Brief Definitive Report

Oncostatin M, Leukemia Inhibitory Factor, and Interleukin 6 Induce the Proliferation of Human Plasmacytoma Cells Via the Common Signal Transducer, GP130

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Summary

We analyzed the stimulatory effect of oncostatin M (OSM), leukemia inhibitory factor (LIF), interleukin 6 (IL-6), IL-11, and the inhibitory effect of anti-IL-6 antibody (Ab), anti-IL-6 receptor monoclonal antibody (mAb), and anti-gp130 mAb on the growth of human plasmacytoma cells freshly isolated from a patient with multiple myeloma. The purified cells showed a plasmacytoid morphology and expressed CD38, CD54, and CD56 antigens but no CD3, CD5, CD10, CD19, CD20, or very late antigen 5. IL-6 receptor (IL-6R) and its signal transducer, gp130, were expressed on their cell surface at a low level. Dose-dependent proliferation of the cells in response to OSM, LIF, and IL-6, but not to IL-11, was observed using [^{3}H]TdR incorporation in vitro. Both anti-IL-6 Ab and anti-IL-6R mAb inhibited the growth of the cells in the presence or absence of exogenous IL-6. These cells release IL-6 but not OSM or LIF into the culture supernatant during short-term culture. Therefore, an autocrine growth mechanism mediated by IL-6, but not by OSM or LIF, was confirmed. Furthermore, anti-gp130 mAb completely inhibited the proliferation of the cells induced by OSM, LIF, as well as IL-6. These data indicate that OSM, LIF, and IL-6 can act as growth factors of human plasmacytoma cells through a common signal transducer, gp130, on their cell surface, and also suggest the potential therapeutic application of anti-gp130 mAb, as well as anti-IL-6R mAb against myeloma/plasmacytomas.

IL-6, a pleiotropic cytokine with a wide range of biological activities (1), is a potent growth factor for murine plasmacytoma cells and human myeloma cells (2–5). Studies on human myeloma cells freshly isolated from the bone marrow of patients demonstrate that IL-6 is an autocrine as well as paracrine growth factor in human multiple myeloma (4, 5). On the basis of this finding, Klein et al. (6) showed that the in vivo administration of murine anti-IL-6 mAb to a patient with plasma cell leukemia was therapeutically effective.

IL-11 was originally described as a factor that promoted the growth of a murine plasmacytoma cell line (7). It supports the growth of hematopoietic progenitor cells directly and synergistically with IL-3, and also promotes both human and mouse megakaryocyte colony formation (8).

Recently oncostatin M (OSM), leukemia inhibitory factor (LIF), and ciliary neurotrophic factor (CNTF), as well as IL-11, were shown to have multiple biological activities that overlap with one other and with those of IL-6 (8–11). The functional redundancy of these cytokines could be explained by their sharing the same signal transducing molecule, gp130 (12, 13). This hypothesis is supported by the finding that anti-gp130 mAb can block the synthesis of acute phase proteins in hepatocytes induced by either IL-6, LIF, or OSM (14) and also block the growth inhibition of melanoma cells induced by either IL-6 or OSM (15). From this evidence, it seems likely that these cytokines, as well as IL-6, may be growth factors of human myeloma/plasmacytoma cells. In this study, we report that OSM and LIF, as well as IL-6, can induce the growth of freshly isolated plasmacytoma cells through a common signal transducer, gp130, on their cell surface.

Materials and Methods

Case Report. A 53-yr-old male patient initially presented with multiple advanced lytic bone lesions, pathological bone fractures, and marrow plasmacytosis (~35%) with mitotic features. He was diagnosed as having multiple myeloma, although no serum M component was detectable. The patient also had extramedullary plasmacytoma tumors. Biopsy tissue from an intercostal tumor was used for analysis after informed consent was obtained.

Phenotypical Analysis of Plasmacytoma Cells. Plasmacytoma cells were freshly isolated from biopsy tissue and enriched by density

J. Exp. Med. © The Rockefeller University Press • 0022-1007/94/04/1343/05 $2.00
Volume 179 April 1994 1343–1347

gradient centrifugation (16). Isolated cells were centrifuged onto glass slides, fixed, and examined for morphology by May-Grünwald-Giemsa's stain and for cytoplasmic Ig using affinity-purified FITC-labeled goat Abs specific for human α, γ, μ, κ, and λ chains (Cappel Organon Teknica Co., West Chester, PA). Cell surface IL-6R and gp130 were identified using biotinylated mouse PM1 mAb ($\gamma 1\kappa$ isotype [17], provided by Chugai Pharmaceutical Co., Shizuoka, Japan) and AM64 mAb ($\gamma 1\kappa$ [18], provided by Tosoh Co., Kanagawa, Japan), respectively, in conjunction with PE-labeled streptavidin. Antihuman very late antigen 5 (VLA-5) α chain mAb (Seikagaku Co., Tokyo, Japan) was used to identify cell surface VLA-5 and was developed with FITC-labeled goat anti-mouse Ig Ab (Zymed Laboratories, Inc., San Francisco, CA). The other surface antigens were detected with FITC- or PE-conjugated anti-CD3, anti-CD5, anti-CD10, anti-CD19, anti-CD20, anti-CD38, anti-CD54, and anti-CD56 mAbs (Becton Dickinson & Co., Mountain View, CA). 2×10^5 cells from single cell suspensions were incubated with the appropriate Ab at a concentration of 6–50 μg/ml for 20 min on ice, washed in PBS with 10% FCS and, if necessary, incubated with the appropriate second-step reagents. Isotype-matched Abs with irrelevant specificity were used as negative controls. Flow cytometric analysis was performed on a FACScan® (Becton Dickinson & Co.).

In Vitro Cell Proliferation Assay. Purified plasmacytoma cells were cultured at a density of $0.3–1 \times 10^6$ cells/ml on 96-well plates for 72 h in 0.2 ml of RPMI-1640 medium supplemented with 10% FCS and 5×10^{-5} mol/liter 2-ME in the presence or absence of recombinant human OSM, LIF, IL-6, IL-11, anti-IL-6 Ab (goat anti-IL-6 antiserum), humanized anti-IL-6R mAb (rhPM1 [19]), and/or anti-gp130 mAb (gpx22 [20]). In an experiment to test the synergistic effects of the cytokines on cell proliferation, various combinations of the cytokines were added. DNA synthesis was measured after the last 16-h pulse label of a 72-h culture with 18.5 kBq/well of [^{3}H]TdR (370 GBq/mmol; Dupont, Wilmington, DE). OSM was purchased from Genzyme Corp. (Cambridge, MA), and LIF and IL-11 were obtained from R&D Systems, Inc. (Minneapolis, MN). IL-6 was provided by Ajinomoto Co. (Kawasaki, Japan); goat anti-IL-6 antiserum by the National Biological Standards Board of the World Health Organization; rhPM1 by Chugai Pharmaceutical Co.; and gpx22 by Tosoh Co.

Cytokine Assays. Purified plasmacytoma cells were cultured at 10^6 cells/ml on 24-well plates for 48–120 h and the culture supernatants were harvested for the cytokine assays. IL-6 was measured by a dissociation-enhanced lanthanide fluoroimmunoassay (21). OSM and LIF were measured by ELISA (R&D Systems, Inc.).

Results

Characterization of Isolated Plasmacytoma Cells. Greater than 99% of the freshly purified cells showed plasmacytoid morphology which can be classified as plasmablastic type according to Greipp's criteria (22), and expressed very small amounts of intracytoplasmic Ig κ light chains. They expressed CD38, CD54, and CD56 antigens but no detectable CD3, CD5, CD10, CD20, or VLA-5 on their cell surface. Low levels of IL-6R and gp130 were identified on their cell surface.

OSM-, LIF-, and IL-6–dependent Proliferation of the Plasmacytoma Cells. When the plasmacytoma cells were cultured with OSM, LIF, IL-6, or IL-11 at varying concentrations (1–100 ng/ml), dose-dependent growth on OSM, LIF, and IL-6 was observed (Fig. 1). 100 ng of recombinant OSM, LIF, and IL-6 augmented the proliferation by 6.0-, 5.5-, and 1.8-fold,

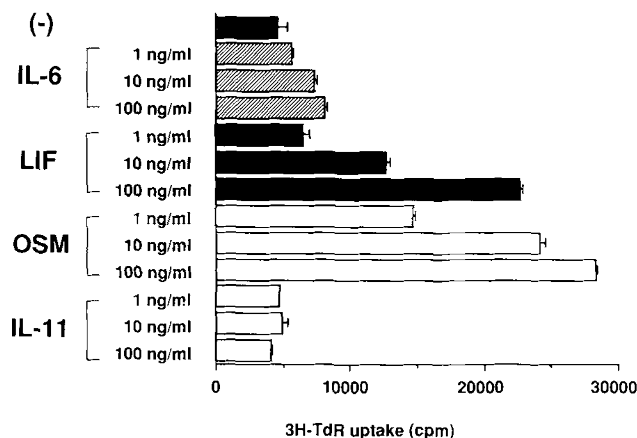

Figure 1. In vitro cell proliferation analysis of freshly isolated plasmacytoma cells. The cells (6×10^4/200 μl/well) were cultured with varying concentrations of recombinant human OSM, LIF, IL-6, of IL-11. DNA synthesis was measured by [^{3}H]TdR incorporation after 3 d of culture. Dose-dependent proliferation with OSM, LIF, and IL-6 but not with IL-11 was observed.

respectively. The stimulatory effect of OSM and LIF was stronger than that of IL-6, although 100 ng of these cytokines was below the optimal dose. IL-11, however, did not induce the growth of plasmacytoma cells as reported in an earlier study (23).

To test for synergistic effects among OSM, LIF, IL-6, and IL-11, various combinations of these cytokines were added. A slight augmentation of [^{3}H]TdR uptake was observed when the cells were cultured with LIF and IL-6 in addition to either OSM or IL-11 (Fig. 2), although it was not significant.

Inhibition of Plasmacytoma Cell Growth by Anti-IL-6 Ab, Anti-IL-6R mAb, and Anti-gp130 mAb. To determine the inhibitory effect of anti-IL-6 Ab or anti-IL-6R mAb on the growth of plasmacytoma cells, the cells were cultured with these Abs in the presence or absence of IL-6. In this experiment, humanized anti-IL-6R mAb (rhPM1) was used for possible ther-

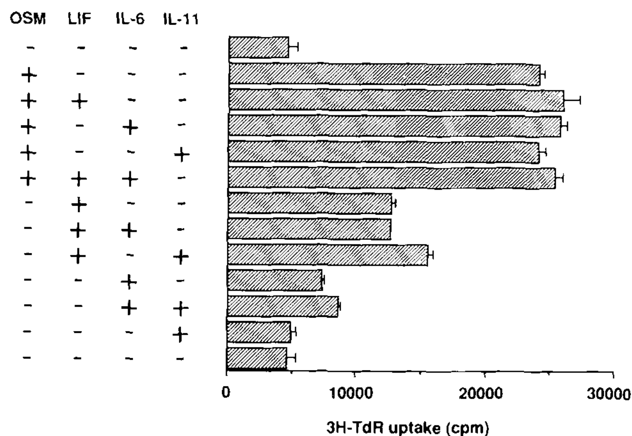

Figure 2. Synergistic effects of cytokines on the proliferation of freshly isolated plasmacytoma cells. DNA synthesis in the cells (6×10^4/200 μl/well) cultured with various combinations of OSM, LIF, IL-6, and IL-11 (10 ng/ml each) was measured. There seemed to be no significant synergistic effects with these cytokines.

apeutic application. rhPM1 as well as goat anti-IL-6 antiserum inhibited the spontaneous growth of plasmacytoma cells (66–80% inhibition, Fig. 3 *A* [−]), suggesting that IL-6 participates in an autocrine growth mechanism. rhPM1 could also inhibit the proliferation of the cells even when rIL-6 was added exogenously (81–87% inhibition, Fig. 3 *A*, *IL-6*).

To confirm that OSM, LIF, and IL-6 stimulate cell growth through a common signal transducer, gp130, anti-gp130 mAb was used for neutralization. Anti-gp130 mAb clearly blocked the OSM-, LIF-, and IL-6–dependent growth of plasmacytoma cells (84–97% inhibition, Fig. 3 *B*). The inhibitory effect of anti-gp130 mAb on spontaneous cell growth was almost as great as that of anti-IL-6 Ab or anti-IL-6R mAb in vitro, suggesting that LIF and OSM were not involved in an autocrine growth of these plasmacytoma cells.

Cytokine Production by Plasmacytoma Cells. In the culture supernatant of the plasmacytoma cells, 125 pg/ml of IL-6 was detected at 48 h and the IL-6 concentration reached 470 pg/ml at 120 h. However, no detectable levels of OSM or LIF were observed in the supernatants either at 48 or 120 hours. (LIF <15 pg/ml; OSM <15 pg/ml). The data confirmed that these cells respond to an autocrine growth mechanism mediated by IL-6 but not by OSM or LIF.

Discussion

The data indicate that an autocrine growth mechanism mediated by IL-6 is involved in the pathogenesis of this plasmacytoma case, as previously reported by Kawano et al. (4). In contrast, Klein et al. (5) demonstrated paracrine but not autocrine regulation of the growth and differentiation of myeloma cells by IL-6 secreted from the stroma of the bone marrow in patients. Since it is very difficult to identify the IL-6–producing cells and the cells responding to IL-6 in the marrow cells of those with multiple myeloma, we took advantage of an extramedullary plasmacytoma to obtain an extremely pure population of plasmacytoma cells in this study. Furthermore, an increased proportion of the plasmacytoma cells undergoing mitosis was observed after in vitro culture both in the presence and absence of exogenous IL-6 (data not shown), thus confirming an autocrine growth mechanism. Although both IL-6R and gp130 were expressed at a low level, the cells clearly responded to IL-6, LIF, and OSM, suggesting that even relatively small number of receptors could transduce enough signals to generate a growth response.

The proliferation of freshly isolated plasmacytoma cells was inhibited in vitro by a humanized anti-IL-6R mAb, rhPM1. The inhibitory effect of rhPM1 was as strong as that of the polyclonal anti-IL-6 Ab even in the presence of additional IL-6. Blockade by anti-IL-6R mAb has been reported previously on myeloma cell lines (19), but not on freshly isolated human cells.

The plasmablastic features and the expression of CD38, CD54, and CD56 but no VLA-5 antigens on these plasmacytoma cells are consistent with a previously reported phenotype of immature myeloma cells that grow in response to IL-6 (24). The loss of the VLA-5 antigen on this extramedullary plasmacytoma suggests that VLA-5 as well as CD54 (intercellular adhesion molecule 1) and CD56 (neural cell adhesion molecule) may play a role in the interactions with marrow stromal cells (24–26).

Functional pleiotropy and redundancy, common characteristics of cytokines, can be explained by interactions between multiple ligand-binding receptors and a common signal transducer that lacks direct ligand-binding activity (12, 13). We demonstrated that OSM and LIF stimulated the growth of freshly isolated human plasmacytoma cells as well as IL-6. Such activity which had never been demonstrated before, had been anticipated since these cytokines share the gp130 protein as a common signal transducer. The blockade of the OSM-, LIF-, and IL-6–dependent growth of the cells by anti-gp130 mAb confirmed that gp130 was actually used as a common signal transducer for all of these cytokines in freshly isolated plasmacytoma cells. The in vitro stimulatory activity on cell growth differed among these cytokines, as shown in Fig. 1. IL-6 appeared less effective than OSM and LIF in this particular case. This may be explained by the number of ligand-binding receptors specific for each cytokine. Another possible explanation may be that the cells were already stimulated by endogenous IL-6 and were therefore less sensitive to exogenous IL-6. On the other hand, IL-11 did not stimulate the growth of the cells in this study. This result is consistent with a report by Paul et al. (23), and is probably due to the absence of IL-11R on the cell surface. Little synergistic effect among these cytokines was observed. This might be explained by the competitive occupation of a common

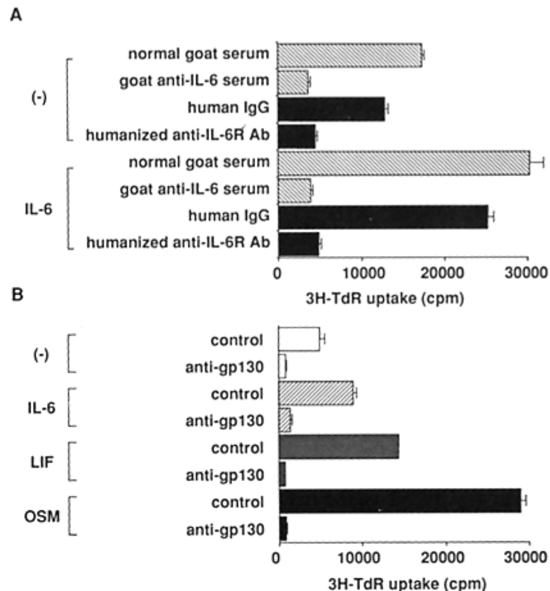

Figure 3. Inhibition of plasmacytoma cell growth by anti-IL-6 Ab, anti-IL-6R mAb, and anti-gp130 mAb. (*A*) The cells (2×10^5/200 μl/well) were cultured with goat anti-IL-6 antiserum (10^{-5} dilution), humanized anti-IL-6R mAb (10 μg/ml), or control Abs in the presence or absence of IL-6 (10 ng/ml). Goat anti-IL-6 antiserum and humanized anti-IL-6R mAb inhibited both the spontaneous and IL-6–induced proliferation of the cells. (*B*) The cells (6×10^4/200 μl/well) were cultured with 10 ng/ml of OSM, LIF, and IL-6 in the presence of anti-gp130 mAb or control mAb (10 μg/ml). Anti-gp130 mAb inhibited the spontaneous and augmented growth of the cells by OSM, LIF, and IL-6.

signal transducer. The evidence that anti-gp130 mAb inhibited the spontaneous growth of these cells as much as anti-IL-6R mAb and that no detectable level of OSM or LIF was observed in the culture supernatant of these cells suggests that no autocrine mechanism by OSM or LIF was active in this case.

Our data suggest the potential therapeutic application of anti-IL-6R mAb and anti-gp130 mAb to this disease, although we do not yet know which cytokine is the major growth stimulator in vivo. Since OSM and LIF are found in vivo (27, 28), anti-gp130 mAb may be therapeutically more effective than anti-IL-6R mAb. In some cases of myeloma/plasmacytoma, no stimulatory effect with any of the cytokines except IL-6 was observed (data not shown). Therefore, we propose that myeloma cases could be classified into one of three groups according to their response to these cytokines: (*a*) myeloma cells whose growth is dependent on IL-6 only; (*b*) myeloma cells whose growth is dependent on OSM, LIF, and IL-6; and (*c*) myeloma cells whose growth is independent of OSM, LIF, and IL-6. We did not test CNTF in this case. CNTF may also have the same activity on plasmacytoma cells, if the CNTF binding receptor is expressed.

Several reports have suggested that the malignant transformation into myeloma cells might occur in immature B cells (29, 30). We do not know whether such precursor cells of myeloma are vulnerable to anti-IL-6R mAb or anti-gp130 mAb, which would influence the therapeutic efficacy of these antibodies. Furthermore, the use of these antibodies in vivo may cause unpredictable effects because of the functional pleiotropy of these cytokines. Nonetheless, these experiments have provided useful information regarding the therapeutic application of these antibodies to human myeloma/plasmacytoma.

We thank Miss A. Nobuhara for her excellent secretarial assistance.

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Received for publication 3 December 1993 and in revised form 21 January 1994.

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