expressed the VLA-4 integrin known to be essen-
tial to B-cell differentiation and more recently for interac-
tion of normal or malignant plasma cells with bone
marrow stromal cells. Seven HMCL also expressed
VLA-5 Ag (a ligand for fibronectin), and 6 a low
density of VLA-6 integrin (the ligand for laminin). All XG HMCL
expressed CD44, an important Ag for adhesion of murine
plasmacytoma cells to stromal cells. Finally, 4 HMCL ex-
pressed CD56 (NCAM), which has been shown to be a fea-
ture of malignant plasma cells.
Mutations of immunoglobulin genes

Follicular dendritic cell

Fig 6. Malignant stem cell in human multiple myeloma.

we found that t(11;14) translocation resulted in overexpression of cyclin D1 gene in some HMCL.49 t(11;14) translocation frequently occurred in centrocytic50 and intermediate lymphocytic lymphomas.51 t(11;14) was also found in 4 previously published HMCL52 and, in a preliminary report,53 in 20% of patients with MM and an abnormal karyotype. The fact that the myeloma stem cell is probably very similar to a centrocytic cell may account for this latter observation. Although patients with t(11;14) in the preliminary report53 were not found to differ in their standard clinical characteristics compared to other patients, the high frequency of t(11;14) translocation in our freshly-obtained cell lines suggests that such translocation and cyclin D1 gene activation may favor extramedullary proliferation and the obtaining of cell lines. Partial loss of chromosome 13 was found in 4 of 8 HMCL and might have involved the retinoblastoma susceptibility gene located in 13q1.4.54

It may be concluded that the possibility of obtaining human myeloma cell lines reproducibly from every patient with extramedullary proliferation offers a unique tool for studying the phenotype, activation antigens, cytokine requirement and gene abnormalities of the tumoral myeloma stem cell in MM.

ACKNOWLEDGMENT

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Reproducible obtaining of human myeloma cell lines as a model for tumor stem cell study in human multiple myeloma

XG Zhang, JP Gaillard, N Robillard, ZY Lu, ZJ Gu, M Jourdan, JM Boiron, R Bataille and B Klein

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