Bcl-2+ Tonsillar Plasma Cells Are Rescued from Apoptosis by Bone Marrow Fibroblasts

By Pierre MerviHe,* Julie Déchanet,* Alexis Desmoulière,‡
Isabelle Durand,* Odette de Bouteiller,* Pierre Garrone,*
Jacques Banchereau,* and Yong-Jun Liu*

From *Schering-Plough Laboratory for Immunological Research, 69571 Dardilly, France; and
‡CNRS URA 1459, Institut Pasteur Lyon, 69365 Lyon

Summary

Plasma cells represent the final stage of B lymphocyte differentiation. Most plasma cells in secondary lymphoid tissues live for a few days, whereas those in the lamina propria of mucosa and in bone marrow live for several weeks. To investigate the regulation of human plasma cell survival, plasma cells were isolated from tonsils according to high CD38 and low CD20 expression. Tonsillar plasma cells express CD9, CD19, CD24, CD37, CD40, CD74, and HLA-DR, but not CD10, HLA-DQ, CD28, CD56, and Fas/CD95. Although plasma cells express intracytoplasmic Bcl-2, they undergo swift apoptosis in vitro and do not respond to CD40 triggering. Bone marrow fibroblasts and rheumatoid synoviocytes, however, prevented plasma cells from undergoing apoptosis in a contact-dependent fashion. These data indicate that fibroblasts may form a microenvironment favorable for plasma cell survival under normal and pathological conditions.

One of the most striking facts of the immune system is the presence of several milligrams of Ig (antibody) in every milliliter of human blood, which represents $10^{16}$ molecules displaying $10^9$ different specificities. Antibodies are secreted by plasma cells at a rate of $10^3$ antibody molecules/cell per s (1, 2). Plasma cells represent the final differentiation stage of B lymphocytes after they have encountered antigens and interacted with various cell types through both membrane molecules and cytokines (for review see reference 3). To avoid overproduction of antibodies, the number of plasma cells in the body must be tightly controlled. Two basic strategies have evolved within the immune system to control plasma cell number: (a) during humoral immune responses, not all activated B cells undergo terminal differentiation into antibody-secreting plasma cells, as many of them differentiate into memory B cells (4); and (b) the life span of plasma cells is tightly regulated. It has been shown that plasma cells generated during primary humoral immune responses are mainly located within the medullary cords of lymph nodes or in the red pulp of the spleen and have a life span of only a few days (5, 6). During secondary humoral immune responses, however, some generated plasma cells will migrate from the secondary lymphoid tissues into the bone marrow (BM)1 or into the lamina propria of mucosa (7), where they survive and secrete large amounts of antibodies for at least 3 wk (5).

Understanding the physiology of normal plasma cell survival represents an important question for both basic immunology and for pathophysiology of malignant myeloma and plasmacytoma. Although plasma cells have a distinct morphology, it has been difficult to isolate them from peripheral lymphoid tissues because of the lack of specific surface markers and their low frequency. After having isolated human B cell subsets representing naive B cells, germinal center (GC) B cells and memory B cells from tonsils (8-10), we examined the isolation and characterization of plasma cells (PC), the end point of B cell differentiation. The isolation was based on the observation that human plasma cells express high levels of CD38 and low levels of CD20 (11-13). As reported herein, human tonsillar plasma cells, which express intracellular Bcl-2 protein and lack surface Fas/CD95, die rapidly by apoptosis, a phenomenon that could not be prevented by antigen receptor or CD40 trig-

1Abbreviations used in this paper: BM, bone marrow; GC, germinal center; PC, plasma cells; SAC, Staphylococcus aureus strain Cowan I; TdT, terminal deoxynucleotidyl transferase; TUNEL, TdT-mediated dUTP-FITC nick end labeling.
ginger. Plasma cells, however, were rescued from apoptosis when cultured in contact with bone marrow fibroblasts.

Materials and Methods

Antibodies and Reagents. The mouse mAbs used for the phenotypic studies were purchased from the following sources: FITC-conjugated anti-CD3 (IOT3), anti-CD19 (IOB4), anti-CD20 (IOB20), anti-CD37 (IOB1), anti-Fas (UB2), unconjugated anti-CD9 (IOTB), and biotinylated anti-CD24 (IOB3) (Immunotech, Marseille, France); FITC-conjugated anti-CD10 (Calla), anti-CD14 (Leu M3), anti-HLA-DR, anti-HLA-DQ, and PE-conjugated anti-CD38 (Leu 17) (Becton Dickinson Monoclonal Center, Mountain View, CA); FITC-conjugated anti-CD28 (CLB, Amsterdam, The Netherlands); FITC-conjugated anti-Bcl-2 (Dako, Glostrup, Denmark); unconjugated anti-CD56 (Coulter Immunology, Hialeah, FL); unconjugated anti-CD74 (BU45) (Binding Site, Birmingham, UK). FITC-conjugated anti-CD40 (mAb 89) was produced in the laboratory, as previously described (19). For viable cell recovery, cells were counted from acquisition data.

Electron Microscopy. Plasma cells were enriched in the pellet. Cells were then labeled with GC and resting B cells used as comparison, were sorted with a FACStar Plus® (Becton Dickinson & Co., Sunnyvale, CA) equipped with a 4-W argon laser. Sorting was carried out at 4°C.

Flow Cytometric Analysis. Phenotypic analysis of plasma cells was performed by immunofluorescence flow cytometry on sorted CD38+ PC (stained with anti-CD38-PE), using either mouse mAbs conjugated with FITC or biotinylated or unconjugated mouse mAbs followed by FITC-conjugated sheep anti-mouse IgG(ab')2 or FITC-conjugated streptavidin. Negative controls were performed with isotype-matched, unrelated mAbs. For intracellular detection of Bcl-2 protein, cells were permeabilized by 15-min incubation at 4°C in saponin (0.5 mg/ml) before staining. Fluorescence was analyzed on a FACScan® flow cytometer (Becton Dickinson & Co.). Gating was set according to forward and right angle light scatter parameters to exclude subcellular particles from acquisition data.
sections (60–80 nm) were obtained with an ultramicrotome (Ultracut; Reichert, Vienna, Austria) contrasted with methanolic uranyl acetate and lead citrate, and observed with a transmission electron microscope (model CM 120; Philips Technologies, Cheshire, CT).

TdT-mediated dUTP–FITC Nick End Labeling. DNA fragmentation in apoptotic cells was detected according to the method described before (20). Briefly, 5 × 10⁵ cells obtained immediately after sorting or after 4 h of culture were fixed with 200 μl of PBS, 1% paraformaldehyde, for 10 min, then washed in PBS and permeabilized with 500 μl of 70% ethanol at −20°C overnight. After washing with PBS, the TdT-mediated dUTP–FITC nick end labeling (TUNEL) reaction was carried out by incubating cells at 37°C for 1 h with 0.3 nmol FITC-12-dUTP, 3 nmol dATP, 2 μl 25 mM CoCl₂, 25 U TdT, and TdT buffer (30 mM Tris, pH 7.2, 140 mM sodium cacodylate) in a total reaction volume of 50 μl (all reagents from Boehringer Mannheim Biochemicals, Indianapolis, IN). The reaction was stopped by adding 2 μl 0.5 M EDTA for 10 min at 4°C. After washing twice in PBS, 1% BSA, samples were analyzed by flow cytometry. Control staining was performed on aliquots of the same cells treated with the staining mixture without TdT.

Immunohistology. Tonsil pieces were snap frozen in liquid nitrogen and stored at −70°C. 5-μm frozen sections were cut and mounted on glass slides. They were dried at room temperature and fixed in cold acetone at −20°C for 15 min. Sections were washed in PBS and were incubated with peroxidasen conjugated anti-IgA (4 mg/ml; Southern Biotechnology Associates, Bir-

**TOTAL TONSILLAR CELLS**

![Diagram](image-url)

**Figure 1.** Isolation of the CD38⁺⁺⁺CD20⁻⁻⁻ tonsillar PC. Total tonsillar cells obtained either directly after tonsil digestion (A) or after three centrifugations over a 1.5% BSA solution (B) were stained with FITC-conjugated anti-CD20 (horizontal axis, log scale) and PE-conjugated anti-CD38 mAbs (vertical axis, log scale). CD38⁺⁺⁺CD20⁻⁻⁻ PC (C), CD38⁺⁺⁺CD20⁺⁺⁺ resting B cells (D), and CD38⁺⁺⁺CD20⁺⁺⁺ GC B cells (E) were sorted with a FACStar plus®. Numbers indicate the percentages of cells obtained in the corresponding inset (mean ± SEM of seven independent experiments).
mingham, AL) and unconjugated mouse anti-CD38 mAb (Im-
munotech) diluted 1:100 in PBS. After washes, slides were incu-
bated with sheep anti-mouse Ig (Binding Site) diluted 1:20 in
PBS containing 10% human serum. Slides were then washed
and incubated with mouse mAbs against alkaline phosphatase and al-
akine phosphatase complexes (APAAP; Dako). After a final
wash, peroxidase was developed with 3-amino-9-ethylcarbazole,
which gives a red color, and alkaline phosphatase was developed
by fast blue substrate, which gives a blue color.

Results

The CD38$_{\text{high}}$ CD20$_{\text{low}}$ Fraction of Tonsillar Cells Represents
Terminally Differentiated PC. Previous studies have charac-
terized human PC as CD38$_{\text{high}}$- and CD20$_{\text{low}}$-expressing
cells (11, 13). Cells with this phenotype were found to re-
represent only 1–2% of tonsillar cells (Fig. 1 A). Centrifuga-
tions of the tonsil cell suspension over a 1.5% BSA solution
at 10 g permitted us to enrich CD38$_{\text{high}}$CD20$_{\text{low}}$ cells up to
~8% (Fig. 1 B). This enrichment step subsequently al-
lowed us to isolate CD38$_{\text{high}}$CD20$_{\text{low}}$ cells by FACS® sort-
ing (Fig. 1 C). From the same tonsillar preparation (Fig. 1,
D and E), CD38$_{\text{medium}}$CD20$_{\text{high}}$ GC B cells and CD38$_{\text{low}}$
-CD20$_{\text{medium}}$ resting B cells (8, 9) were also collected and
analyzed in parallel with PC.

After Giemsa staining, CD38$_{\text{high}}$CD20$_{\text{low}}$ cells display a
typical PC morphology, including an eccentric nucleus, a
dark basophilic cytoplasm, and a pale Golgi compartment
(Fig. 2 A). In accordance with the morphological features
of PC, immunoenzymatic staining with anti-Igκ and anti-
Igλ antibodies showed that the sorted CD38$_{\text{high}}$CD20$_{\text{low}}$
cells contained high levels of intracytoplasmic Ig (Fig. 2 B),
in contrast to GC B cells and resting B cells (data not shown).

Electron microscopic study of sorted CD38$_{\text{high}}$CD20$_{\text{low}}$
cells reveals ultrastructural characteristics of PC showing
parallel arrays of rough endoplasmic reticulum, a distinct
juxtanuclear Golgi area, and many mitochondrias (Fig. 2 C).

CD38$_{\text{high}}$CD20$_{\text{low}}$ Tonsillar Plasma Cells Have a Distinct
Surface Phenotype. To determine tonsillar PC surface phe-
notype, these cells were sorted according to their very high
expression of CD38 (fluorescence intensity above log 10$^3$
Fig. 1) and were further stained with FITC-conjugated
mAbs. PC expressed B cell markers such as CD9, CD19,
CD24, CD37, CD40, CD74, and HLA-DR, but did not
express CD10 nor HLA-DQ (Fig. 3). By contrast to plas-
macytoma cells (21) and myeloma cells (22), CD28 and
CD56 were not detectable on tonsillar PC. The absence of
CD3$^+$ and CD14$^+$ cells confirmed the lack of T cells and
monocytes in the PC population.

Human Tonsils Contain Many IgA-secreting PC beneath the
Mucosal Epithelium. To functionally prove that the sorted
CD38$_{\text{high}}$CD20$_{\text{low}}$ cells are PC, they were cultured for 12 h,
and the spontaneous Ig secretion into culture medium was
measured by ELISA. As shown in Fig. 4, only PC were
able to produce significant amounts of IgA (mean ± SEM =
214 ± 37 ng/ml, n = 3) and IgG (293 ± 89 ng/ml), but
low levels of IgM (29 ± 9 ng/ml). The comparable levels
of secreted IgG and IgA were surprising since the majority
of tonsillar B cells express slgG but not slgA (10). Never-
theless, the isotype distribution in the culture supernatant
 correlated with that obtained after intracytoplasmic staining
of sorted PC (data not shown). Furthermore, double im-

![Image A](https://via.placeholder.com/150)

![Image B](https://via.placeholder.com/150)

![Image C](https://via.placeholder.com/150)
munohistological staining of tonsil sections with red anti-IgA and blue anti-CD38 further confirmed that ~40–50% of tonsillar CD38^{high} PC contained intracellular IgA (Fig. 5). Whereas most IgA⁺ PC (dark purple) were found beneath the mucosal epithelium (Fig. 5 A) and occasionally in the interfollicular areas (not shown), CD38^{high} PC (dark blue) found in GC were IgA⁻ (Fig. 5 B). The biological significance of such an anatomical distribution of PC in relation to their life span and survival is discussed later.

**Tonsillar PC Die Rapidly by Apoptosis During In Vitro Culture.** During our attempts to estimate survival of normal PC, we found that they died very rapidly in culture medium at 37°C. As shown in Fig. 6, PC lost their viability even more rapidly than did GC B cells, since only 22% of viable PC were recovered after 6 h and <5% after 24 h of culture. As expected (23), >60% of resting B cells remained viable after 24 h of culture. After 4 h of culture, numerous PC displayed apoptotic figures, with typical chromatin condensation and nuclear fragmentation, as revealed by Giemsa staining (Fig. 7 A) and electron microscopy (Fig. 7 B). The presence of parallel arrays of rough endoplasmic reticulum within the cytoplasm indicated that the apoptotic cells indeed derived from PC (Fig. 7 B). To provide molecular evidence that PC undergo apoptosis, a TUNEL method was used to detect DNA fragmentation (20). As shown in Fig. 8, only a small proportion of freshly sorted cells including resting B cells, GC B cells, and PC were labeled by dUTP-FITC. Strikingly, after only 4 h of culture, the majority of GC B cells (66.7%) and PC (80.3%) were labeled by dUTP-FITC, whereas only 12.2% of resting B cells were labeled.

**Tonsillar PC Express Bcl-2 but Not Fas/CD95.** The Bcl-2 gene product (24) and Fas/CD95 (25, 26) have been documented as playing important roles in the regulation of cell survival and death. Since the rapid onset of apoptosis in GC B cells correlates with their low expression of Bcl-2 (27) and high expression of Fas/CD95 (10, 28), the expression of intracellular Bcl-2 and surface Fas/CD95 was assessed by flow cytometry in PC in parallel with that of GC B cells and resting B cells. Interestingly, a large proportion of PC expressed Bcl-2 without displaying Fas/CD95 (Fig. 9), a finding apparently in contrast with their propensity to rapidly enter apoptosis.
Figure 5. Anatomical localization of tonsillar IgA⁺ PC and IgA⁻ PC. Tonsil sections were double stained with red anti-IgA and blue anti-CD38. (A) many double stained (dark purple) IgA⁺CD38⁺⁺ PC were found within the fibroblast networks beneath the basal mucosal epithelial cells. (B) the PC cells within a GC of the same section contain many single-stained (dark blue) IgA⁻CD38⁺⁺ presumptive PC. ×100.

Plasma Cells Are Rescued from Apoptosis by Contact with BM Fibroblasts but Not by Antigen Receptor or CD40 Triggering. Table 1 shows that SAC particles and anti-CD40 mAb, which markedly enhance the viability of GC B cells (23), are unable to sustain PC viability. Similarly, CD40 ligand-transfected L cells, anti-Ig antibodies, and cytokines involved in B cell activation (IL-2, IL-3, IL-4, IL-10, and TNF-α) were unable to maintain the viability of PC (data not shown). In addition, IL-6 at a concentration up to 250 ng/ml did not prevent PC death (Table 2). Together with the different patterns of Bcl-2 and Fas/CD95 expression of GC B cells and PC, these results indicate that the apoptosis/survival of both cell types is regulated through different mechanisms.

In vivo experiments, however, have shown that a proportion of PC generated within the secondary lymphoid organs migrates into BM (29) or lamina propria of the mucosa, where they survive for 3 wk (3). This suggests that survival signals for PC may be dependent on these microenvironments. In accordance with this hypothesis, purified human BM fibroblasts greatly improved the viability of cultured plasma cells (82.7% on fibroblasts vs. 16.3% in medium alone, Table 1) as well as GC B cells (89.5% of viable cells on fibroblasts vs. 27.5% in medium alone, Table 1). Since the inflammatory rheumatoid synovium is an ectopic site for PC accumulation (30), synovial fibroblasts (synoviocytes) were compared with BM fibroblasts for their

Figure 6. Tonsillar PC die rapidly in culture. 2 × 10⁴ sorted resting GC B cells and PC were cultured in medium alone for 24 h. Percentage of viable cells was determined at various time points by trypan blue dye exclusion. Results are expressed as mean ± SD of culture triplicates.

Figure 7. The dead PC show apoptotic figures. 2 × 10⁴ tonsillar PC were cultured for 4 h in culture medium. (A) Giemsa staining showing apoptotic PC with nuclear condensation and fragmentation. ×1,000. (B) An apoptotic PC with chromatin condensation shows arrays of rough endoplasmic reticulum under the electron microscope. ×11,800.
Figure 8. Detection of DNA fragmentation within apoptotic PC. PC, GC B cells, and resting B cells isolated from the same tonsils were cultured for 4 h in culture medium. The DNA fragmentation within these cells was analyzed by the TUNEL method (see Materials and Methods) before and after 4 h of culture. The maximal dUTP–FITC labeling of cells without TdT reaction are indicated by the dotted lines as negative controls. Horizontal and vertical axes illustrate log of fluorescence and relative cell number, respectively. Data shown are from one representative of three experiments.

Figure 9. Tonsillar PC express intracytoplasmic Bcl-2 but not surface Fas/CD95. PC, GC B cells, and resting B cells were stained with FITC-conjugated anti-Fas/CD95 mAb or with FITC-conjugated anti-Bcl-2 mAb after permeabilization of the cells with saponin. Histograms corresponding to each mAb (solid line) are superimposed on that of the negative control (dashed line) performed with an isotype-matched unrelated mAb. Horizontal and vertical axes illustrate log of fluorescence and relative cell number, respectively.

ability to maintain PC survival. Table 1 also shows that synoviocytes were able to potently rescue GC B cells and PC from apoptosis. The maintainance of PC survival by fibroblasts was dependent upon cell–cell contact, since PC died rapidly when separated from fibroblasts by a semipermeable membrane (data not shown).

To further investigate if the fibroblast-dependent PC survival is specific to BM and synovial fibroblasts, PC were also cultured on a monolayer of human lung fibroblasts or a monolayer of murine fibroblasts (L cells) for 12 h. Table 1 shows that human lung fibroblasts significantly enhance the viability of both PC and GC B cells, whereas the murine L cell line did not. The survival effect of human lung fibroblasts on PC is, however, always lower than that of either BM fibroblasts or synoviocytes.

In contrast with the recent study showing that IL-6 protects human BM PC from apoptosis (31), IL-6 and a wide range of cytokines used in the present study (IL2, IL3, IL4, IL10, TNF-α) did not protect tonsillar PC from apoptosis. To further investigate if fibroblast-dependent PC survival was mediated by IL-6 released by fibroblasts during a close cell–cell interaction, functional blocking antibodies against the IL-6 receptor gp80 chain were added into the coculture of PC and fibroblasts. Table 2 shows that anti–IL-6 re-
Table 1.  PC Are Rescued from Apoptosis by Fibroblasts

| Percentage of viable cells |
|---------------------------|
| Resting B Cells | GC B cells | PC |
|-----------------|------------|----|
| Medium          | 81.6 ± 3.8 | 27.5 ± 0.5 | 16.3 ± 0.5 |
| SAC             | 70.2 ± 10.9 | 45.3 ± 5.2 | 17.7 ± 1.0 |
| Anti-CD40       | 74.3 ± 6.2 | 44.2 ± 5.9 | 17.2 ± 0.2 |
| BM fibroblasts  | 97.4 ± 0.9 | 89.5 ± 0.7 | 82.7 ± 5.3 |
| Synoviocytes    | 97.2 ± 0.7 | 72.5 ± 12.2 | 63.4 ± 8.6 |
| Murine fibroblasts | ND       | 31.2 ± 2.6 | 21.6 ± 1.1 |
| Lung fibroblasts | ND       | 76.4 ± 1.7 | 51.6 ± 0.3 |

2 x 10^6 resting B cells, GC B cells, or PC were cultured in medium alone for 12 h or in the presence of SAC particles (0.05%), anti-CD40 mAb (G28.5, 5 µg/ml), and/or a monolayer of fibroblasts. Percentages of viable cells were determined by trypan blue dye exclusion.

*Mean ± SEM of three experiments.

Table 2.  The Fibroblast-dependent Plasma Cell Survival Is Independent of IL-6

| Percentage of viable cells |
|----------------------------|
| PC | GC |
|---------------------------|
| Plastic                    |
| Medium                     | 23.6 ± 2.9 | 44.4 ± 3.7 |
| IL-6                       | 27.3 ± 0.2 | 44.2 ± 0.1 |
| Synoviocytes               |
| Medium                     | 76.5 ± 3.8 | 86.8 ± 2.2 |
| Anti-IL-6 R                | 70.5 ± 4.1 | ND |

Culture conditions are described in the legend for Table 1. IL-6 was used at a final concentration of 40 ng/ml. Anti-IL-6 receptor gp80 antibody BR6 was used at 10 µg/ml. Data shown are from one representative of two independent experiments.

Discussion

Differentiation of B lymphocytes into PC mostly occurs within secondary lymphoid organs, where they represent a minor population compared with naïve B cells or GC B cells. Thus, our knowledge about PC is mainly derived from the phenotypic and functional studies of PC tumors, such as myelomas and plasmacytomas. Herein, we designed a procedure to prepare PC from human tonsils by sorting CD38<sup>high</sup>CD20<sup>low</sup> cells after enrichment by repeated centrifugations through a BSA gradient.

Surface phenotypic analysis shows that tonsillar PC express several pan-B cell markers such as CD19, CD37, and CD40. The expression of CD19 on PC is in contrast with the reported lack of CD19 on myeloma and plasmacytoma cell lines, which had led to a conclusion that normal PC may not express CD19 (32). CD19, however, was also recently found on normal PC isolated from BM (22, 33) and on those generated in vitro (17, 34). Thus, normal PC can be distinguished from malignant PC by their expression of CD19. In the same line, CD28 is expressed on plasmacytoma (21) and myeloma cells (33), but not on normal PC. Accordingly, CD28 may represent a potential marker for diagnosis of malignant PC tumors, and its function on malignant PC remains to be established. The expression of CD40 antigen on tonsillar PC complements the similar observation on BM PC (33) and myeloma cells (35). The function of CD40 antigen on PC remains to be determined as its ligation failed to rescue them from apoptosis (36).

A major finding of this study is the propensity of PC to undergo spontaneous apoptosis. The molecular regulation of PC apoptosis/survival appears different from that of GC B cells. First, PC express the intracytoplasmic Bcl-2 protein at a level comparable to that of resting B cells, whereas GC do not. The spontaneous apoptosis of Bcl-2<sup>+</sup> PC suggests that other survival/death genes may be involved (25). The Bc1-2 gene family presently contains at least seven genes (37), which represent control elements of the first checkpoint of apoptosis (38). A second checkpoint of apoptosis has recently been identified which is controlled by members of the IL-1β-converting enzyme gene family, including ICE, ICH-1, and CPP32 (38-40). It will therefore be interesting to determine the expression of these genes in PC either undergoing apoptosis or being rescued by fibroblasts. Second, the surface triggers involved in PC apoptosis/survival are obviously different from that of GC B cells. In contrast to GC B cells, PC do not express surface Fas/CD95, which triggering has been shown to induce apoptosis of activated B cells and T cells (41-43) and accelerate spontaneous apoptosis of GC B cells (10). Signals known to rescue GC from apoptosis, such as antigen receptor and CD40 triggering, had no effect on PC. However, direct contact of PC with BM or synovial fibroblasts represents an efficient survival signal for these cells. Interestingly, human lung fibroblasts, which are efficient to maintain GC B cell survival (44), are able to enhance PC survival to a limited extent. In addition, IgA<sup>+</sup> PC were found mostly within the connective tissue beneath the mucosa (Fig. 5). All these data suggest that fibroblasts and/or stromal cells may provide the microenvironments beneath the mucosa, within the BM and the rheumatoid synovium, that are favorable for the survival of plasma cells (45, 46). This finding is in accordance with a number of studies showing that GC B cells, peritoneal B cells, thymocytes, and T cells can be protected from rapid apoptosis by stromal cells or fibroblasts (18, 44, 47, 48). Although the molecular mechanism of stroma/fibroblast-mediated cell survival is currently unknown, these data suggest that PC survival depends on direct cell–cell contact, but not on cytokines such as IL-2, IL-3, IL-4, IL-6, IL-10, and TNF-α.

In conclusion, we have isolated normal tonsillar PC that...
display a rapid onset of apoptosis and a unique regulation of apoptosis/survival. Analysis of the surface and intracellular molecules that control PC survival may ultimately permit us to understand the pathogenesis of myeloma and plasmacytoma.

We thank J. Reyes and E. Garcia for FACS® sorting, I. Berger for preparation of ultrathin sections, G. Joly for ultrastructural pictures, S. Peyrol for expert help with electron microscopy, C. Van Kooten for CD40 ligand–transfected L cells, F. Brière and C. Arpin for helpful discussions, and N. Courbière and M. Vatan for editorial assistance.

P. Merville and J. Déchanet are the recipients of a grant from the Fondation Mérieux (Lyon, France).

Address correspondence to Y. J. Liu, Schering-Plough Laboratory for Immunological Research, 27 Chemin des Peupliers, B.P. 11, 69571 Dardilly Cedex, France. The present address for P. Merville and J. Déchanet is CNRS URA 1456, Université Bordeaux II, 33076, Bordeaux, France.

Received for publication 10 July 1995 and in revised form 21 August 1995.

References

1. Jerne, N.K. 1984. Idiotypic networks and other preconceived ideas. *Immunol. Rev.* 79:5-24.
2. Jerne, N.K. 1989. Opening speech for the 7th International Congress of Immunology. In *Progress in Immunology*. F. Nelchers et al., editors. Springer-Verlag. Berlin. 7:XXXIII–XXXIV.
3. Banchereau, J., and F. Rousset. 1992. Human B lymphocytes: phenotype, proliferation and differentiation. *Adv. Immunol.* 52:125-251.
4. Kosco, M.H., A.K. Szakal, and J.G. Tew. 1988. In vivo obtained antigen presented by germinal center B cells to T cells in vitro. *J. Immunol.* 140:354-360.
5. Ho, F., J.E. Lortan, I.C.M. MacLennan, and M. Khan. 1986. Distinct short-lived and long-lived antibody-producing cell populations. *Eur. J. Immunol.* 16:1297-1301.
6. Mäkelä, O., and G.J.V. Nossal. 1962. Autoradiographic studies on the immune response. II. DNA synthesis among single antibody-producing cells. *J. Exp. Med.* 115:231-244.
7. Benner, R., W. Hjømmsen, and J.J. Haaheim. 1981. The bone marrow: the major source of serum immunoglobulins, but still a neglected site of antibody formation. *Clin. Exp. Immunol.* 46:1-8.
8. Pascual, V., Y.-J. Liu, A. Magalski, O. de Bouteiller, J. Banchereau, and J.D. Capra. 1994. Analysis of somatic mutation in five B cell subsets of human tonsil. *J. Exp. Med.* 180:329-339.
9. Liu, Y.-J., O. de Bouteiller, C. Arpin, I. Durand, and J. Banchereau. 1995. Five human mature B cell subsets. In *In Vivo Immunology*. E. Heenen, M.P. Defresne, J. Boniver, and V. Geenen, editors. Plenum Press, New York. 289-294.
10. Liu, Y.-J., C. Barthélémy, O. de Bouteiller, C. Arpin, I. Durand, and J. Banchereau. 1995. Memory B cells from human tonsils colonize mucosal epithelium and directly present antigen to T cells by rapid upregulation of B7.1 and B7.2. *Immunity.* 2:238-248.
11. Stashenko, P., L.M. Nadler, R. Hardy, and S.F. Schlossman. 1981. Expression of cell surface markers after human B lymphocyte activation. *Proc. Natl. Acad. Sci. USA.* 78:3848-3852.
12. Anderson, K.C., M.P. Bates, B.L. Slughenhoupt, G.S. Pinkus, S.F. Schlossman, and L.M. Nadler. 1984. Expression of human B cell-associated antigens on leukemias and lymphomas: a model of human B cell differentiation. *Blood.* 63:1424-1433.
13. Ling, N.R., I.C.M. MacLennan, and D. Mason. 1987. B-cell and plasma cell antigens: new and previously defined clusters. In *Leucocyte Typing III*. A.J. McMichael, editor. Oxford University Press. Oxford, UK. 302-335.
14. Vallé, A., C.E. Zuber, T. Defrance, O. Djossou, M. De Rie, and J. Banchereau. 1989. Activation of human B lymphocytes through CD40 and interleukin 4. *Eur. J. Immunol.* 19:1463-1467.
15. Clark, E.A., and J.A. Ledbetter. 1986. Activation of human B cells mediated through two distinct cell surface differentiation antigens, Bp35 and Bp50. *Proc. Natl. Acad. Sci. USA.* 83:4494-4498.
16. Moreau, I., V. Duvert, C. Caux, M.-C. Galmiche, P. Charbord, J. Banchereau, and S. Saeland. 1993. Myofibroblastic stromal cells isolated from human bone marrow induce the proliferation of both early myeloid and B lymphoid cells. *Blood.* 82:2396-2405.
17. Déchanet, J., P. Merville, I. Durand, J. Banchereau, and P. Miossec. 1995. The ability of synovocytes to support terminal differentiation of activated B cells may explain plasma cell accumulation in rheumatoid synovium. *J. Clin. Invest.* 95:456-463.
18. Gregory, C.D., C.F. Edwards, A. Milner, J. Wiels, M. Lipinski, M. Rowe, T. Tursz, and A.B. Rickinson. 1988. Isolation of a normal B cell subset with a Burkitt-like phenotype and transformation in vitro by Epstein-Barr virus. *Int. J. Cancer.* 42:213-220.
19. Defrance, T., B. Vanbervliet, J.P. Aubry, and J. Banchereau. 1988. Interleukin 4 inhibits the proliferation but not the differentiation of activated human B cells in response to interleukin 2. *J. Exp. Med.* 168:1321-1337.
20. Sgonc, R., G. Boeck, H. Dietrich, J. Gruber, H. Recheis, and G. Wick. 1994. Simultaneous determination of cell surface antigens and apoptosis. *Trends Genet.* 10:37-67.
21. Kozbor, D., A. Moreta, H.A. Messner, L. Moretta, and C.M. Croce. 1987. Tp44 molecules involved in antigen-independent T cell activation are expressed on human plasma cells. *J. Immunol.* 138:4128-4132.
22. Harada, H., M.M. Kawano, N. Huang, Y. Harada, K. Iwato, O. Tanabe, H. Tanaka, A. Sakai, and A. Kuramoto. 1993. Phenotypic difference of normal plasma cells from manure myeloma cells. *Blood.* 81:2658–2663.

23. Liu, Y.-J., D.E. Joshua, G.T. Williams, C.A. Smith, J. Gordon, and I.C.M. MacLennan. 1989. Mechanisms of antigen-driven selection in germinal centers. *Nature (Lond).* 342:929–931.

24. Reed, J.C. 1994. Mini-review: cellular mechanisms of disease series. Bcl-2 and the regulation of programmed cell death. *J. Cell Biol.* 124:1–6.

25. Korsmeyer, S.J., J.R. Shutter, D.J. Veis, D.E. Merry, and Z.N. Oltvai. 1993. bcl-2 protein expression is widespread in the developing nervous system and retained in the adult PNS. *Semin. Cancer Biol.* 4:327–332.

26. Nagata, S., and T. Suda. 1995. Fas and Fas ligand: apoptosis regulator of cell death and survival. *Annu. Rev. Immunol.* 12:881–922.

27. Kurosaka, M., and M. Ziff. 1994. The Bcl-2 family of proteins: regulators of cell death and survival. *Trends Cell. Biol.* 4:399–404.

28. Müller, P., C. Henne, F. Leithäuser, A. Eichelmann, A. Schmidt, S. Brüderlein, J. Dhein, and P.H. Krammer. 1993. Coregulation of the APO-1 antigen with intercellular adhesion molecule-1 (CD54) in tonsillar B cells and coordinate expression in follicular center B cells and in follicle center and mediastinal B-cell lymphomas. *Blood.* 81:2067–2075.

29. Tew, J.G., R.-M. DiLosa, G.F. Burton, M.H. Kosco, L.I. Kupp, A. Masuda, and A.K. Szakal. 1992. Germinal centers and antibody production in bone marrow. *Immunol. Rev.* 126:99–112.

30. Kurosaka, M., and M. Ziff. 1983. Immunoelectron microscopic study of the distribution of T cell subsets in rheumatoid synovium. *J. Exp. Med.* 158:1191–1210.

31. Kawano, M.M., K. Mihara, N. Huang, T. Tsujimoto, and A. Kuramoto. 1995. Differentiation of early plasma cells on bone marrow stromal cells requires interleukin-6 for escaping from apoptosis. *Blood.* 85:487–494.

32. Nadler, L.M., K.C. Anderson, G. Marti, M. Baytes, E. Park, J.F. Daley, and S.F. Schlossman. 1983. B4, a human B lymphocyte-associated antigen expressed on normal, mitogen activated, and malignant B lymphocytes. *J. Immunol.* 131:244–250.

33. Paglia, C., B. Bataille, N. Robillard, J.-L. Harousseau, M.-J. Rapp, N. Uge-Morinoue, J. Wijdenes, and M. Amiot. 1994. Expression of CD28 and CD40 in human myeloma cells: a comparative study with normal plasma cells. *Blood.* 84:2597–2603.

34. Merville, P., J. Dechanet, G. Grouard, I. Durand, and J. Banchereau. 1995. T cell-induced B cell blasts differentiate into plasma cells when cultured on bone marrow stroma with IL3 and IL10. *Int. Immunol.* 7:635–643.

35. Westendorf, J.J., G.J. Ahmann, R.J. Armitage, M.K. Spriggs, J.A. Lust, P.R. Greipp, J.A. Katzmann, and D.F. Jelinek. 1994. CD40 expression in malignant plasma cells. Role in stimulation of autocrine IL-6 secretion by a human myeloma cell line. *J. Immunol.* 152:117–128.

36. Banchereau, J., F. Bazan, D. Blanchard, F. Brière, J.-P. Galizzi, C. van Kooten, Y.-J. Liu, F. Roussset, and S. Saeland. 1994. The CD40 antigen and its ligand. *Annu. Rev. Immunol.* 12:881–922.

37. Nunez, G., and M.F. Clarke. 1994. The Bcl-2 family of proteins: regulators of cell death and survival. *Science (Wash. DC).* 254:305–315.

38. Oltvai, Z.N., and S.J. Korsmeyer. 1994. Checkpoints of development and cell death. *Science (Wash. DC).* 267:28–37.

39. Wang, L., M. Muria, L. Bergeron, H. Zhu, and J. Yuan. 1994. Ich-1, and lce/ced-3-related gene, encodes both positive and negative regulators of programmed cell death. *Cell.* 78:739–750.

40. Fernandes-Alnemri, T., G. Litwack, and E.S. Alnemri. 1994. CPP32, a novel human apoptotic protein with homology to Caenorhabditis elegans cell death protein Ced-3 and mammalian interleukin-1β-converting enzyme. *J. Biol. Chem.* 269:30761–30764.

41. Trauth, B.C., C. Klas, A.M. Peters, S. Matzku, P. Moler, W. Falk, K.M. Debatin, and P.H. Krammer. 1989. Monoclonal antibody-mediated tumor regression by induction of apoptosis. *Science (Wash. DC).* 245:301–305.

42. Garrone, P., E.M. Neidhardt, E. Garcia, L. Calibert, C. van Kooten, and J. Banchereau. 1995. Fas ligation induces apoptosis of CD40-activated human B lymphocytes. *J. Exp. Med.* 182:1265–1273.

43. Lagresle, C., C. Bella, T. Daniel, P.H. Krammer, and T. DeFrance. 1995. Regulation of germinal center B cell differentiation. Role of the human APO-1/Fas (CD95) molecule. *J. Immunol.* 154:5746–5756.

44. Holder, M.J., Y.-J. Liu, T. DeFrance, L. Flores-Romo, I.C.M. MacLennan, and J. Gordon. 1991. Growth factor requirements for the stimulation of germinal center B cells: evidence for an interleukin 2-dependent pathway of development. *Int. Immunol.* 3:1243–1251.

45. DiLosa, R.M., K. Maeda, A. Masuda, A.K. Szakal, and J.G. Tew. 1991. Germinal center B cells and antibody production in the bone marrow. *J. Immunol.* 146:4071–4077.

46. Hibi, T., and H.-M. Dosch. 1986. Limiting dilution analysis of the developing nervous system and retained in the adult PNS. *Cell.* 79:189–192.

47. Eck, L.M., and M. Ziff. 1983. Ich-1, and Ich/ced-3-related gene, encodes both positive and negative regulators of programmed cell death. *Cell.* 78:739–750.

48. Scott, S., F. Pandolfi, and J.T. Kurnick. 1990. Fibroblasts mediate T cell survival: a proposed mechanism for retention of primed T cells. *J. Exp. Med.* 172:1873–1876.