Inorganic polyphosphate and specific induction of apoptosis in human plasma cells

Laura Hernandez-Ruiz
Inés González-García
Carmen Castro
José A. Brieva
Felix A. Ruiz

Background and Objectives. Inorganic polyphosphate (polyP), a ubiquitous phosphate polymer with ATP-like bonds, has recently been related to a variety of functions including blood coagulation and cell proliferation. We investigated the effects of polyP in the biology of human plasma cells (PC), responsible for the production and maintenance of antibodies in response to antigens.

Design and Methods. The U266 myeloma cell line was used to study whether polyP affects immunoglobulin (Ig) secretion and survival. Different human cell lines were used to test the specificity of polyP on viability. We analyzed Ig secretion of PC from bone marrow and peripheral blood after polyP addition. A conventional tetanus toxoid booster immunization was used to increase the proportion of PC in order to examine the *ex vivo* effects of polyP. We also tested the effects of polyP on primary myeloma cells. Ig secretion and apoptosis were determined by ELISA and FACS respectively.

Results. Addition of polyP to human PC produced an unexpected inhibition of Ig secretion and stimulation of apoptosis. PolyP generated apoptosis specifically in PC, myeloma (malignant PC) cell lines, primary myeloma cells, and B lymphoid cell lines. Normal B cells, T cells, total blood mononuclear cells, and non-lymphoid cell lines were not affected by polyP. In the U266 myeloma cell line, polyP induced externalization of phosphatidylserine, activation of caspase-3, and arrest of the cell cycle. The protective effects of interleukin-6 did not overcome the polyP-induced apoptosis

Interpretation and Conclusions. Taken together, our results suggest for the first time the relevance of the use of polyP to the humoral immune response and open prospects for polyP as a novel therapy for myeloma.

Key words: polyphosphate, plasma cells, myeloma, apoptosis, cytotoxicity.

Haematologica 2006; 91:1180-1186

From the Unidad de Investigacion (LH-R, IG-G, FAR) and Servicio de Immunologia (JAB), Hospital Universitario Puerta del Mar, Cadiz, Spain; and Facultad de Medicina (CC, FAR), Universidad de Cadiz, Cadiz, Spain..

Correspondence:
Felix A. Ruiz, Unidad de Investigacion, 9ª Planta, Hospital Universitario Puerta del Mar, Avenida Ana de Viya 21, 11009 Cadiz, Spain.
E-mail: felix.ruiz@uca.es

Human plasma cells (PC) are ultimately responsible for human humoral immune responses. PC represent the last stage of B-cell differentiation controlling the formation and maintenance of antibodies in response to antigens. Malignant PC, known as myeloma cells, proliferate in patients with multiple myeloma producing the complications of this incurable disease. More efficient drugs are urgently needed for the management of multiple myeloma. In this context, there is particular interest in compounds that can overcome the effect of interleukin-6 (IL-6), one of the most important growth factors for myeloma, which prevails over most of the apoptotic signals mediated by cytotoxic agents.[1-3]

Inorganic polyphosphate (polyP) is a polymer formed by phosphate (Pi) residues linked by high-energy phosphoanhydride bonds. Existing since prebiotic times,[4] polyP has been present in all cells from early in evolution.[5] PolyP has been detected in human gingival fibroblasts, osteoblasts, erythrocytes, and peripheral blood mononuclear cells.[6] We have recently reported that human platelets have 10 to 20 times more polyP than other human tissues investigated.[7] Despite its widespread presence, only recently have a variety of associated functions been reported for polyP in mammalian cells. It has been found that this polyanion can modulate blood coagulation,[8] induce osteoblast calcification,[9] and enhance cellular proliferation in breast cancer[10] and human fibroblasts.[11] It has also been shown that polyP possesses antibacterial[6,12] and antiviral activities,[13] but no apoptotic or cytotoxic effects have been reported for mammalian cells. In this work we investigated the effects of extracellular polyP in the biology of human PC. We determined antibody secretion, cell survival and/or apoptosis of myeloma cell lines, non-lymphoid cell lines, normal human blood cells, and myeloma primary cells in the presence of polyP.

Design and Methods

Reagents

RPMI 1640 culture medium, L-glutamine, fetal calf serum (FCS), penicillin and streptomycin were purchased from Gibco BRL Life Technologies (Paisley, Scotland). Cycloheximide, 1,4-diazabicyclo[2.2.2]octane (Dabco), $PolyP_{25}$, $PolyP_{45}$, $PolyP_{75}$, and Sephadex G25 were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Annexin-V-fluoroscein isothiocyanate (FITC) was provided by Roche (Penzberg, Germany). Allophycocyanin (APC)-labeled monoclonal antibodies against CD19, phycoerythrin (PE)-labeled monoclonal antibodies against CD38, FITC-labeled monoclonal antibodies against CD138, and PE-labeled monoclonal antibodies against active caspase-3 were provided by Becton Dickinson (San Jose, CA, USA). Biosource (Camarillo, CA, USA) provided peroxidase-conjugated goat anti-human IgG and IgE, used for determining human Ig by enzyme linked immunosorbent assay (ELISA). The Intrastain kit was from Dako (Glostrup, Denmark). Heparin was from Rovi Pharmaceutical Laboratories (Madrid, Spain). Purified tetanus toxoid was a generous gift from Llorente Laboratorios (Madrid, Spain). *Escherichia coli* strain CA38 *pTrcPPX1* was kindly provided by Prof. Arthur Kornberg, Stanford University School of Medicine (Stanford, CA, USA). All other reagents were analytical grade.

Cell lines and cell cultures

The cell lines U266, IM9, DAUDI, RAJI, and HEp-2 were obtained from the European Collection of Cell Cultures (London, UK). Plasma cells and cell lines were grown in RPMI 1640 medium (GIBCO) supplemented with 10% FCS, 10 mM glutamine, 100 IU/mL penicillin, and 100 μg/mL streptomycin. The human neuroblastoma NB69 cell line was kindly donated by Dr. Carmen Estrada (University of Cadiz, Spain) and cultured as previously described[14] in DMEM supplemented with 2 mM glutamine, 1 g/L glucose and 15% FCS. HeK293 cells were obtained from Invitrogen (San Diego, CA, USA) and cultured following the manufacturer's instructions in DMEM supplemented with 4.5 g/L glucose, 2 mM L-glutamine, 10% FCS and 0.1 mM MEM non-essential amino acids.

Preparation of blood and bone marrow mononuclear cells

Heparinized peripheral blood was obtained from volunteers 0 or 6 days after a conventional tetanus toxoid booster immunization (5 LF).[15] Peripheral blood mononuclear cells were obtained after Ficoll/Hypaque density-gradient centrifugation of peripheral blood from volunteers as previously described.[16] Remaining material from bone marrow aspirates was obtained from patients after the diagnosis of multiple myeloma. Bone marrow mononuclear cells were obtained as previously reported and prepared by Ficoll/Hypaque density-gradient centrifugation.[16] Approval for this study was obtained from the institutional review board (*Comisión de Ética*) and informed consent was provided according to the Declaration of Helsinki.

Secretion of Ig

Human PC and myeloma cell lines were cultured at 1.2×10^6 cell/mL in 96-well plates. Cell-free supernatants were collected after 3, 6, 16, or 120 hours, and total IgG or IgE secretion was determined by ELISA as previously reported.[17] Addition of millimolar concentrations of $polyP_{75}$ to isolated control cell supernatants did not affect the sensitivity of the ELISA assay (*results not show*).

Cell labeling for apoptosis and cell cycle analysis

Apoptosis was determined by cell labeling with annexin-V-FITC (Roche) following the manufacturer's procedure. Briefly, 6×10^5 control or polyP-treated cells were washed twice with phosphate-buffered saline (PBS), resuspended in isotonic calcium buffer, and then stained with annexin-V-FITC and/or propidium iodide (PI) (5 μM). Early apoptosis was discriminated from late apoptosis by the combination of annexin and PI staining. Apoptosis was confirmed by caspase-3 intracellular staining; PBS-washed cells were fixed and permeabilized using the Intrastain kit (Dako) and incubated with PE-labeled monoclonal antibodies against active caspase-3 in a 1/9 dilution. For cell cycle measurements, 1.5×10^6 cells were fixed with 70% ethanol at 4°C, washed twice with PBS, and re-suspended in PBS with 100 μg/mL RNase and 50 μg/mL of PI. Samples were analyzed by flow activated cell sorting (FACS).

Flow cytometry analysis

FACS analysis was performed on a FACScalibur cytometer (Becton Dickinson). Green fluorescence (FITC, FL1), red/orange fluorescence (PE and PI, FL2), and red fluorescence (APC, FL4) were collected with 530/30 nm, 585/42 nm, and 661/16 nm bandpass filters respectively. Cell analysis was performed with CELLQUEST software (Becton Dickinson). Data were collected from 10,000 cells per sample.

Fluorescence microscopy analysis of cellular apoptotic bodies

Control or polyP-treated cells were washed twice with PBS and fixed at 3×10^6 cells/mL with 4% formaldehyde in PBS. Then, 4',6-diamidino-2-phenylindole (DAPI) was added at 1 μg/mL and after 5 min the samples were mounted on a slide and observed in an epifluorescence microscope (Olympus model BX60). Images were recorded with an Olympus DP70 digital camera. Percentages of cells with apoptotic bodies were calculated from at least 500 cells for each experiment and condition.

PolyP isolation and measurement

PolyP$_{25}$, PolyP$_{45}$, or PolyP$_{75}$ (Sigma) were partially isolated using spin column purification with Sephadex G25[18] to remove orthophosphate, pyrophosphate, and lower chain polyP. Final concentrations of polyP were measured using purified recombinant yeast exopolyphosphatase (*scPPX1*) as described before.[19] In all experiments described here, polyP, and PPi concentrations are expressed in terms of phosphate residues.

Cell viability

Percentages of dead cells in either the HeK293 or the NB69 cell line were determined by cell counting after trypan blue staining. In other cells lines, percentages of dead cells were resolved by FACS analysis of PI incorporation.

Results

Effects of polyP on Ig secretion and survival of the U266 myeloma cell line

We used the U266 myeloma cell line to study whether extracellular polyP affects Ig secretion and survival. The Ig secretion of U266 cells was inhibited proportionally when they were cultured in the presence of increasing concentrations of PolyP$_{75}$ (Figure 1A). Around 3 mM of PolyP$_{75}$ (in terms of phosphate residues) produced a 50% decrease in Ig secretion. The addition of increasing concentrations of orthophosphate (up to 5 mM) did not produce any effect on the active Ig secretion of U266 cells (Figure 1A), suggesting that the effect of polyP was not due to the increase of the phosphate concentration or the ionic force in the culture media. The Ig secreted from U266 cells was actively produced, since cycloheximide, which blocks protein synthesis, depleted the Ig secretion (Figure 1A).

Inhibition of the Ig secretion was dependent on the duration of incubation with polyP and maximum inhibition was reached after 16 hours of incubation with polyP (Figure 1B). In a similar fashion, polyP decreased myeloma survival (*data not show*). The alteration of Ig secretion and cell survival depended on the length of the polyP chain (Figure 1C). Only PolyP$_{75}$ had a significant effect on the survival (Figure 1C) and Ig secretion (*data not shown*) of U266 cells: shorter chain polyphosphates, pyrophosphate or phosphate did not.

Addition of heparin (up to 150 μg/mL) did not produce any changes in the secretion and survival of U266 cells (*data not shown*). Total negative charges added with either heparin (at 150 μg/mL) or polyP (at 1^{-5} mM) were both in the same molar range, suggesting that the addition of any negatively-charged polymer was not sufficient to induce changes in survival and Ig secretion of myeloma cells.

PolyP-mediated apoptosis in the U266 myeloma cell line

Flow cytometry analysis of U266 cells labeled with PI and extracellular annexin-V was used to determine apoptosis in the presence or absence of polyP (Figure 2A). After

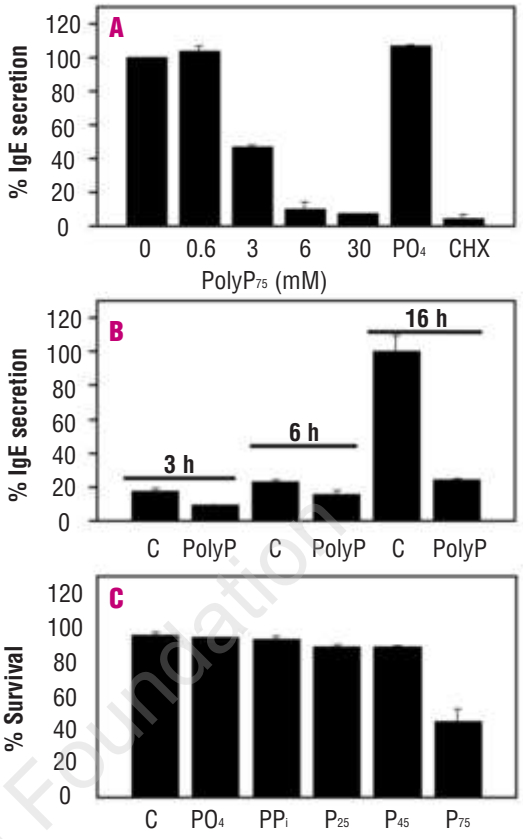

Figure 1. PolyP inhibits Ig secretion and reduces cell survival of the U266 myeloma cell line. (A) IgE secretion after 16 hours' treatment with different concentrations of PolyP$_{75}$, 3 mM orthophosphate (PO$_4$), or 10 μg/mL cycloheximide (CHX): 100% of the secretion represents 0.73 μg IgE/mL. (B) IgE secretion after 3, 6, or 16 hours of incubation with 6 mM PolyP$_{75}$. (C) Cell survival after 16 hours' treatment with 3 mM of orthophosphate (PO$_4$), pyrophosphate (PPi), PolyP$_{25}$ (P$_{25}$), PolyP$_{45}$ (P$_{45}$), or PolyP$_{75}$ (P$_{75}$). Results are expressed in percentages, considering the control sample as 100%. Values represent the mean±S.E from three separate experiments. PolyP, and PP$_i$ concentrations are expressed in terms of phosphate residues.

16 hours of incubation with polyP, the percentage of early apoptotic cells and late apoptotic cells increased by two and six times, respectively (Figure 2A). As in the secretion experiments (Figure 1), the addition of orthophosphate (3 mM) did not produce any effect on the apoptosis of myeloma U266 cells (Figure 2A). After the addition of polyP, the proportion of early and late apoptotic cells increased over time and a plateau was reached after 6 hours (Figure 2B). We then analyzed the activation of caspase-3, the key enzyme in mammalian cell apoptosis. The addition of polyP produced activation of caspase-3 in four times more U266 cells (Figure 2C). We also studied the effect of polyP on the PC cell cycle by measuring nuclear DNA by PI. As before, treatment of U266 cells with polyP produced a 3-fold increase in the proportion of apoptotic cells (Figure 2D) This increase in apoptosis was accompanied by a decrease in the proportion of cells in S/G2/M stages, suggesting an arrest of the cell cycle (Figure 2D).

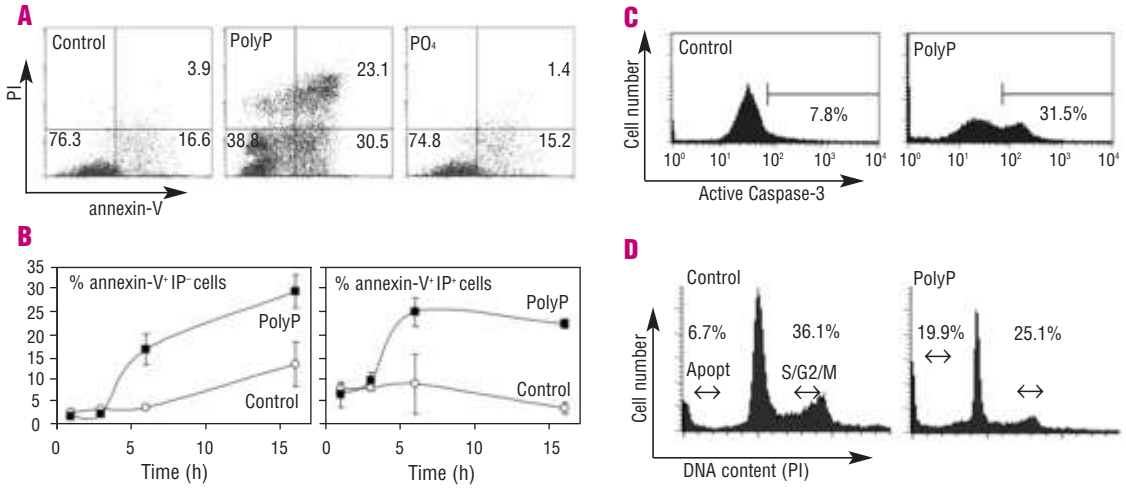

Figure 2. PolyP induces apoptosis and suppresses cell cycling in the U266 myeloma cell line. (A) Flow cytometry analysis of U266 cells labeled with PI and annexin-V FITC. Cells were cultured for 6 hours in the absence (control) or presence of 3 mM $PolyP_{75}$ (PolyP), or orthophosphate (PO_4). Percentages of viable cells (annexin V^-/PI^-), early apoptotic cells (annexin V^+/PI^-) and late apoptotic cells (annexin V^+/PI^+) in each plot are indicated in the corresponding quadrants. (B) Percentages of U266 early (annexin V^+/PI^-) and late apoptotic cells (annexin V^+/PI^+) after 1, 3, 6, or 16 hours in the absence (control) or presence of 3 mM $PolyP_{75}$ (PolyP). (C) PolyP induces activation of caspase-3. Flow cytometry analysis of U266 cells labeled with an FITC-specific antibody against the active form of caspase-3. Cells were cultured for 16 hours in the absence (control) or presence of 3 mM $PolyP_{75}$ (PolyP). Percentages of cells with active caspase-3 are indicated. (D) Percentages of cells in each stage of the cell cycle were determined by PI staining of nuclei and flow cytometry analysis. Cells were incubated in the presence (control) or absence of 3 mM $PolyP_{75}$ for 6 hours. The number in the left corner represents the apoptotic fraction, and the number in the right corner represents the combined S and G2/M stages of the cell cycle. Data shown are from a representative experiment except in (B), where results represent the mean±S.E from three separate experiments.

Table 1. Effect of polyP on survival of different human cell lines.

	Percentages of non-viable cells	
	Control	*PolyP*
B lymphoid cell lines		
U266[a] (myeloma)	4.92±0.4	22.72±0.8
IM9[a] (lymphoblast)	27.44±1.5	92.79±1.6
DAUDI[a] (Burkitt's lymphoma)	10.41±1.7	22.63±2.5
RAJI[a] (Burkitt's lymphoma)	5.01±0.7	14.18±1.4
Non-B lymphoid cell lines		
HEp-2[a] (adenocarcinoma)	3.30±0.1	2.75±0.4
NB69[b] (neuroblastoma)	3.22±0.0	4.25±1.8
HeK293[b] (embryonic kidney)	3.61±0.0	4.82±2.1

Cells were incubated in the absence (control) or presence of 3 mM $PolyP_{75}$ for 16 hours. Percentages of dead cells were determined by [a]propidium iodide or [b]trypan blue incorporation. Data shown are representative of three independent experiments.

Morphological analysis of the apoptotic bodies clearly confirmed the specific increment of apoptosis of polyP-treated cells (Figure 3).

Specificity of polyP in the viability of human cell lines

Previous studies in some cell lines of fibroblasts[9,11] did not report any cytotoxic effects after the addition of polyP. We, therefore, tested the specificity of polyP in the viability of different human cell lines (Table 1). In B lymphoid cell lines, such as U266, IM9, DAUDI, and RAJI, addition of $PolyP_{75}$ significantly increased the proportion of non-viable cells after 16 hours. In contrast, non-lymphoid cell lines, such as HEp-2, NB69, and HeK293, did not suffer any significant loss in viability in the presence of polyP (Table 1).

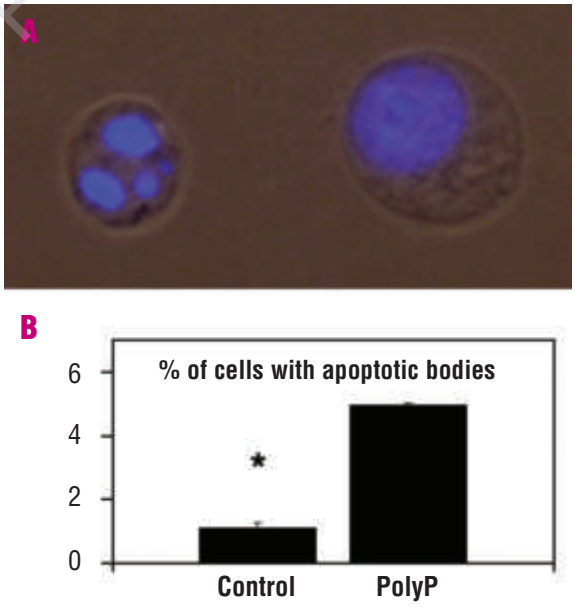

Figure 3. PolyP increases the percentage of cells with apoptotic bodies in the U266 myeloma cell line. (A) Microscopy overlapping image of fluorescent DNA-DAPI (blue) and bright field of cells treated with polyP. An apoptotic cell and a non-apoptotic cell are shown. (B) Percentages of cells with apoptotic bodies are indicated. Cells were cultured for 16 hours in the absence (control) or presence of 3 mM $PolyP_{75}$ (PolyP). Results represent the mean±S.E from two separate experiments. The t-test showed that the percentages were significantly different ($p<0.05$).

Curiously, the addition of polyP in cell cultures produced a decrease of cell adhesion. After 16 hours in the

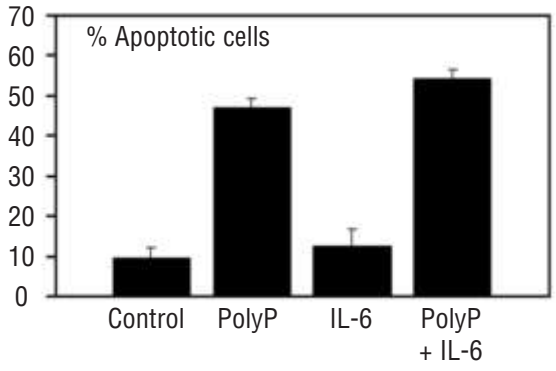

Figure 4. The protective effects of IL-6 do not overcome polyP-induced apoptosis. Percentages of apoptotic U266 cells determined by annexin-V FITC labeling and flow cytometry analysis. Cells were cultured for 16 hours without FCS in the absence (control) or presence of 3 mM PolyP$_{75}$ (PolyP), 10 ng/mL IL-6 or a combination of these. Results represent the mean±SE from three separate experiments.

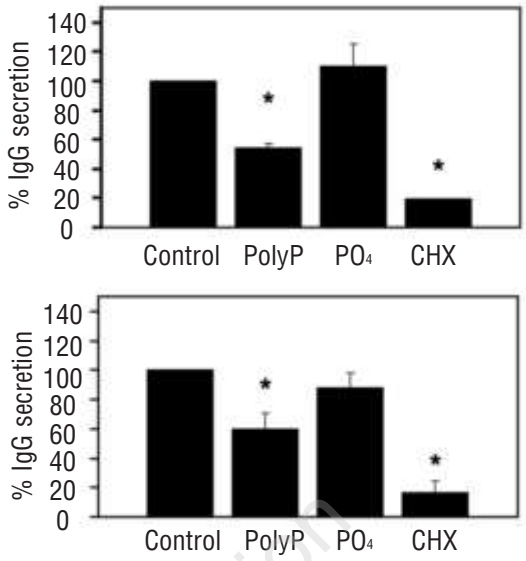

Figure 5. PolyP reduced Ig secretion of human normal PC from peripheral blood or from bone marrow. IgG secretion of isolated peripheral blood mononuclear cells (A) and bone marrow mononular cells (B) after 120 hours' treatment with 3 mM PolyP$_{75}$, 3 mM orthophosphate (PO_4), or 10 μg/mL cycloheximide (CHX). Values are expressed as the percentage of Ig secretion with respect to control cultures. 100% of the secretion represents 0.88 μg IgG/mL. Results represent the mean±S.E from three separate experiments. Asterisks indicate results that were significantly different from the control, by the t-test ($p<0.05$).

presence of 3 mM PolyP$_{75}$, adherent non-lymphoid cells were less attached to the culture flasks and lymphoid cells presented fewer cell aggregates (*data not shown*). However, the impairment of adherence did not appear to be associated with apoptosis. The presence of PolyP$_{75}$ did not produce any change in early and late apoptosis of the non-lymphoid HEp-2 cultures, as verified by labeling with PI and extracellular annexin-V (*data not shown*).

Interleukin-6 did not counteract polyP-induced apoptosis

It has been described that IL-6 protects myeloma cells against apoptosis induced by compounds such as dexamethasone, IL-4, or γ-interferon.[1-3] We studied the effect of IL-6 on the polyP-induced apoptosis of U266 cells. Addition of 10 ng/mL IL-6, a concentration known to inhibit dexamethasone-induced apoptosis, failed to reverse the apoptotic effects of polyP (Figure 4). This result indicates that polyP overcomes or bypasses the protective effects of IL-6.

PolyP inhibited Ig secretion and stimulated apoptosis in ex vivo normal human PC and primary myeloma cells

The addition of 3 mM polyP$_{75}$ (in terms of phosphate residues) produced a significant reduction of the active Ig secretion of cultured human mononuclear cell fractions from peripheral blood or bone marrow (Figure 5). The addition of a similar concentration of orthophosphate (3 mM) did not produce any effect on Ig secretion (Figure 5).

To study whether polyP produced a general effect on blood cell viability, we estimated apoptosis of the total mononuclear cell fraction from peripheral blood, by using PI and annexin-V labeling ((Table 2). The presence of 3 mM polyP$_{75}$ in the culture of blood cells did not produce any differences in the proportion of early and late apoptotic cells (Table 2).

We then measured the effect of polyP on the apoptosis of different cell populations of peripheral blood by annexin-V and surface marker labeling with CD19 and CD38 (Figure 6). In non-immunized volunteers, in whom PC are very scarce (less than 0.05%), attempts to measure apoptosis in blood PC failed (no results were obtained). For this reason, we used peripheral blood from volunteers given a tetanus toxoid booster immunization, which increases the proportion of PC by around 10-fold after 6 days.[20,21] In a CD19 CD38 dot plot, we were able to differentiate T-cell, B-cell, and PC populations (Figure 6A) and to estimate the proportion of annexin V$^+$ cells (apoptotic cells) in each population. The addition of polyP to the culture media produced a significant increase in the proportion of apoptotic PC, but did not change the proportions of the apoptotic B or T cells (Figure 6B). Similar results were obtained using

Table 2. Effect of polyP on apoptosis of total peripheral blood mononuclear cells.

	Control	*PolyP*
Viable cells	95.48±0.6	94.28±1.2
Early apoptotic cells	2.58±0.4	4.13±0.8
Late apoptotic cells	1.36±2.7	1.23±2.4

Isolated total human peripheral blood mononuclear cells were incubated in the absence (control) or presence of 3 mM PolyP$_{75}$ for 16 hours. Percentages of viable cells (annexin V$^-$/PI$^-$), early apoptotic cells (annexin V$^+$/PI$^-$) and late apoptotic cells (annexin V$^+$/PI$^+$) were determined by flow cytometry.

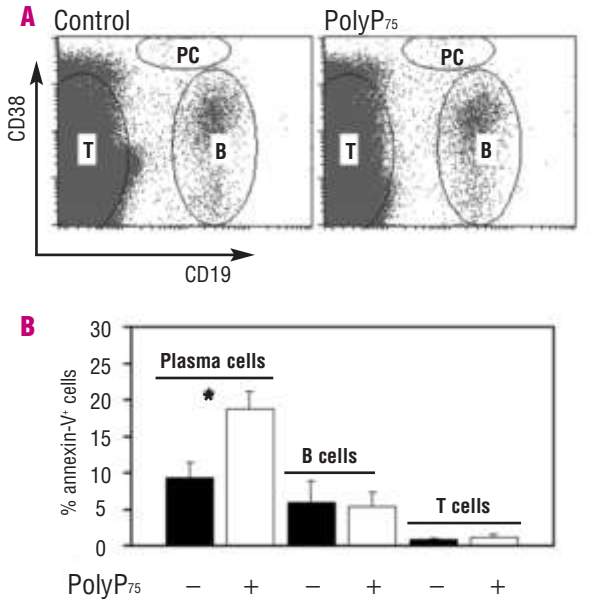

Figure 6. PolyP increases apoptosis specifically in human plasma cells from peripheral blood. Isolated peripheral blood mononuclear cells from volunteers immunized with a tetanus toxoid booster, were cultured for 16 hours in the absence or presence of 3 mM PolyP75. Cells were analyzed by flow cytometry after simultaneous labeling with anti-CD19 APC antibodies, anti-CD38 PE antibodies, and annexin-V FITC. (A) CD19 CD38 dot plots showing mononuclear cell populations: T cells (T), B cells (B), and plasma cells (PC). (B) Percentage of annexin V+ cells of cell populations represented in (A). *White bars*, control samples; *black bars*, PolyP-treated samples. Results represent the mean±SE from four separate experiments. The asterisk indicates a result that was significantly different, by the t-test ($p<0.05$).

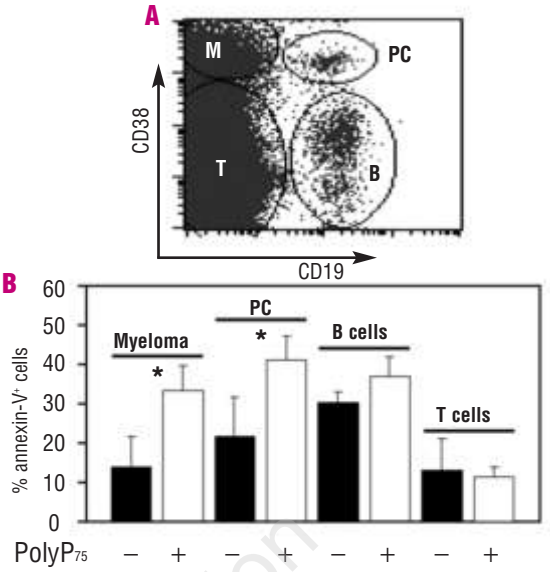

Figure 7. PolyP increases apoptosis specifically in myeloma primary cells. Isolated bone marrow mononuclear cells from four myeloma multiple patients were cultured for 16 hours in the absence or presence of 3 mM PolyP75. Cells were analyzed by flow cytometry after simultaneous labeling with anti-CD19 APC antibodies, anti-CD38 PE antibodies, and annexin-V FITC. (A) Representative CD19 CD38 dot plot showing mononuclear cell populations: T cells (T), B cells (B), plasma cells (PC), and myeloma cells (M). (B) Percentage of annexin V+ cells of cell populations represented in (A). *White bars*, control samples; *black bars*, PolyP-treated samples. Results represent the mean ± S.E from four separate experiments. Asterisks indicate results that were significantly different, by the T-test ($p<0.05$).

bone marrow aspirates from multiple myeloma patients (Figure 7). As described in the previous paragraph, we differentiated PC and myeloma populations using labeling with CD19 and CD38 (Figure 7A). Annexin labeling of these cells showed that polyP produced a significant and specific increase in the proportion of apoptotic PC and myeloma cells (Figure 7B).

Discussion

In this study we demonstrate that polyP inhibits Ig secretion and stimulates apoptosis specifically in human PC and myeloma cells. The apoptotic effects of polyP were specific for PC and myeloma cells (Figures 6, 7 and Table 1). Since total peripheral blood mononuclear cells, T cells, B cells, and non-lymphoid cell lines were not affected, we believe that polyP (or polyP-related molecules) could potentially be useful in the design of new anti-myeloma drugs.

Among several mechanisms of myeloma polyP-dependent apoptosis, we found that polyP induced externalization of phosphatidylserine, activation of caspase-3, arrest of the cell cycle in U266 cells (Figure 2), and an increment in the proportion of cells with apoptotic bodies (Figure 3). Nitrogen-bisphosphonates are currently used in the management of multiple myeloma,[21-23] and we hypothesized that the combination of bisphosphonates with polyP-related molecules could increase the effectiveness of conventional treatments. A potential clinical use for polyP was confirmed by the finding that the phosphate polymers were able to overcome the protective effects of IL-6 (Figure 4).

We have discovered that polyP polymers of 75 P_i residues appear to modify the survival of myeloma cells (Figure 1C). This size of polyP appears commonly in mammalian cell signaling, and it has been reported to stimulate mTOR kinase in breast cancer cells,[10] interact with fibroblast growth factors,[11] to be released by activated platelets,[7] and to modulate blood coagulation.[24] In addition, DdPPK2, the only protein found exclusively in eukaryotes that synthesizes polyP, produces molecules of around this size.[25]

Levels of plasma polyP may modulate humoral immune response during some pathological conditions or during bacterial infections. PolyP can accumulate massively in platelet granules,[7] and therefore plasma polyP concentrations could be increased by abnormal platelet activation or at the periphery of platelet-rich thrombi. We believe that basal plasma polyP could also have a considerable effect

on circulating PC in some specific physiologic conditions. In mammals, basal levels of PC in circulating blood are very low (around 0.05% of peripheral blood mononuclear cells) and the levels increase temporarily for only a few hours in response to antigens.[21] We propose that polyP could contribute in part to maintaining low levels of PC and/or to eradicating residual circulating PC after these have increased.

In conclusion, our results indicate, for the first time, the relevance of polyP to the humoral immune response and suggest the possible use of polyP as a novel therapy for myeloma. Further studies are necessary to decipher the mechanisms through which polyP affects the survival of normal and malignant PC.

LH-R performed the experiments; IG-G provided samples from tetanus toxoid-immunized individuals; CC performed experiments on the non-lymphoid lines; JAB assisted in the design of the study and manuscript preparation; FAR designed the study, wrote and submitted the manuscript. We thank Arthur Kornberg for the gift of E. coli CA38 pTrc PPX1, Carmen Segundo and Francisco Medina for assistance with the flow cytometry measurements and Veronica Rivas for advice on plasma cell cultures. This work was supported in part by the Spanish Ministerio de Ciencia y Tecnología (Grant BFU2004-06097), the Fondo de Investigacion Sanitaria (Grant PI052357), and the Plan Andaluz de Investigacion (Cod. CTS-554). The authors declare that they have no potential conflict of interest. Manuscript received February 15, 2006. Accepted June 26, 2006.

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