Osteoclasts support the survival of human plasma cells in vitro

Alexandrine Geffroy-Luseau, Gaëtan Jégo, Régis Bataille, Loïc Campion and Catherine Pellat-Deceunynck

1INSERM U601, Department of Cancer Research, Biology Institute, 9 Quai Moncousu, F-44000 Nantes, France
2Université de Nantes, Nantes Atlantique Universités, Nantes, France
3Centre de Lutte Contre Le Cancer Nantes Atlantique, Saint-Herblain, France

Keywords: dendritic cells, osteoclasts, plasma cells, survival

Abstract

The aim of this in vitro study was to evaluate if osteoclasts (OCs) and dendritic cells (DCs), both of monocyte origin, can support the survival of normal human plasma cells (PCs). PCs differentiate from plasmablasts (PBs) arising from activated B cells, essentially memory B cells. To study the survival of both PBs (CD20lowCD38highCD138neg) and PCs (CD20negCD38brightCD138bright), we generated pre-PBs (CD20lowCD38posCD138neg) from CD40-activated B cells (CD20highCD38negCD138neg) and cultured them on DCs or OCs in the presence of added IL-6. By quantitative and qualitative study, we showed that DCs support the survival of PBs and early PCs, but not that of PCs. In contrast, OCs support the survival of PBs, early PCs and PCs. PCs surviving on OCs 12 days after pre-PB input display phenotypic features of bone marrow PCs, CD138brightCD38brightHLA-DRlowCD45dim. The ability for OCs to support the survival of PCs was fully dependent on cell–cell contact and not inhibited by BCMA-Fc suggesting that secreted BAFF and APRIL were not involved.

Introduction

Plasma cells (PCs) are terminally differentiated B cells (1–4). PCs differentiate from plasmablasts (PBs) that arise from activated B cells, essentially memory B cells (5). Memory B cells are activated in secondary immune organs and differentiate into PBs that migrate to inflamed tissues, lamina propria and bone marrow, where they finally differentiate into PCs. Long-lived PCs that are the source of protective antibodies for extended periods of time are mainly located within the bone marrow (BM). Their number is stable, ~0.2% of mononuclear cells, revealing a tight regulation of their survival. There is growing evidence that in vivo PC survival depends on the ability of PCs to find a suitable niche and to remain in it (1, 2). Stromal cells and synoviocytes, all of mesenchymal origin, have been reported to support the survival of PBs and/or PCs independently or partly through an IL-6 secretion (6–10). BM stromal cells were more able to support PC survival than spleen or liver (8, 10). The molecules bases of this property of BM stromal cells have not been elucidated yet (10). On the other hand, dendritic cells (DCs) and T cells, both types of hematopoietic origin, have been reported to support the growth of murine lymph node PBs and the survival of human tonsil PCs (11, 12). Multiple myeloma (MM) cells, which are malignant-switched PCs, are mainly located within the bone marrow. Interaction of MM cells with bone marrow environment, stromal and osteoclast (OC) cells is essential for myeloma growth and survival (13). More recently, DCs were reported to support myeloma survival and DCs were found within myeloma infiltrates (14). In vitro studies have further proved that OCs support the long-term survival of myeloma cells through cell–cell contact (15–16). OCs that are of monocyte origin are located within the bone marrow where they control bone homeostasis hand in hand with osteoblasts (17). However, in rheumatoid arthritis, OCs are found within the inflamed synovial effusions, where they destroy the joints (18). Arthritic synovial effusions also contain an abnormally high number of immune cells, including differentiated PCs (19). Myeloid DCs, as OCs of monocyte origin, have also been reported to support myeloma cell growth in vitro (20). Since myeloma cells keep some bone marrow PC features, in particular that their survival depends on the bone marrow environment, OCs are very likely to support PC survival. The aim of this in vitro study was to evaluate if monocyte-derived cells, namely DCs and OCs, were able to support PB and/or PC survival.
Methods

Antibodies and reagents
Anti-CD20-FITC, anti-CD1a–APC, anti-CD38–APC and IgG1-APC control were from BD Biosciences (San Jose, CA, USA). Anti-CD45-FITC, anti-CD14–PE, anti-CD138–PE, anti-HLA-DR–PE, FITC-IgG1 control and PE-IgG1 control were from Beckman Coulter (Roissy, France). Blocking anti-CD40L mAb, rat anti-BCMA mAb, rat anti-TACI mAb and PE-conjugated polyclonal antibody to rat IgG were from Sigma–Aldrich (L’Isle d’Abeau Chesnes, France). The recombinant human IL-2, IL-10, RANKL, M-CSF, granulocyte macrophage colony-stimulating factor (GM-CSF), IL-4, IgG1-Fc and BCMA-Fc were obtained from R&D (Minneapolis, MN, USA). The recombinant IL-6 was from Novartis Pharmaceuticals (Basel, Switzerland). The neutralizing mAbs directed against CD11a were from Diaclone (Besançon, France), CD11b from Serotec (Cergy Saint-Christophe, France) and CD49d, CD50, CD54 and CD102 from R&D.

Generation of DCs and OCs
DCs and OCs were derived from peripheral blood of two different normal donors, obtained in agreement with current French laws and provided by the Etablissement Français du Sang. PBMCs were isolated by ficoll–hypaque centrifugation and cryopreserved. Thawed cells were seeded in 24-well plates (2.5 × 10^6 cells/10% FCS α-MEM) in 10% FCS α-MEM. For DC generation, adherent PBMCs were cultured 5 days with 10 ng ml^-1 IL-4 and 100 ng ml^-1 GM-CSF (21). After 5 days, cells displayed morphological and phenotypic characteristics of DCs (CD1a^posCD14^neg). For OC generation, PBMCs were cultured 10 days with 50 ng ml^-1 RANKL, 25 ng ml^-1 M-CSF and 10 nM dexamethasone (15). After 10 days, most cells showed the characteristics of OCs; they were large multinucleated cells and positive for TRAP staining. As described, purities of DC and OC population were always >90% as monitored by FACS analysis or TRAP staining, respectively (15, 21).

In all experiments comparing OC and DC effect on PB and PC survival, DCs and OCs were derived from the same PBMC donor but were performed from two badges since OC generation requires 5 days more than DC generation.

Generation of pre-PBs and co-cultures
B cells were isolated from tonsils obtained from children and adults undergoing routine tonsillectomy. Following isolation by ficoll–hypaque centrifugation and adherence, lymphocytes were cryopreserved. After thawing, T cells were removed by sheep rosetting (22) and CD27^+ B cells were sorted (MACS, Miltenyi Biotec, Paris, France).

Pre-PBs were generated in vitro from purified CD27^+ B cells, as initially described by Arpin et al. and slightly modified (23). Briefly, CD27^+ B cells were activated 3 days over a mitomycin-C-treated (45 min, 75 μg ml^-1) CD40L-expressing fibroblasts (kind gift of T. Defrance, Lyon, France) in presence of 50 U ml^-1 IL-2 and 100 ng ml^-1 IL-10. The cells were thereafter re-cultured for 2 days without fibroblasts, in presence of IL-2, IL-10 and blocking anti-CD40L mAb (2 μg ml^-1). Pre-PBs were purified by CD20 immunomagnetic depletion of remaining CD20^high B cells (Miltenyi Biotec).

Identification and count of PBs and PCs
PBs and PCs were identified by CD20, CD38 and CD138 expression with a FACScalibur (Becton Dickinson), as previously described (22, 24). A known number of CaliBRITE beads (BD biosciences) was added into each well before harvesting and the number of living PBs and PCs was calculated as a function of the bead ratio to live cells (25), see legend of Fig. 2. Enumeration of PCs rather than proportion of living PCs was required since in the presence of OCs, but not of DCs, no dead cells were found in the culture (suggesting that dead cells had been eliminated by OCs).

Co-culture assay, conditioned media and Ig secretion
Purified PBs (0.4 × 10^6 per well in 1 ml) were cultured in 10% FCS α-MEM supplemented with 10 ng ml^-1 IL-6, alone or co-cultured with DCs or OCs in their respective media. At the end of the co-cultures, PBs were recovered by thorough washing that occasionally detached OCs. Conditioned media (CM) of OCs and OC/PC co-cultures were, respectively, obtained by culturing OCs 2 days with RANKL and MScF with or without PCs (in presence of IL-6). To investigate contact dependency, PBs were cultured in 3-μm pore transwell inserts (Nunc) in OC-containing wells. IgG concentrations were determined by ELISA (22).

Statistical analysis
Group comparisons were performed by Wilcoxon rank-sum test or Student’s t-test and paired comparisons were performed by Wilcoxon signed-rank test or Paired Student’s t-test.

Results

Generation of DCs, OCs, pre-PBs and PCs
DCs and OCs were derived from monocytes as described within the Method. Monocytes cultured with GM-CSF and IL-4 displayed morphological and phenotypic characteristics of DCs (CD1a^posCD14^neg) (Fig. 1A). OCs were derived from monocytes cultured 10 days with RANKL, M-CSF and dexamethasone, as described (15). Indeed, most cells showed the characteristics of OCs; they were large multinucleated cells and positive for TRAP staining (Fig. 1A).

Pre-PBs were generated from tonsil CD27^+ B cells, as previously described (19, 22, 23). To obtain a pure population of pre-PBs (CD20^lowCD38^pos), remaining undifferentiated B cells (CD20^highCD38^neg) were removed by CD20 depletion (Fig. 1B).

Pre-PBs (CD20^lowCD38^pos) differentiated then into PBs (CD20^lowCD38^high), pre-PCS (CD20^+/CD38^highCD138^int to bright) and PCs (CD20^weakCD38^brightCD138^bright). As shown in Fig. 2(A), the survival of PBs and the appearance of pre-PCs were monitored through enumeration of CD38^high CD138^neg and CD38^brightCD138^int to bright, respectively...
Pre-PB differentiation was monitored in each culture to determine the cytokines required for DCs or OCs from playing any role. Pre-PB differentiation was monitored in each culture condition. GM-CSF and IL-4 or M-CSF and RANKL addition significantly increased: the MFIR of TACI and BCMA on PCs were 2.3 and 1.6, respectively. These data showed a residual expression of all three receptors that could account for BAFF–APRIL involvement of the survival of PCs on OCs. To further explore this hypothesis, BCMA-Fc was added to PB/OC co-cultures in order to neutralize both BAFF and APRIL. BCMA-Fc was added at day 2 or 5 and survival of PBs and PCs was analyzed at day 5 or 12, respectively. As shown in Table 1 and Fig. 2(A), OCs allowed pre-PBs to survive and mature into PCs that look like bone marrow PCs.

**BAFF–APRIL were not involved in OC-mediated PC survival**

Interestingly, OCs have been reported to produce high amounts of both APRIL and BAFF (28). BAFF binds to BAFF-R, APRIL to TACI and both cytokines bind to BCMA, the expression of which is reported to be essential for long-term PC survival in mice (29). Moreover, APRIL is known to interact with CD138 (30). In humans, BAFF was reported to increase survival of PBs (25). Therefore, we investigated BAFF-R, TACI and BCMA kinetic expression during PC generation from resting B cells up to PCs (Fig. 3A). BAFF-R expression peaked, respectively, on activated B cells (MFIR = 21 ± 4.7) and on pre-PCs (MFIR = 4.8 ± 1.8) and thereafter decreased: the MFIR of TACI and BCMA on PCs were 2 ± 1 and 1.6 ± 0.4, respectively. These data showed a residual expression of all three receptors that could account for BAFF–APRIL involvement of the survival of PCs on OCs. To further explore this hypothesis, BCMA-Fc was added to PB/OC co-cultures in order to neutralize both BAFF and APRIL. BCMA-Fc was added at day 2 or 5 and survival of PBs and PCs was analyzed at day 5 or 12, respectively. As shown in

**Osteoclasts support plasma cell survival**

Phenotypic analysis of surviving cells at day 5 (Fig. 2A) showed that both co-cultures enhanced the survival of both PBs (CD138\textsuperscript{neg}) and pre-PCs (CD138\textsuperscript{int} to bright). However, after 12 days, surviving cells expressed quite all brightly CD138, suggesting that surviving cells were now PCs (CD20\textsuperscript{neg}CD138\textsuperscript{bright}CD38\textsuperscript{int}). As shown in Fig. 2(B), in good agreement with high syndecan expression, CD38, CD20 and HLA-DR expression confirmed PC differentiation: CD38 was high, CD20 was negative, HLA-DR expression was very low and CD45 was reduced (Fig. 2B). This phenotype was similar to that of bone marrow PCs, but not to that of tonsil PCs, CD20\textsuperscript{pos}CD138\textsuperscript{neg}HLA-DR\textsuperscript{pos}, or of peripheral blood PCs, CD20\textsuperscript{neg}CD138\textsuperscript{neg}HLA-DR\textsuperscript{pos} (26, 27). Bone marrow PCs are considered to be the prototype of differentiated PCs in vivo. Our data show that OCs allowed pre-PBs to survive and mature into PCs that look like bone marrow PCs.

**OCTs were more efficient than DCs to support the survival of PCs**

We then investigated whether DCs and OCs were able to support the survival of differentiating PCs. Co-cultures of pre-PBs with DCs or OCs were performed in presence of IL-6 and analyzed after 5–12 days after pre-PB input. To prevent the cytokines required for DCs or OCs from playing any role, pre-PB differentiation was monitored in each culture condition. GM-CSF and IL-4 or M-CSF and RANKL addition to IL-6 did not modify the survival of PBs and PCs cultured alone (data not shown).

As shown in Table 1 and Fig. 2(A), co-culture of pre-PBs with DCs (in the presence of GM-CSF and IL-4) significantly enhanced the number of both PBs (CD138\textsuperscript{neg}) and pre-PCs (CD138\textsuperscript{int} to bright) at day 5 (median survival: 17%, range 4–55%, \(P = 0.018\)). Interestingly, DCs significantly enhanced cell survival over a short period of time, since at day 12 no surviving cells were recovered, 0% (0–0.1%), \(P = 0.0861\). In accordance with enhanced cell survival at day 5, IgG production was significantly higher in co-cultures with DCs, 80 \(\mu\)g ml\(^{-1}\) (range 40–109 \(\mu\)g ml\(^{-1}\)), than for pre-PBs alone, \(P = 0.028\). In contrast to DCs, OCs were able to enhance the number of surviving PBs and pre-PCs at day 5 (median survival: 20%, range 12–53%, \(P = 0.0077\)) and to sustain survival for at least 12 days in all experiments (median survival: 4%, range 0.4–10%, \(P = 0.018\)), Table 1 and Fig. 2(A). As expected, IgG production was significantly higher in co-cultures with OCs, 115 \(\mu\)g ml\(^{-1}\) (range 75–182 \(\mu\)g ml\(^{-1}\)), than for PBs alone (\(P = 0.018\) and than in co-cultures with DCs (\(P = 0.018\)).

**BAFF–APRIL were not involved in OC-mediated PC survival**

Interestingly, OCs have been reported to produce high amounts of both APRIL and BAFF (28). BAFF binds to BAFF-R, APRIL to TACI and both cytokines bind to BCMA, the expression of which is reported to be essential for long-term PC survival in mice (29). Moreover, APRIL is known to interact with CD138 (30). In humans, BAFF was reported to increase survival of PBs (25). Therefore, we investigated BAFF-R, TACI and BCMA kinetic expression during PC generation from resting B cells up to PCs (Fig. 3A). BAFF-R was expressed on resting B cells [mean fluorescence intensity ratio (MFIR) = 9 ± 1.4] and thereafter decreased (MFIR = 2.3 ± 0 on PCs). In contrast, TACI and BCMA expression peaked, respectively, on activated B cells (MFIR = 21 ± 4.7) and on pre-PCs (MFIR = 4.8 ± 1.8) and thereafter decreased: the MFIR of TACI and BCMA on PCs were 2 ± 1 and 1.6 ± 0.4, respectively. These data showed a residual expression of all three receptors that could account for BAFF–APRIL involvement of the survival of PCs on OCs. To further explore this hypothesis, BCMA-Fc was added to PB/OC co-cultures in order to neutralize both BAFF and APRIL. BCMA-Fc was added at day 2 or 5 and survival of PBs and PCs was analyzed at day 5 or 12, respectively. As shown in

**Osteoclasts support plasma cell survival**

Phenotypic analysis of surviving cells at day 5 (Fig. 2A) showed that both co-cultures enhanced the survival of both PBs (CD138\textsuperscript{neg}) and pre-PCs (CD138\textsuperscript{int} to bright). However, after 12 days, surviving cells expressed quite all brightly CD138, suggesting that surviving cells were now PCs (CD20\textsuperscript{neg}CD138\textsuperscript{bright}CD38\textsuperscript{int}). As shown in Fig. 2(B), in good agreement with high syndecan expression, CD38, CD20 and HLA-DR expression confirmed PC differentiation: CD38 was high, CD20 was negative, HLA-DR expression was very low and CD45 was reduced (Fig. 2B). This phenotype was similar to that of bone marrow PCs, but not to that of tonsil PCs, CD20\textsuperscript{pos}CD138\textsuperscript{neg}HLA-DR\textsuperscript{pos}, or of peripheral blood PCs, CD20\textsuperscript{neg}CD138\textsuperscript{neg}HLA-DR\textsuperscript{pos} (26, 27). Bone marrow PCs are considered to be the prototype of differentiated PCs in vivo. Our data show that OCs allowed pre-PBs to survive and mature into PCs that look like bone marrow PCs.

**OCTs were more efficient than DCs to support the survival of PCs**

We then investigated whether DCs and OCs were able to support the survival of differentiating PCs. Co-cultures of pre-PBs with DCs or OCs were performed in presence of IL-6 and analyzed after 5–12 days after pre-PB input. To prevent the cytokines required for DCs or OCs from playing any role, pre-PB differentiation was monitored in each culture condition. GM-CSF and IL-4 or M-CSF and RANKL addition to IL-6 did not modify the survival of PBs and PCs cultured alone (data not shown).

As shown in Table 1 and Fig. 2(A), co-culture of pre-PBs with DCs (in the presence of GM-CSF and IL-4) significantly enhanced the number of both PBs (CD138\textsuperscript{neg}) and pre-PCs (CD138\textsuperscript{int} to bright) at day 5 (median survival: 17%, range 4–55%, \(P = 0.018\)). Interestingly, DCs significantly enhanced cell survival over a short period of time, since at day 12 no surviving cells were recovered, 0% (0–0.1%), \(P = 0.0861\). In accordance with enhanced cell survival at day 5, IgG production was significantly higher in co-cultures with DCs, 80 \(\mu\)g ml\(^{-1}\) (range 40–109 \(\mu\)g ml\(^{-1}\)), than for pre-PBs alone, \(P = 0.028\). In contrast to DCs, OCs were able to enhance the number of surviving PBs and pre-PCs at day 5 (median survival: 20%, range 12–53%, \(P = 0.0077\)) and to sustain survival for at least 12 days in all experiments (median survival: 4%, range 0.4–10%, \(P = 0.018\)), Table 1 and Fig. 2(A). As expected, IgG production was significantly higher in co-cultures with OCs, 115 \(\mu\)g ml\(^{-1}\) (range 75–182 \(\mu\)g ml\(^{-1}\)), than for PBs alone (\(P = 0.018\) and than in co-cultures with DCs (\(P = 0.018\)).
Fig. 2. OCs, but not DCs, supported PC survival. (A) Enumeration of surviving PBs and PCs 5 or 12 days after input of pre-PBs. Pre-PBs were seeded alone or with DCs or OCs as indicated. At the end of co-cultures, PCs were detached by thorough washing of wells in order to recover all PCs (a significant proportion of OCs or DCs was recovered too during this process, gate R4). Enumeration of CD38<sup>high</sup> cells was performed by an addition of a known number of CaliBRITE beads (BD Biosciences) added in each well before harvesting. Cells were stained with anti-CD38–APC and anti-CD138–PE mAbs. To compare the number of PCs in the cultures, acquisition was stopped once 1000 events were accumulated in gate R2 (beads). FSC and SSC were in log scale in order to acquire simultaneously beads and cells. The number of CD38<sup>high</sup> cells was calculated as a function of the ratio of beads (R2) to live cells (R1). PBs (CD38<sup>high</sup>CD138<sup>neg</sup>) and PCs (CD38<sup>high</sup>CD138<sup>high</sup>) were identified in double staining with anti-CD38–APC and anti-CD138–PE mAbs. Dead PCs were identified by their altered morphology, reduced FSC and enhanced SSC defined by gate R3 (44). In the presence of OCs, but not of DCs, no dead PCs were found within the co-culture suggesting that OCs eliminated dead PCs (lack of PCs in gate R3). (B) Evolution of the phenotype of pre-PBs during differentiation and survival on OCs. The phenotype of PBs and PCs surviving on OCs was analyzed at days 5 and 12 in comparison to the one of pre-PBs (D0). The level of expression was defined as the MFIR (specific fluorescence/control fluorescence). When MFIR was inferior to 1.5, expression was considered as negative.

Fig. 3(B), the addition of 10 μg ml<sup>−1</sup> BCMA-Fc only slightly reduced the number of surviving cells at day 12 (30%), but this inhibition did not reach statistical significance (P = 0.10). Similarly, the production of IgG was not modified, P = 0.46 and P = 0.65 at days 5 and 12, respectively (Fig. 3C). Chimeric BCMA-Fc was efficient to neutralize both autocrine and paracrine activity of BAFF: indeed, BCMA-Fc strongly reduced (42%) the growth of RPMI8226 multiple myeloma cell line that is BAFF autocrine (31) and fully prevented the survival of PBs induced by 200 ng ml<sup>−1</sup> of BAFF as described by Avery <i>et al.</i> (25) (data not shown). These data strongly suggest that secreted APRIL and BAFF were not implied in the survival of PB/PC on OCs.

**Direct interactions were required between PBs and OCs**

To investigate whether the survival of progenitors and precursors of PCs in the presence of OCs depends on cell–cell
interaction and/or on soluble factors, pre-PBs were co-cultured either with OCs in a transwell system or in an OC CM. As shown in Fig. 4(A), the culture of pre-PBs without OC contact fully abrogated OC-mediated survival. After 5 days, the number of CD38bright cells was highly reduced (116% ± 25% of inhibition of PC survival, P < 0.05, n = 4), suggesting that close interactions are required. In good agreement with this finding, CM of OCs was not more potent than control medium: the number of CD38bright was 101% ± 34% of control, P > 0.05 (Fig. 4B). In order to further exclude that survival is supported by soluble factors released after contact between OCs and PBs, we further performed CM of OC/PB co-culture. Here again, OC/PC CM was not more potent than control medium, 80% ± 15%, P > 0.05 (Fig. 4B). Altogether, these experiments indicated that only close interactions with OCs allow pre-PBs to survive and differentiate.

Discussion

The aim of this in vitro study was to evaluate if OCs and DCs, both of monocyte origin, can support the survival of normal human PCs. PCs differentiate from PBs arising from activated B cells, essentially memory B cells (1–5). Differentiation of B cells into PCs is linked to proliferation (22, 32–34). Proliferation is induced either by antigen binding or by triggering CD40 or TLR. We used the classical CD40/IL-2 + IL-10 system to induce the proliferation and differentiation of tonsil CD27+ B cells. We generated pre-PBs (CD20highCD38brightCD138neg) from CD40-activated B cells (CD20highCD38brightCD138neg) and cultured them on DCs or OCs. To study the differentiation process, we monitored the appearance and survival of all successive types of cells, PBs (CD20lowCD38highCD138neg), pre-PCs (CD20negCD38brightCD138int to bright), and PCs (CD20negCD38brightCD138bright). We show that DCs supported the survival of ongoing differentiating cells but during a short period only. Indeed, DCs were unable to support the survival of PCs suggesting that DC-mediated cell survival was restricted to the PB and pre-PC stages. This ability of monocyte-derived DCs to increase PC differentiation had never been reported in humans. Our results are in good agreement with in vivo data in mice showing that CD11c DCs support the survival of PBs (11), although a more recent study reported that DC–PB interactions were dispensable for PC differentiation in T-independent type 2 immune responses (35).

Table 1. Compared survival of PBs and PCs on DCs and OCs

<table>
<thead>
<tr>
<th>CD38high cells, % of pre-PB input</th>
<th>Day 5</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Medium</td>
<td>DCs</td>
</tr>
<tr>
<td>Median</td>
<td>11</td>
<td>17</td>
</tr>
<tr>
<td>Range</td>
<td>4–17</td>
<td>4–55</td>
</tr>
<tr>
<td>P</td>
<td>0.018</td>
<td>0.0077</td>
</tr>
</tbody>
</table>

Survival of PBs and PCs was monitored, respectively, at days 5 and 12 as described in the Method and within the legend of Fig. 2. The median (and range) number of CD38high cells was expressed as the percentage of pre-PB input. Paired comparisons to control were performed by Wilcoxon signed-rank test (mean of two wells, seven independent experiments).

Fig. 3. BCMA-Fc did not prevent OC-mediated PC survival. Kinetics of BAFF-R, TACI and BCMA expression. BAFF-R, TACI and BCMA expression was analyzed by flow cytometry during the PC generation process, on resting CD27+ B cells (day –5), activated B cells (day –2), pre-PBs (day 0), PBs (day 2) and PCs (day 5). Cells were either directly (BAFF-R) or indirectly stained (TACI and BCMA). The level of expression was defined as the MFIR. Mean of five independent experiments. (B and C) Survival and Ig secretion of PBs and PCs in the presence of BCMA-Fc. Pre-PBs were cultured 5 (B) or 12 (C) days with OCs in the presence of 10 μg ml−1 control IgG1-Fc or 10 μg ml−1 BCMA-Fc. BCMA-Fc or IgG1-Fc was added at day 2 (B) or 5 (C). The total number of surviving CD38high cells was enumerated as described in the legend of Fig. 2. IgG secretion was determined by ELISA at day 5 (B) or 12 (C). Mean of three independent experiments performed in duplicate wells.
CD138neg cells were more frequent in the presence of DCs of proportion of CD138 neg versus CD138 pos indicates that display both shared and specific properties. Careful analysis of tonsil PCs or of peripheral blood PCs (26, 27). was clearly similar to that of bone marrow PCs, but not to that maintain minimal survival of pre-PBs cultured alone. Anyway, IL-6 alone (22,24). Exogenous IL-6 was added in order to main-
educed their proliferation with IL-2 but was poorly efficient involved in different contexts. We previously pointed out the essential role of IL-6 in the We previously pointed out the essential role of IL-6 in the survival mediated by synoviocytes or stromal cells also depends on cell–cell contact (6, 9). The molecules involved in the survival of PCs mediated through cell–cell contact have not been identified. Only CD49d–fibronectin interactions were reported to be essential for stromal-mediated survival of PCs (6). We observed that CD54 expression was increased on PCs surviving on OCs and that OCs expressed CD11b (data not shown), one of the counter receptors for CD54 (41). Increased expression of CD54 was mediated survival of PCs (6). We observed that CD54 expression peaked, respectively, on activated B cells and on pre-PBs. Pre-PCs displayed a residual expression of all three receptors. However, we showed that addition of BCMA-Fc did not modify significantly the survival of PBs and pre-PCs on OCs. It was also reported that bone marrow PCs did not express BAFF–APRIL receptors and that stromal cells do not secrete any BAFF or APRIL (36). The lack of effect of OC CM on PC survival, the weak expression of all BAFF–APRIL receptors on PCs and the lack of significant inhibition of PC survival by BCMA-Fc converged to suggest that OC secretion of BAFF–APRIL was not involved in PC survival. This observation is consistent with previous studies (25, 36, 37). In our hands, the survival of PB/pre-PCs mediated by DC did not seem to be supported through BAFF–APRIL too since addition of BCMA-Fc (at day 0) did not modify PB and pre-PC survival at day 5 (data not shown).

Since BCMA has been reported to be essential for in vivo PC survival in mice (29), our data could suggest that BAFF–APRIL might be implicated in PC retention within the bone marrow, rather than in direct survival. The treatment of patients with multiple myeloma with TACI–Ig induced a high reduction of polyclonal PCs (38). On the other hand, mobilization of resident bone marrow PCs was observed in physiological conditions, during secondary immune response to tetanus toxin in humans, showing that PCs are actively maintained within the bone marrow (39, 40).

Our experiments have demonstrated that only cellular interactions with OCs allow PBs to survive and differentiate. Similarly, it was reported that OCs support the survival of myeloma cells through cell-cell contact (15, 16) and that PC survival mediated by synoviocytes or stromal cells also depends on cell–cell contact (6, 9). However, the molecules involved in the survival of PCs mediated through cell–cell contact have not been identified. Only CD49d–fibronectin interactions were reported to be essential for stromal-mediated survival of PCs (6). We observed that CD54 expression was increased on PCs surviving on OCs and that OCs expressed CD11b (data not shown), one of the counter receptors for CD54 (41). Increased expression of CD54 was reported on bone marrow PCs as compared with PBs (4).

However, neutralizing antibodies directed against CD11a, CD11b, CD49d, CD50, CD54 and CD102 were unable to prevent PC survival on OCs, even in presence of BCMA-Fc (data not shown). On the other hand, CD44–hyaluronic acid interactions have been reported to increase the survival of murine PCs (42). However, the survival of murine or human PCs was not affected by anti-CD44 mAbs (9). In humans, CD44 was also not involved in the survival of PCs on stromal cells and one of the myeloma cells on OCs (6, 10, 15). We did not find too any effect of both hyaluronic acid and anti-CD44 mAb on the survival of human PCs (data not shown).

Since our data did not cover involvement of these molecules, other approaches are further required to identify the interactions involved in survival on DCs and OCs. One approach could be to identify the survival pathways of PC and by this way the molecules that could trigger the pathways.
Partner cells for PCs appear to be multiple, stromal cells, synoviocytes, T cells, DCs and OCs. PCs are located in different organs, bone marrow, lymphoid organs, inflamed tissues and lamina propria. It is tempting to speculate that PC partner will be different depending on the PC location. In all cases, direct cell–cell interactions are strictly required. The multiplicity of the type of PC partner could suggest either a specificity of partners depending on the nature of PCs or on the contrary a shared mechanism involving the same molecules. This question remains pending and will require further investigations. Interestingly, the survival of myeloma cells which are malignant PCs also requires close interactions with different partner cells including stromal cells, OCs and DCs (13–16, 18). This suggests that survival of myeloma cells and PCs could imply the same interactions.

Our data raise the hypothesis that OC could play a role in PC homeostasis within the bone marrow. To date, direct interactions between stromal cells and PC in mouse bone marrow have been reported by Tokoyoda et al. (43) suggesting that stromal cells are involved in the PC niche as they are in the B cell niche. Osteoblasts and OCs, which do regulate each other, are critical not only for bone homeostasis but also for hematopoiesis, especially for B cells. They could also play a role in PC long-term survival.

Funding
Ligue Nationale Contre Le Cancer (équipe labélisée 2005); Association pour la Recherche sur le Cancer to A.G.L.

Abbreviations
CM conditioned media
DC dendritic cell
GM-CSF granulocyte macrophage colony-stimulating factor
MFIR mean fluorescence intensity ratio
OC osteoclast
PB plasmablast
PC plasma cell
TRAP tartrate-resistant acid phosphatase

References
Osteoclasts support plasma cell survival


